

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/263042906>

Pyrroloquinoline Quinone-Dependent Enzymatic Bioanode: Incorporation of the Substituted Polyaniline Conducting Polymer as a Mediator

ARTICLE in ACS CATALYSIS · JUNE 2014

Impact Factor: 9.31 · DOI: 10.1021/cs500442b

CITATIONS

4

READS

47

2 AUTHORS:



Shelley Minteer

University of Utah

244 PUBLICATIONS 4,572 CITATIONS

[SEE PROFILE](#)



Shuai Xu

University of Utah

14 PUBLICATIONS 143 CITATIONS

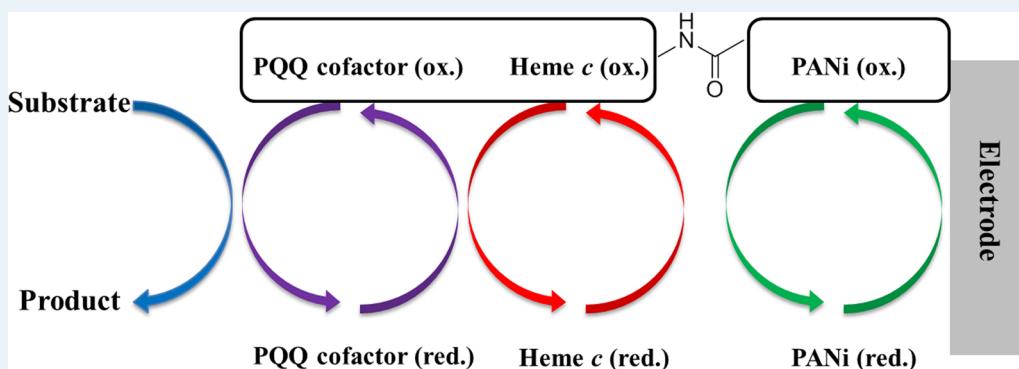
[SEE PROFILE](#)

Pyrroloquinoline Quinone-Dependent Enzymatic Bioanode: Incorporation of the Substituted Polyaniline Conducting Polymer as a Mediator

Shuai Xu and Shelley D. Minteer*

Departments of Chemistry and Materials Science & Engineering, University of Utah, 315 South 1400 East, Room 2020, Salt Lake City, Utah 84112, United States

 Supporting Information



ABSTRACT: One of the main technological issues with enzymatic biofuel cells and biosensors is improving the electron transfer between the enzyme and the current collector to improve current densities. In this study, we show the use of a conducting polymer to mediate pyrroloquinoline quinone-dependent enzymatic bioelectrocatalysis. A self-doped polyaniline (PANi) film is electropolymerized on a Toray carbon paper electrode surface to covalently bond enzymes to this three-dimensional interface. Sulfonic acid groups are introduced into the PANi backbone structure to increase the polymer conductivity at neutral pH via a self-doping process, and the carboxyl groups can be activated to covalently bond to enzymes. The electropolymerization of 2-methoxyaniline-5-sulfonic acid and 3-aminobenzoic acid is optimized with respect to the rate of the bioelectrocatalytic conversion of enzyme substrates. Comparing this PANi conducting copolymer enzyme immobilization technique with the hydrophobically modified Nafion encapsulation-based enzyme immobilization method showed a 9.8-fold increase in current density.

KEYWORDS: *conducting polymer, polyaniline, bioelectrocatalysis, PQQ-dependent dehydrogenases, enzyme immobilization, biofuel cell*

INTRODUCTION

Conjugated polymers have been extensively studied for application in diverse fields such as electronics,¹ energy storage,² catalysis,³ chemical sensing,⁴ and corrosion control.⁵ The biocompatible nature of conjugated electro-active polymers, such as polyphenylene, polyaniline, and polypyrrole, also provides a suitable basis for the construction of electrodes with efficient electrochemical communication with biocatalysts.⁶ Conjugated polymers have shown many unique electronic properties, such as electric conductivity,⁷ low ionization potential,⁸ and high electron affinity,⁹ due to the π -electron rich backbone structure of the polymers. Polyanilines (PANis), a member of the family of polyaromatics, are a popular conducting polymer, because of their simple synthesis, unique conduction mechanism, and good environmental stability in the presence of oxygen and water.¹⁰

Conducting polymer functionalization allows for the introduction of anionic and cationic groups linked to the polymer backbone, which alter the functionality of the polymeric materials. For example, via the introduction of functional groups such as

hydroxyl or sulfonic acid directly or bound with a spacer to the aromatic ring of polyaniline structure, so-called “self-doped” polyanilines are formed.¹¹ In those self-doped polyaniline structures, the protonation of the parent polyemeraldines can be performed via the exchange of a proton from the functional groups instead of the external chemical environment and thus greatly expands the pH range over which the polymers remain conductive. Among those self-doped polyanilines, sulfonated polyaniline is popular because the sulfonic acid group is a strong acid, and because there is a large variety of sulfonated aromatic amines commercially available.¹² Via the introduction of sulfonic groups into polyaniline parent structures, the polymers show stable electrical conductivity when treated with aqueous solutions at pH ≥ 4 .¹³

Stable and convenient immobilization of enzymatic biocatalysts on conducting surfaces (electrodes) with complete retention of

Received: April 3, 2014

Revised: May 28, 2014

Published: June 2, 2014



their catalytic properties is a crucial problem for the commercial development of biosensors or biofuel cells.¹⁴ Self-doped polyanilines that provide fair conductivity (1–10 S/cm) at neutral pH are capable of forming an appropriate environment for enzyme immobilization at an electrode surface and providing electrical interactions with metallic or carbon electrode surfaces. Most of the conventional procedures for biomolecule immobilization such as entrapment and encapsulation in gels or membranes suffer from a low bioelectrocatalytic rate,¹⁵ because the process is limited by the rate of transfer of an electron from the biocatalyst to the electrode surface. This problem can be solved by introducing carboxyl groups into the PANi parent structure that can be activated to covalently bond to the primary amine groups of the enzyme molecules. Schubart et al. have prepared a polyaniline copolymer that contains both sulfonyl groups and carboxyl groups via a potentiostatic method.¹⁶ This copolymer has displayed the ability to electrochemically communicate with pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase (PQQ-GDH) through a self-doping process. PQQ-GDH is a commercially available quinoprotein but contains no heme for improving communication with the electrode.

