1.2 Fuel Cell Development

The effort to develop novel fuel cell components included two major activities: biocarbons for fuel cells, and bioactive fuel cells. Each of these topics is addressed in the following paragraphs.

1.2.1 Biocarbons for Fuel Cells

1.2.1.1 Technical Accomplishments

Under this subtask, biocarbons were produced using the HNEI Flash Carbonization™ reactor, with various feedstocks, including corn cobs, oak wood and sweet gum wood. The results of the corn cob work are described in a recent publication. The following is the abstract of this publication:

*Elevated pressure secures the highest fixed-carbon yields of charcoal from corncob.* Operating at a pressure of 0.8 MPa a Flash-Carbonization reactor realizes fixed-carbon yields that range from 70 to 85% of the theoretical thermochemical equilibrium value from Waimanalo corncob. The fixed-carbon yield is reduced to a range from 68 to 75% of the theoretical value when whole Waimanalo corncobs are carbonized under nitrogen at atmospheric pressure in an electrically heated muffle furnace. The lowest fixed-carbon yields are obtained by the standard proximate analysis procedure for biomass feedstocks: this yield falls in a range from 49 to 54% of the theoretical value.

A round-robin study of corncob charcoal and fixed-carbon yields involving three different thermogravimetric analyzers (TGAs) revealed the impact of vapor-phase reactions on the formation of charcoal. Deep crucibles that limit the egress of volatiles from the pyrolyzing solid greatly enhance charcoal and fixed-carbon yields. Likewise, capped crucibles with pinholes increase the charcoal and fixed-carbon yields compared with values obtained from open crucibles. Large corncob particles offer much higher yields than small particles. These findings show that secondary reactions involving vapor-phase species (or nascent vapor-phase species) are at least as influential as primary reactions in the formation of charcoal.

*Our results offer considerable guidance to industry for its development of efficient biomass carbonization technologies.* Size reduction handling of biomass (e.g., tub grinders and chippers), which can be a necessity in the field, significantly reduces the fixed-carbon yield of charcoal. Fluidized bed and transport reactors, which require small particles and minimize the interaction of pyrolytic volatiles with solid charcoal, cannot realize high yields of charcoal from biomass. When a high yield of corncob charcoal is desired, whole corncobs should be carbonized at elevated pressure. Under these circumstances, carbonization is both efficient and quick.

The oak wood and sweet gum wood were supplied to us by the Dow-Corning Corporation. Dow-Corning is interested in the use of charcoals produced from these woods as reductants in the manufacture of pure silicon from quartz. We have completed our study of these woods and their charcoals, and are now preparing a paper on this topic for publication. We expect to submit the paper to *Energy & Fuel* before the end of the year.

We supplied biocarbons for testing to Ben Thien of Scientific & Research Associated, Inc. (SARA). For many years SARA has had Department of Defense support for its development of a direct carbon fuel cell.
This subtask also enabled us to complete and publish a study of the thermodynamic properties of water at high pressures and temperatures. These properties are relevant to the development of aqueous-alkaline-carbonate direct carbon fuel cells.

HNEI’s development of an aqueous-alkaline-carbonate direct carbon fuel cell with primary support from the National Science Foundation is ongoing and will be completed next year. This subtask has been supportive of the NSF activity. Results will be reported next year.

References cited

1.2.1.2 References Cited


1.2.2 Bioactive Fuel Cells

Bioactive fuel cells or enzymatic bio-fuel cells (EBFCs) are fuel cells that use enzymatic biocatalysts to convert chemical energy directly to electricity as power sources. They are promising alternatives to complement conventional fuel cell technologies that rely on transitional metal oxides or noble metals as catalysts for conversion of chemical energy, typically stored in hydrogen or other biofuels, to useful electrical energy. An EBFC exhibits some promising technical merits as follows:

- **Selectivity** – Enzyme catalysts are fuel specific and capable of handling complex fuels in the liquid phase, which can simplify fuel logistics and cell design;
- **Abundance in fuels and catalysts supply** – Unlike conventional Pt-based catalysts, enzymatic catalysts can be produced via biological or chemical methods, thus promising a potentially low-cost mass production and unlimited supply; similarly, the biofuels, as diverse as we can be in choosing proper biocatalysts to convert the chemical energy, can be produced from photosynthesis or alternative refinement pathways, thus considered renewable and green as abundant supplies;
- **Wider range of operation** – Due to their selectivity, enzymes are generally more adaptive to extreme conditions and tolerant to contaminants;
- **Reformulation** – If the gene coding for the enzyme is obtained, a suite of directed evolution techniques exists to create mutants that are more effective in catalysis; and
- **Self-assembly** – Unique in biological systems, to simplify fabrication processes for micro-devices in situ.