Quinohemoproteins from acetic acid bacteria (e.g., *Gluconobacter*) have been shown to be interesting anodic bioelectrocatalysts because of their high level of promiscuity.¹⁷ This allows them to be used in minimal enzyme cascades for deep oxidation of biofuels, such as glycerol and glucose, which are common fuels being considered for enzymatic biofuel cells. To investigate the electrochemical communication between the carbon electrode and quinohemoproteins, such as PQQ-dependent alcohol dehydrogenase and PQQ-dependent aldehyde dehydrogenase, and compare this enzyme immobilization technique with the previously used encapsulation method, we prepared enzymatic bioanodes with a polyaniline linking layer. One sulfonated aniline derivative [2-methoxyaniline-5-sulfonic acid (MASA)] and one carboxylated aniline derivative (3-aminobenzoic acid) were co-electropolymerized on a carbon fiber electrode via cyclic voltammetry, as shown in Figure 1. The electropolymerization was characterized and

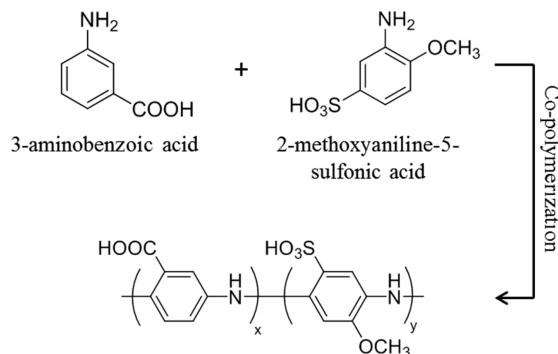


Figure 1. Schematic illustration of the copolymerization of 3-aminobenzoic acid (ABA) and 2-methoxyaniline-5-sulfonic acid (MASA).

optimized; the electron transfer mechanism was studied, and the enzyme-modified bioanode was characterized and compared to a commonly used encapsulation method [tetrabutylammonium bromide (TBAB)-modified Nafion immobilization method] to show the improved performance from this self-doped conducting polymer matrix.

EXPERIMENTAL SECTION

PQQ-Dependent Enzyme Extraction. *Gluconobacter* sp. (DSM 3504) was cultivated aerobically in a basal medium

containing yeast extract, D-mannitol, (NH₄)₂HPO₄, and MgSO₄·7H₂O; 5 g of *Gluconobacter* was suspended in 50 mM MES buffer (pH 6.5), 1 mM CaCl₂, 10% CHAPS (to a final concentration of 0.5%), and 1 mL of lysozyme [10 mg of lysozyme in 1 mL of 0.2 M phosphate buffer (pH 7.2) with 1 mM CaCl₂]. The solution was incubated at 4 °C while being gently stirred for 1 h followed by ultrasonication using a sonic dismembrator for 2 min at 4 °C. The insoluble materials in suspension were removed by centrifugation. The resulting enzyme lysate was used directly for enzyme immobilization, because this is the form in which these promiscuous enzymes are frequently used in biofuel cells,^{17,18} because they are more stable than purified individual enzymes.

Preparation of a Polymer Film on a Carbon Electrode.

Toray paper electrodes (1 cm × 1 cm) were cleaned by sonication in methanol for 45 min (3 × 15 min) and dried in air. Cleaned electrodes were equilibrated in a 1 M H₂SO₄ solution containing 0.1 M KNO₃ before polymerization. Electropolymerization was performed via cyclic voltammetry with different numbers (5, 15, and 30) of scans and scan windows (0–0.8 and 0–0.9 V). Cyclic voltammetry was performed in a solution with a total concentration of 0.1 M aniline derivatives with a scan rate of 100 mV/s. A Ag/AgCl electrode was used as reference electrode, and a platinum mesh electrode was used as counter electrode.

Copolymer Carboxyl Group Activation and Enzyme Immobilization.

For covalent coupling with the PQQ-dependent enzymes, the carboxyl groups of 3-aminobenzoic acid were activated with 25 mM N-hydroxysuccinimide (NHS) and 100 mM N-[3-(dimethylamino)propyl]-N'-ethylcarbodiimide hydrochloride

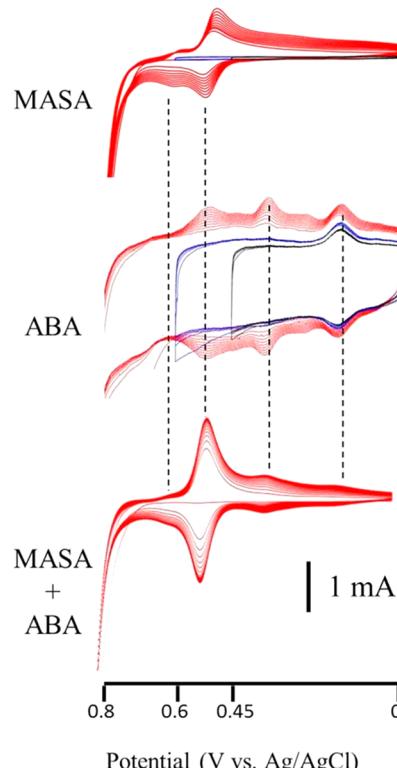


Figure 2. ABA and MASA electrochemical copolymerization study. Black lines represent the 0–0.45 V scan window, blue lines the 0–0.6 V scan window, and red lines the 0–0.8 V scan window. All measurements were performed in a 1 M H₂SO₄ solution with 0.1 M KNO₃, at a scan rate of 100 mV/s.

(EDC) in 50 mM MES buffer (pH 6.5) for 15 min. Electrodes were transferred to a 5 mg/mL enzyme solution immediately after the activation process and incubated overnight. Finally, the electrodes were washed three times with 50 mM MES buffer containing 1 mM CaCl_2 and stored at 4 °C until they were used.

Activity Assay of Immobilized Enzymes. A dichlorophenolindophenol (DCPIP) assay was used to investigate the activity of the immobilized enzyme on modified Toray paper electrodes. Assays were conducted as described previously.¹⁸ Ethanol, glucose, glycerol, acetaldehyde, and glyceraldehyde were used as substrates. Fully modified electrodes were immersed in the reaction mixture for 4 min, and the absorbance change at 600 nm (ΔA_{600}) was recorded. Enzyme activities per unit area were calculated.

Tetrabutylammonium Bromide (TBAB)-Modified Nafion Enzyme Immobilization. TBAB-modified Nafion membrane suspensions were prepared as described previously.¹⁹ TBAB-modified Nafion electrodes were prepared by drop-casting 100 μL of a 1:1 (v:v) enzyme solution/TBAB-modified Nafion suspension (final protein concentration of 10 mg/mL, which is the TBAB-modified Nafion protein capacity limit) to a Toray paper electrode. Electrodes were dried under a fan overnight before being tested.

Electrochemical Measurements. Electropolymerization was performed on a Digi-Ivy DY2300 potentiostat, and

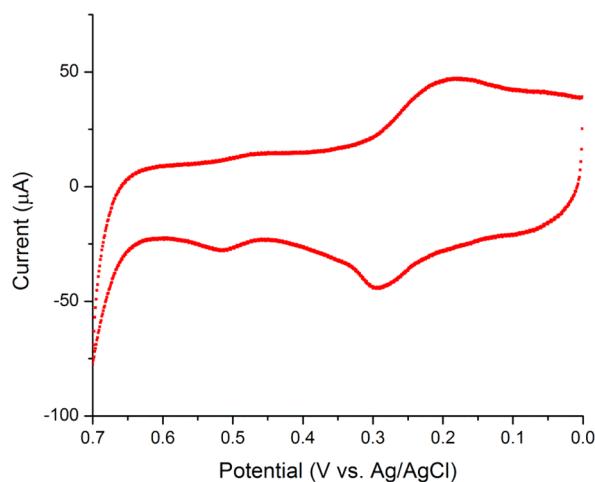


Figure 3. Representative cyclic voltammogram of a MASA and ABA polyaniline-polymerized Toray electrode in neutral pH buffer [50 mM MES buffer (pH 6.5) and 0.1 M KNO_3], at a scan rate of 100 mV/s.

Table 1. Results of Immobilized Activity Assays with Electrodes with the Polyaniline Immobilization Technique and the TBAB-Modified Nafion Enzyme Immobilization Technique with Different Substrates^a

substrate	PANI (units/cm ²)	TBAB-modified Nafion (units/cm ²)
control	0.1 ± 0.04	0.05 ± 0.02
EtOH	0.9 ± 0.03	1.5 ± 0.2
glucose	0.9 ± 0.03	1.3 ± 0.1
glycerol	0.3 ± 0.06	0.5 ± 0.04
acetaldehyde	0.6 ± 0.03	0.9 ± 0.1
glyceraldehyde	1.1 ± 0.04	1.3 ± 0.1

^aControl is the polymerized electrode without enzyme loading. EtOH was used as a substrate in the control. An enzyme unit is defined as the amount of enzyme that catalyzes the conversion of 1 μmol of substrate/min.

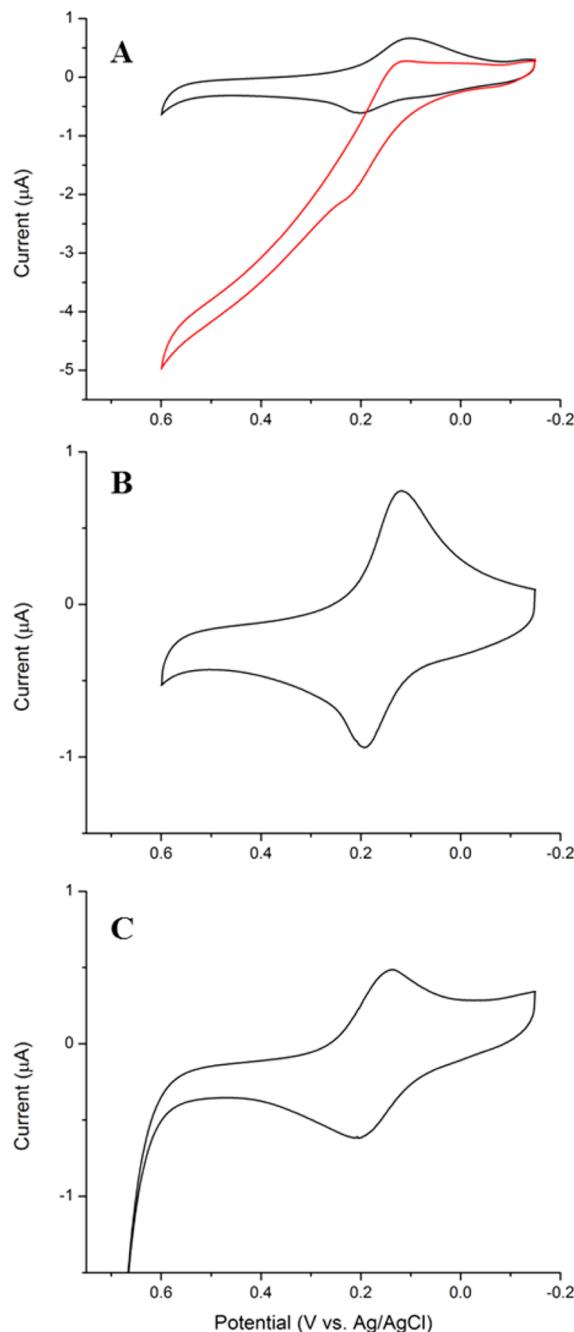


Figure 4. (A) Cyclic voltammogram of the enzyme-immobilized electrode in MES buffer (black) and cyclic voltammogram of the same electrode upon addition of 10 mM ethanol (red). (B) Cyclic voltammogram of the BSA-immobilized electrode in buffer. (C) Cyclic voltammogram of the NHS/EDC-activated electrode in buffer. Measured in 50 mM MES buffer (pH 6.5) and 0.1 M KNO_3 , at a scan rate of 0.5 mV/s.

electrochemical measurements of the modified electrodes were performed on a CH Instruments 1030 potentiostat. Platinum and Ag/AgCl electrodes were used as the counter and reference electrodes, respectively. All polymerizations were performed in a 1 M H_2SO_4 solution containing 0.1 M KNO_3 , and all electrochemical measurements of modified electrodes were performed in 50 mM MES buffer (pH 6.5) containing 0.1 M KNO_3 . All experiments were performed in triplicate, and reported uncertainties correspond to one standard deviation. All figures show representative data.