The continuation of support has enabled us to execute fundamental studies that have (i) elucidated charge transfer limitations in enzyme-catalytic electrodes, (ii) developed preferred immobilization matrices for enhancing enzyme activity and stability, (iii) designed and fabricated standardized test cells for performance testing, and (iv) developed some unique in situ characterization techniques to understand the immobilization process of mediators on carbon or metal electrode. We have transferred this knowledge into improved engineering designs of practical bio-fuel cells including, more recently, microbial fuel cells. To this end, the following long-term objectives have been achieved under this program:

- Established an array of quantitative in situ characterization techniques, test cells, and modeling capabilities to determine limitations to bioelectrocatalysis [1-7],
- Developed a test bed modular cell that has allowed us to test cell performance of enzyme-based bioelectrocatalysis operation [8, 9];
- Developed macroporous flow-through immobilization matrices permitting improved catalyst performance (activity and lifetime) [10-13]
- Developed working enzyme fuel cells [14, 15]

1.2.2.1 Scope of Work and Approach

Over the course of this project, three major tasks in the EBFC work have been focused on:

1) Developing platform fabrication technology to control the resulting multidirectional pore structure of three-dimensional electrodes, and immobilization techniques;
2) Developing qualitative and quantitative fluorescence as a characterization technique for enzyme fuel cells; and

3) Developing in situ interface characterization techniques utilizing imaging ellipsometry with quartz crystal microbalance and electrochemical techniques to facilitate fabrication and testing of bio-fuel cells and to understand the interfacial charge transfer mechanism for future optimization of the charge transfer efficiency.

For this specific funding period, we were funded to pursue the following:

1) Continue to develop in-situ characterization techniques using fluorescence to explore protein aggregation in the immobilized state, as a means to characterize chitosan based immobilization of multiple enzymes that can more fully oxidize complex energy fuels, and

2) Continue to apply in situ characterization techniques based on spectroscopic imaging ellipsometry with microgravimetric and electrochemical techniques to study the enzyme-electrode interaction on the electrode surface and the associated dynamic behavior, aiming to understanding the charge transfer process with more direct measurements and correlation.

1.2.2.2 Technical Accomplishments

Summary of work from previous project periods. In past reports, we have presented several prototype cells constructed to deliver gas (e.g., hydrogen) fuels. A final working hydrogenase enzymatic bio-fuel cell was constructed and tested (Figure 1.2.1). The design considerations and test applications were described in Final Technical Report, ONR Grant N00014-01-1-0928, June 2005. Complete details on this work have been published [1]. From this work we determined, and reported previously, that the use of gaseous fuels is limited by the solubility of the gaseous fuel in the aqueous buffer that is required to maintain enzyme activity.

In past reports we have also commented on the development of a suite of characterization techniques, including potentiostatic DC polarization, dynamic potentiometry, and electrochemical impedance spectroscopy (EIS) combined with spectrophotometric detection of enzyme activity, in order to characterize electrode performance, and to differentiate between the relative contributions towards charge transfer efficiency. Among the results of our efforts, in particular, we believe we were one of the first groups to report charge transfer efficiency for bound enzyme [1] and to report a mass transport modeling effort that can be combined with DC-polarization data to yield information valuable for future electrode development [2]. A detailed description of the technique application, data and results can be found in the literature. A summary can also be found in Final Technical Report, ONR Grant N00014-07-1-1094, August 2010 (Section 3.3.2).
To characterize the distribution of enzymes within the polymer films used to immobilize the enzymes, we have applied the technique of fluorescence. Electrode fabrication methodologies using the immobilization process with polymer films inherently assume that the immobilized enzymes are homogeneously distributed. Our work, which has tagged ethanol-oxidizing enzymes with various fluorescent probes, has used laser-scanning confocal microscopy to image the spatial distribution of the enzyme within the film. Our results, which have been published [3] (see Figure 1.2.2), have clearly demonstrated that this is not necessarily the case, and that the tagged enzymes may not be homogenously distributed within polymer films. To investigate how the charge-charge interaction between the enzyme and the polymer affect the immobilization process, we have also studied the steady-state and dynamic polarization of fluorescent probes when placed in solution with charged polymers. This work has shown that the enzymes are retained in the micelles of the hydrophobically-modified polymer as it dries, but are not entrapped within those polymers while mixed in free solution [4].

The premise of conducting in situ investigation is to characterize and understand charge transfer process and its limitations in a bio-fuel cell operation, which is strongly and critically dependent on the interfacial property of the electrode surface, and where the charge transfer occurs and high efficiency matters. The interfacial properties need to be characterized in situ in the environment where the electrocatalytic reaction occurs. To study such property and behavior of the interface, we have developed a unique capability which employs an advanced spectroscopic imaging ellipsometry with complementary tools, such as microgravimetric and electrochemical techniques to assist us understand the nature of such charge transfer process on the electrode surface.

Mediator films immobilized on electrode surface have been suggested to be an effective method to promote charge transfer. Such mediator immobilization has been pursued in our laboratory. Two major pathways are considered. One is chemical route and another electrochemical.