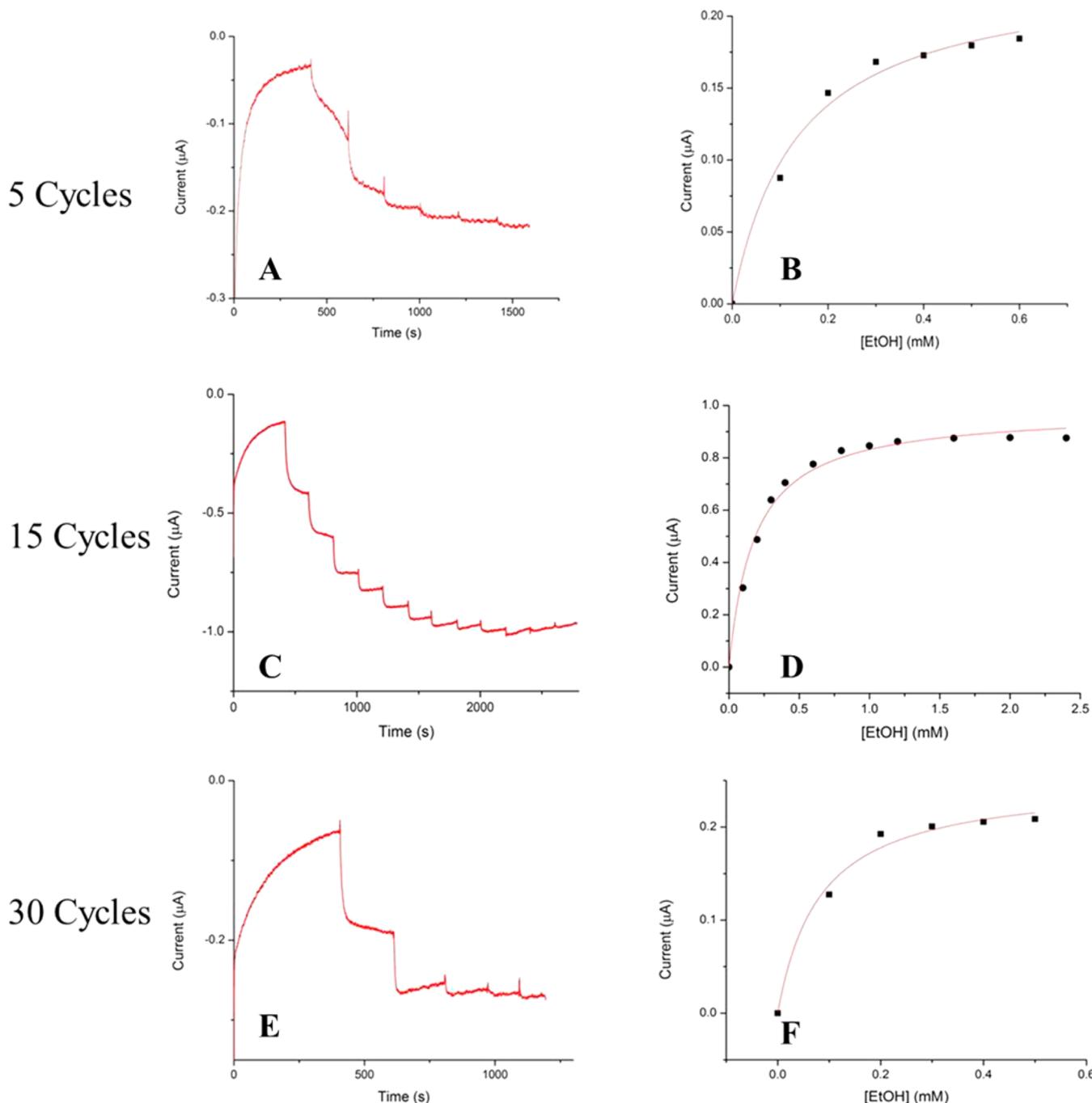


Figure 5. Amperometric measurement results of electrodes with (A) 5-cycle polymerization after injections of ethanol that increase the concentration of ethanol by 100 μM each at 400, 600, 800, 1000, 1200, and 1400 s. (C) Fifteen-cycle polymerization after injections of ethanol that increase the concentration of ethanol by 100 μM each at 400, 600, 800, and 1000 s, by 200 μM each at 1200, 1400, 1600, and 1800 s, and by 400 μM each at 2000, 2200, 2400, and 2600 s. (E) Thirty-cycle polymerization after injections of ethanol that increase the concentration of ethanol by 100 μM at 400, 600, 700, 900, and 1100 s. Calibration curves of (B) 5-cycle polymerization, (D) 15-cycle polymerization, and (F) 30-cycle polymerization.

RESULTS AND DISCUSSION

Electropolymerization of Aniline Derivatives. The polymerization of aniline derivatives is initiated by an oxidation step that leads to the radical cation.²⁰ This has previously been shown to be the rate-limiting process.²⁰ Thus, finding the appropriate cyclic voltammetric scan window to efficiently form radicals is crucial for electropolymerization. Before the copolymerization of two aniline derivatives was studied, MASA and ABA were polymerized individually to find their significant

polymerization peaks (Figure 2). Different cyclic voltammetric scan windows were applied; results have shown that efficient formation of aniline cation radicals starts at 800 mV versus Ag/AgCl in the oxidation scan, and both MASA and ABA showed two reversible polymerization peaks (at 600 and 500 mV for MASA and at 500 and 350 mV for ABA vs Ag/AgCl) with a scan window of 0–800 mV versus Ag/AgCl. Then two aniline monomers were co-electropolymerized with this scan window. A polymerization solution containing 80 mM MASA and 20 mM

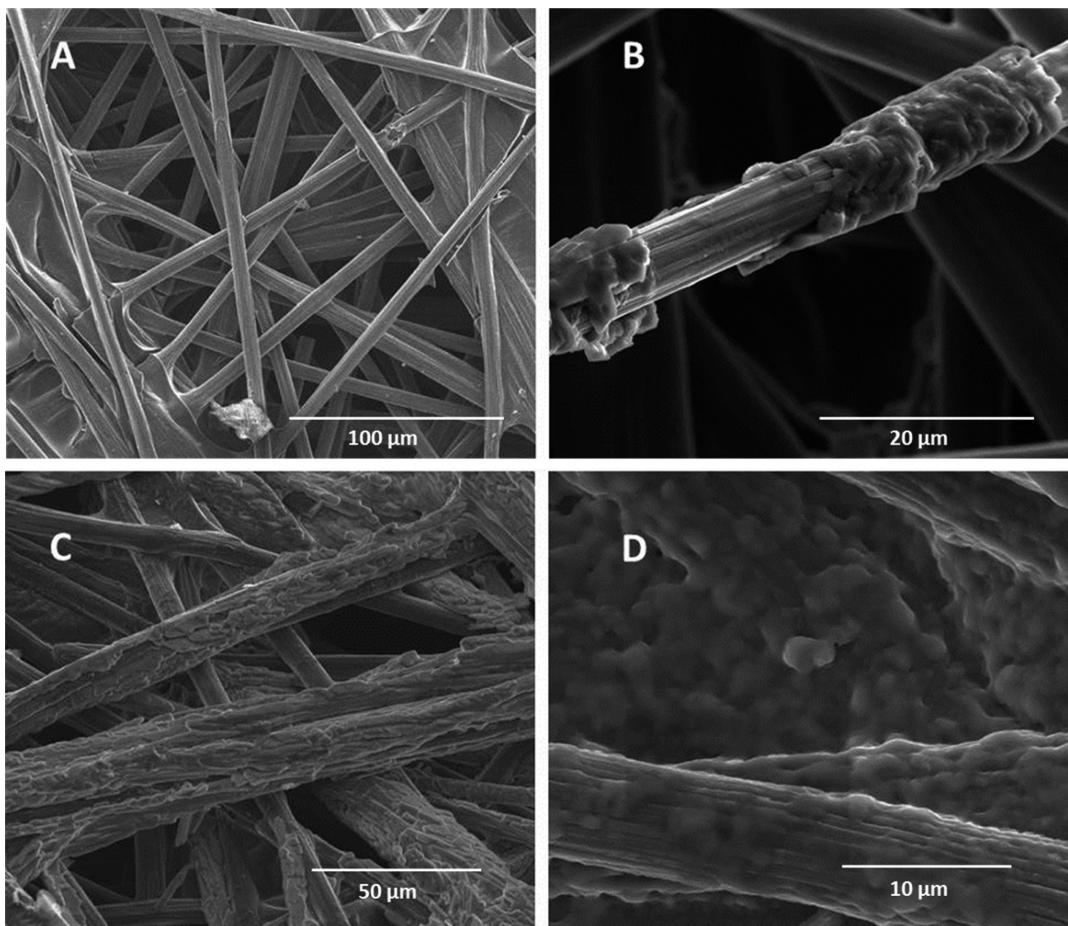


Figure 6. SEM images of (A) a bare Toray paper electrode, (B) 5-cycle polymerization, (C) 15-cycle polymerization, and (D) 30-cycle polymerization.

ABA in 1 M H₂SO₄ was used for co-electropolymerization. The cyclic voltammograms showed significant polymerization peaks from both aniline derivatives (Figure 2), indicating that MASA and ABA have been successfully polymerized to form a copolymer film on a Toray paper electrode surface. Further study showed that when we extend the oxidation scan to 900 mV versus Ag/AgCl, the polymerization peak heights increased by 25-fold compared to those at 800 mV with the same scan cycles (30 cycles) (Figure S1 of the Supporting Information). With this knowledge, the optimized polymerization window has been set to be 0–900 mV versus Ag/AgCl.

Cyclic voltammetry was also performed with polymerized electrodes at neutral pH. In MES buffer (pH 6.5), two distinct redox peaks at 300 and 500 mV can be observed (Figure 3). These two peaks represent the conversion of polyaniline in the fully reduced form (polyleucoenmeraldine) to a mixed state (polyemeraldine) and the conversion between polyemeraldine and the fully oxidized form of polyaniline (polypernigriline).¹⁶ The results suggest that this polyaniline copolymer film formed on a Toray paper electrode surface is electrically conductive at neutral pH.

Enzyme Immobilization. For the covalent coupling of PQQ-dependent enzymes to the polymerized electrode, the carboxyl groups in the polymer film should be activated. EDC and NHS are commonly used activators that can form amine-reactive esters of carboxylate groups for cross-linking. Immobilized enzyme activity assays were performed with fully modified PANi electrodes and electrodes modified with the

TBAB-modified Nafion enzyme encapsulation technique. As shown in Table 1, significant absorbance changes were observed for ethanol, glyceraldehyde, and glucose as the substrate, indicating that PQQ-dependent alcohol dehydrogenase (PQQ-ADH), PQQ-dependent aldehyde dehydrogenase (PQQ-AIDH), and PQQ-dependent glucose dehydrogenase (PQQ-GDH) in the *Gluconobacter* lysate were successfully immobilized on electrodes with both immobilization techniques and retained their catalytic activities. PQQ-dependent dehydrogenases were identified as the biocatalyst responsible for the oxidations of the substrates because only PQQ- and NAD(P)-dependent enzymes extracted from *Gluconobacter* are known to be able to oxidize glucose, ethanol, and glyceraldehyde²¹ and there is no NAD(P) cofactor in the experimental system, so the oxidation of substrates can only be catalyzed by PQQ-dependent dehydrogenases.

Electrochemical Measurements. The study of the polyaniline-modified bioelectrode started with the investigation of the role of the conducting polymer in the bioelectrocatalysis process. Heme-containing PQQ-dependent enzymes such as PQQ-ADH and PQQ-AIDH have been demonstrated to be capable of performing direct electron transfer (DET).²² Electrons generated in these biosystems can arrive at the electrode surface via an electron tunneling process without the need for electron mediators. Therefore, there are two potential roles that the polyaniline film can be playing. The polymer may act as a conductive bridge for the gap between the biocatalyst (PQQ-dependent enzymes) and the current collector (electrode).

In this case, the polymer acts like any conductive material such as carbon or metal. The electrons travel through the conjugated system within the polymer without performing any redox reactions. Alternatively, the polymer may act as a mediator for the electron transfer process. In this case, the polyaniline is reduced when it receives the electrons generated at the enzyme active site, shuttles the electrons to the electrode surface through electron hopping within the polymer system, and releases the electrons to the electrode surface by being oxidized to its original form (mediator-regenerated). Whether the polyaniline is acting as a conductive bridge or a mediator can be determined by cyclic voltammetry to determine whether the redox peak is from the enzyme or the polymer.

First, control experiments were conducted to rule out the possibility of the PANi catalyzing redox reaction of the enzyme substrate. Polymerized Toray paper electrodes without the carboxylate group activation process were tested via cyclic voltammetry with and without the addition of 10 mM ethanol. Results showed that there is no catalytic current appearing upon addition of ethanol, which indicates no catalytic property of polyaniline toward ethanol substrate (Figure S2 of the Supporting Information). Then, a fully modified electrode with PQQ-dependent enzyme loading was tested via cyclic voltammetry in MES buffer. An oxidation peak at 200 mV was observed (Figure 4A, black line), and this peak was not observed in the polymer electrodes without enzyme loading. A catalytic current was observed when 10 mM ethanol was added (Figure 4A, red line). This result demonstrated the ability of immobilized PQQ-dependent alcohol dehydrogenase (PQQ-ADH) to perform bioelectrocatalysis. To answer the question of whether this electrochemical system is mediated electron transfer (MET) or direct electron transfer (DET), an electrochemical measurement was performed with an activated polyaniline electrode immobilized with bovine serum albumin (BSA). BSA is a commonly used protein that is not electrochemically active within the electrochemistry test window. Cyclic voltammetry results with the BSA electrode showed the same peak at 200 mV (Figure 4B), which indicates that this peak is from polyaniline and is not from the PQQ-dependent enzymes. Cyclic voltammetry was also performed with an activated polyaniline polymer electrode without enzyme loading (Figure 4C). A peak at 200 mV was observed, and this result revealed the fact that the activation step shifted the peak to a lower potential and the electrochemical signal observed from the fully modified electrode is from the polyaniline, which indicated that the electron transfer is mediated by the polyaniline polymer and the mechanism is MET. In 2012, Schubart et al. reported that direct electron transfer was observed with PQQ-GDH in a polyaniline-modified bioelectrode.¹⁶ In that report, they observed catalytic current at ~200 mV (vs Ag/AgCl) with the addition of 10 mM glucose but did not discuss the origin of the redox peak in detail. To investigate this individual enzyme, we incorporated the polyaniline system with purified PQQ-GDH, and cyclic voltammetric results show the same peak at 200 mV versus Ag/AgCl that we observed with the PQQ-ADH system. This indicates that the polyaniline/PQQ-GDH system follows the mediated electron transfer mechanism (Figure S3 of the Supporting Information) for both quinoproteins and quinohemoproteins.

Electrodes with different polymerization cycles were tested to optimize the electropolymerization process. Electrodes prepared with 5, 15, and 30 cyclic voltammetry scan cycles were modified with the same batch of PQQ-dependent enzyme lysate. Results are shown in Figure 5. During amperometry with an applied potential of 250 mV versus Ag/AgCl, all electrodes

showed electrocatalytic responses to the ethanol substrate, and the calibration curves showed that they all follow Michaelis–Menten kinetics, indicating enzymatic electrocatalysis. For each electrode system, the curves were fit to determine the Michaelis–Menten kinetic parameters I_{\max} and K_m , where I_{\max} is the theoretical maximal current density that can be generated by the electrode surface. Five-cycle polymerization showed that the electrode has a maximal current response (I_{\max}) of $0.23 \pm 0.03 \mu\text{A}$ with a K_m value of $0.14 \pm 0.02 \text{ mM}$. Fifteen-cycle polymerization yielded a maximal current response (I_{\max}) of $0.98 \pm 0.12 \mu\text{A}$ with a K_m value of $0.18 \pm 0.02 \text{ mM}$. With 30-cycle polymerization, the electrode amperometry results showed an I_{\max} of $0.25 \pm 0.07 \mu\text{A}$ with a K_m value of $0.08 \pm 0.03 \text{ mM}$. The results have shown that the 15-cycle polymerization has a 4.2-fold increase in the maximal current response compared to that with 5-cycle polymerization and 3.9-fold increase compared to that of 30-cycle polymerization. To improve our understanding of the results, an electrode surface study was conducted via scanning electron microscopy (SEM) (Figure 6). SEM results show that with a 5-cycle

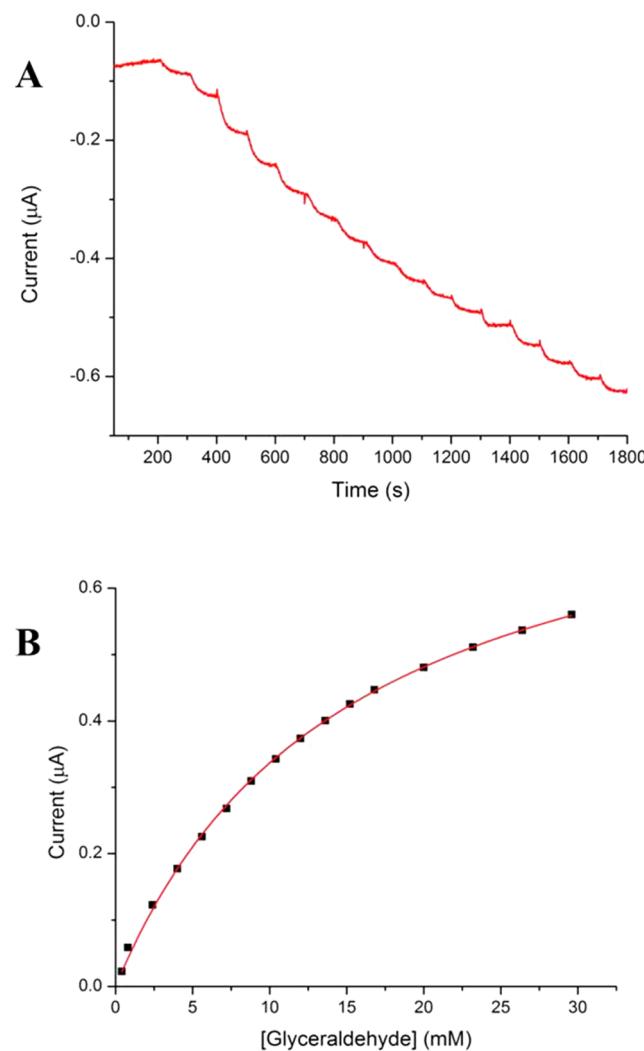


Figure 7. Amperometric measurement results (A) and calibration curve (B) of a modified electrode with glyceraldehyde as a substrate. The injections of glyceraldehyde increased the concentration by $400 \mu\text{M}$ at 200 s, by $800 \mu\text{M}$ at 300 s, by 1.6 mM each at 400, 500, 600, 700, 800, 1000, 1100, 1200, and 1300 s, and by 3.2 mM each at 1400, 1500, 1600, and 1700 s.

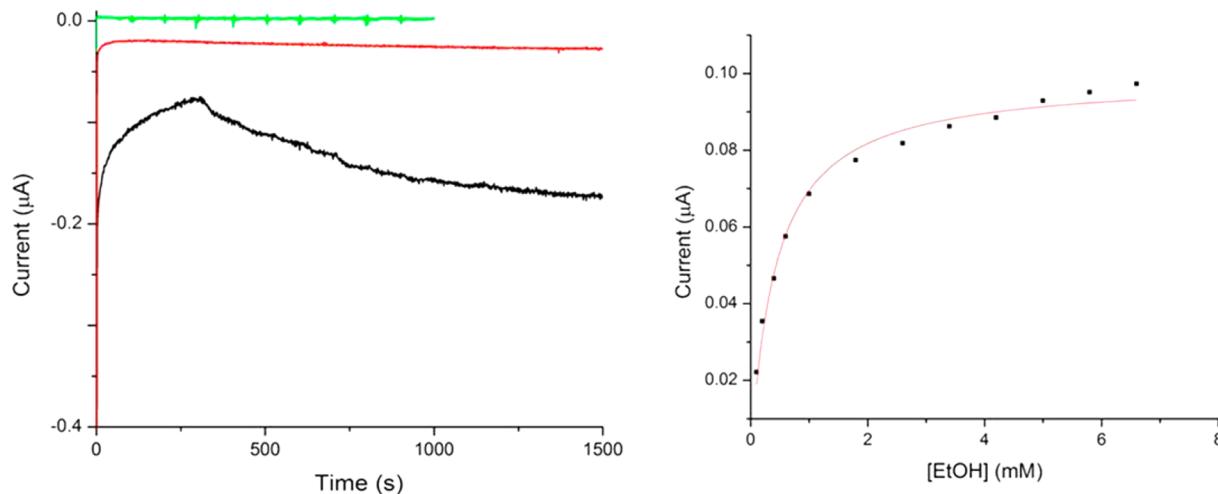


Figure 8. Amperometric results (A) and calibration curve (B) of the TBAB-modified Nafion electrode. The green line represents data from the control experiment where the TBAB–Nafion polymer without enzyme loading was tested with ethanol injections, the red line data from the blank experiment in which the enzyme-immobilized electrode was tested and MES buffer was injected, and the black line data from ethanol injections. Injections were made to increase the ethanol concentration by 0.1 mM each at 300 and 400 s, by 0.2 mM each at 500 and 600 s, by 0.4 mM ethanol at 700 s, and by 0.8 mM each at 800, 900, 1000, 1100, 1200, 1300, and 1400 s.

polymerization, carbon fibers were incompletely coated with PANi. The carbon fiber surface coverage was significantly increased with 15-cycle polymerization. When the polymerization cycle number increased to 30, PANi clusters were formed in the space between carbon fibers and filled the space within the carbon fiber matrix. With this knowledge, we understand that the current density increase from 5-cycle to 15-cycle polymerization was due to increasing electrode surface coverage with PANi and the current density decrease between 15-cycle and 30-cycle polymerization was due to the fact that the electrode surface coverage was saturated and the excessive formation of PANi in the latter case was blocking the mass transfer of substrate within the carbon fiber matrix system.

To investigate the bioelectrocatalytic property of PQQ-dependent aldehyde dehydrogenase (PQQ-ADH) immobilized on a polyaniline film, glyceraldehyde was used as a substrate in electrochemical measurements. Results are shown in Figure 7. The catalytic current was observed in amperometry experiments with an I_{\max} of $0.84 \pm 0.10 \mu\text{A}$ and a K_m value of $15 \pm 2 \text{ mM}$. It is important to note that immobilization has little effect on enzyme stability and the current density over time still decreases 50% in the first 12 h.

To compare the TBAB-modified Nafion encapsulation enzyme immobilization technique and the polyaniline cross-linking technique, TBAB-modified Nafion bioanodes were made with the same PQQ-dependent enzymes at the maximal enzyme capacity. Cyclic voltammetry was performed with TBAB-modified Nafion-modified electrodes in 50 mM MES buffer with 10 mM ethanol. An oxidation peak was observed at 350 mV versus Ag/AgCl (Figure S4 of the Supporting Information). Amperometry measurements were performed on the TBAB-modified Nafion electrodes with an applied potential of 360 mV versus Ag/AgCl. The results and calibration curve are shown in Figure 8. The calculated K_m value for the TBAB-modified Nafion electrode is $0.43 \pm 0.09 \text{ mM}$, and the I_{\max} is $0.1 \pm 0.02 \mu\text{A}$. Via comparison of the current densities of optimized 15-cycle polymerization PANi electrodes ($I_{\max} = 0.98 \pm 0.12 \mu\text{A}$) from Figure 5 with the maximal loaded TBAB-modified Nafion entrapment data in Figure 8, we see a 9.8-fold increase in current density, which indicates an order of magnitude performance improvement when used in a biofuel cell system, assuming the anodic bioelectrocatalysis is the limiting process.

CONCLUSION

In this work, we have demonstrated that by utilizing PANi functionalized with sulfonic and carboxylic substitute groups, we can fabricate a quinohemoprotein-based bioanode where the conducting copolymer acts as both an enzyme immobilizer and an electron mediator. The polyaniline conducting polymer successfully bonds to enzymes while retaining their biological catalytic properties, efficiently receives the electrons generated at the enzyme active sites, and rapidly passes the electrochemical signal to the electrode surface to produce a catalytic current. Mediated bioelectrocatalysis was demonstrated with PQQ-ADH and PQQ-ALDH, and the results showed a significant 9.8-fold increase in current density when compared to commonly used entrainment enzyme immobilization technique.

ASSOCIATED CONTENT

S Supporting Information

Voltammetry of electropolymerization optimization, PQQ-GDH immobilization, and TBAB-modified Nafion immobilization. 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. Phone: (801) 587-8325.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge the National Science Foundation (Grant 1158943) for funding.

REFERENCES

- (1) Brédas, J. L., Chance, R. R., Eds. *Conjugated polymeric materials: Opportunities in electronics, optoelectronics, and molecular electronics*; Springer: Berlin, 1990; Vol. 182.
- (2) Gurunathan, K.; Murugan, A. V.; Marimuthu, R.; Mulik, U.; Amalnerkar, D. *Mater. Chem. Phys.* **1999**, *61*, 173.

- (3) Hasik, M.; Turek, W.; Stochmal, E.; Lapkowski, M.; Pron, A. *J. Catal.* **1994**, *147*, 544.
- (4) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537.
- (5) Tallman, D. E.; Spinks, G.; Dominis, A.; Wallace, G. G. *J. Solid State Electrochem.* **2002**, *6*, 73.
- (6) Sarauli, D.; Xu, C.; Dietzel, B.; Stiba, K.; Leimkühler, S.; Schulz, B.; Lisdat, F. *Soft Matter* **2012**, *8*, 3848.
- (7) Coleman, J. N.; Curran, S.; Dalton, A.; Davey, A.; McCarthy, B.; Blau, W.; Barklie, R. *Phys. Rev. B* **1998**, *58*, R7492.
- (8) Janietz, S.; Bradley, D.; Grell, M.; Giebel, C.; Inbasekaran, M.; Woo, E. *Appl. Phys. Lett.* **1998**, *73*, 2453.
- (9) Samuel, I.; Rumbles, G.; Collison, C. *Phys. Rev. B: Condens. Matter Mater. Phys.* **1995**, *52*, R11573.
- (10) Kumar, D.; Sharma, R. *Eur. Polym. J.* **1998**, *34*, 1053.
- (11) Malinauskas, A. *J. Power Sources* **2004**, *126*, 214.
- (12) Wei, X.-L.; Wang, Y.; Long, S.; Bobeczko, C.; Epstein, A. *J. Am. Chem. Soc.* **1996**, *118*, 2545.
- (13) Maželkienė, R.; Tomkutė, V.; Kuodis, Z.; Niaura, G.; Malinauskas, A. *Vib. Spectrosc.* **2007**, *44*, 201.
- (14) (a) Moehlenbrock, M. J.; Minteer, S. D. *Chem. Soc. Rev.* **2008**, *37*, 1188. (b) Halámková, L.; Halámek, J.; Bocharova, V.; Szczupak, A.; Alfönta, L.; Katz, E. *J. Am. Chem. Soc.* **2012**, *134* (11), 5040. (c) Katz, E.; MacVittie, K. *Energy Environ. Sci.* **2013**, *6*, 2791. (d) MacVittie, K.; Halamek, J.; Halamkova, L.; Southcott, M.; Jemison, W. D.; Lobel, R.; Katz, E. *Energy Environ. Sci.* **2013**, *6*, 81.
- (15) (a) Cooney, M. J.; Svoboda, V.; Lau, C.; Martin, G. P.; Minteer, S. D. *Energy Environ. Sci.* **2008**, *1*, 320. (b) Besic, S., Minteer, S. D., Eds. *Micellar Polymer Encapsulation of Enzymes*. In *Enzyme Stabilization and Immobilization*; Springer: Berlin, 2011; Vol. 679.
- (16) Schubart, I. W.; Göbel, G.; Lisdat, F. *Electrochim. Acta* **2012**, *82*, 224.
- (17) (a) Arechederra, R. L.; Treu, B. L.; Minteer, S. D. *J. Power Sources* **2007**, *173*, 156. (b) Arechederra, R. L.; Minteer, S. D. *Fuel Cells* **2009**, *9* (1), 63. (c) Xu, S.; Minteer, S. D. *ACS Catal.* **2012**, *2*, 91.
- (18) Xu, S.; Minteer, S. D. *ACS Catal.* **2013**, *3*, 1756.
- (19) Moore, C. M.; Akers, N. L.; Hill, A. D.; Johnson, Z. C.; Minteer, S. D. *Biomacromolecules* **2004**, *5*, 1241.
- (20) Syed, A. A.; Dinesan, M. K. *Talanta* **1991**, *38*, 815.
- (21) Matsushita, K.; Toyama, H.; Adachi, O. *Adv. Microb. Physiol.* **1994**, *36*, 247.
- (22) Treu, B. L.; Arechederra, R. L.; Minteer, S. D. *J. Nanosci. Nanotechnol.* **2009**, *9*, 2374.