In the chemical pathway, we have initiated an enzyme immobilization effort to consider the feasibility of developing a common platform for apo-enzyme reconstitution. This approach is of particular interest with prosthetic group that contains pyrroloquinoline quinine (PQQ) and PQQ-dependent glucose dehydrogenase (PQQ-GDH), which is attractive for
glucose oxidation. For instance, PQQ and PQQ-GDH have been chemically bound to CHIT-CNT films in the presence of EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydro-chloride), respectively, on glassy carbon electrodes (GCEs). The immobilized PQQ-GDH on CHIT-CNT matrix displays a quasi-reversible electron transfer with a formal potential $E^{\circ'} = -0.110$ V, which is found to be independent of the scan rate. The bioactivity of the immobilized PQQ-GDH was retained.

Interestingly, the enzyme-free bound PQQ exhibits a more facile electron transfer with GCE than immobilized PQQ-GDH, suggesting that PQQ-bound CHIT-CNT films hold promise as a platform for reconstitution of PQQ-dependent apo-enzymes, and greater potential for applications in biosensors and bio-fuel cells. Evidence of electron transfer between bound PQQ-GDH and the GCE has been observed when the PQQ-GDH has been coupled with EDC to a CNT-CHIT film. The experimental results suggest that the presence of CNT in the CHIT film promotes the electron transfer of bound PQQ-GDH to the GCE.

A quasi-reversible electrochemical reaction, as revealed by a pair of well-defined redox peaks, is observed by cyclic voltammetry, with PQQ-bound CHIT-CNT-modified GCE (Figure 1.2.3). Additional experimental results suggest that the activity of PQQ-GDH was retained in PQQ-GDH-bound CHIT-CNT/GCE (Figure 1.2.4), which permits its use as a biocatalyst for a mediated bio-fuel cell or amperometric biosensor for glucose detection (Figure 1.2.5). It should be noted that PQQ bound to CHIT-CNT film also exhibited an effective charge transfer with GCE, suggesting that it can be used as a promising platform for reconstitution of various PQQ-dependent apo-enzymes. This work has been published in *Electrochem. Solid State Letters* [10].

The electrochemical approach of mediator immobilization is demonstrated in the work for NADH-dependent alcohol-based biofuel cell applications. Methylene green (MG) and its polymer forms (poly-MG) have been reported as effective mediators for alcohol partial oxidation. It is also known that poly-MG can be immobilized on GCE using cyclic voltammetric deposition. In order to understand its functionality, it is important to characterize the electrochemical deposition process, the modified electrode surface, the stability and property of such electrochemically immobilized poly-MG films.

![Figure 1.2.5 Cyclic voltammograms of PQQ-GDH-bound CHIT-CNT-modified GCE in 0.1 M PBS and 40 µM of PMS at 5 mV/s in the (a) absence and (b) presence of 10 mM glucose](image)

![Figure 1.2.6 Time-resolved ellipsometric observations of poly-methylene green films and their thickness during electrochemical deposition on Pt electrode](image)
Figure 1.2.6 shows that we can evaluate poly-MG film thickness during electrochemical deposition on a Pt electrode using time-resolved imaging ellipsometry. The time-resolved imaging ellipsometry is an in situ observation and a unique capability in our laboratory. We can perform real time monitoring of film growth and the film’s physico-chemical property on an electrode surface. In this work, we use the imaging ellipsometry and cyclic voltammetric deposition technique to track the thickness of the deposited film with cycle number. This valuable technique may allow us collect new information of the fundamental reaction kinetics and mechanisms underlying the electrochemical deposition of conductive poly-MG films onto electrode surfaces (see Figure 1.2.6). The time resolved observation provides nanometer resolution with film thickness and the imaging results in real time monitoring of the surface morphology and roughness. The work has been published in Ref. [5].

![Diagram of spectroscopic imaging ellipsometry (EP³) with quartz crystal microbalance (QCM) and electrochemical techniques](image)

*Figure 1.2.7* A unique combination of spectroscopic imaging ellipsometry (EP³) with quartz crystal microbalance (QCM) and electrochemical techniques to perform in situ characterization of surface of electrodes

To understand the kinetics of the deposition process, we further combine the imaging ellipsometry with quartz crystal microbalance (QCM) and cyclic voltammetric techniques to characterize the film deposition (Figure 1.2.7). We are able to control the film deposition with accurate thickness and morphology, at the same time correlate the mass, charge, and the film properties (ellipsometric angles, which can be used to estimate film thickness and identify film
chemical composition changes) in the deposition process (Figure 1.2.8). It helps us understand the stepwise underlying mechanism in the deposition and provide unprecedented details in the film formation process involving redox reactions. We intend to use this technique to study the redox kinetics involved in other mediator oxidation.

During this period, these outcomes were also used to leverage two extramurally funded projects. The first one was a grant from the Intelligence Community Postdoctoral Fellow Research Program to support development of bio-fuel cells for micro-power source applications (B.Y. Liaw, PI). The second was an sub-award from the AFOSR Multi-disciplinary University Research Initiative (MURI) program (M.J. Cooney, PI), awarded to the lead institution, the University of New Mexico (P. Atanassov, PI). Both awards have lent national recognition of this EBFC program.

In collaboration with these partner programs, we have explored chitosan and chitosan-composite scaffolds as a material for the fabrication of macroporous electrodes that can support both mediator-based and direct electron transfer. Work accomplished has included the development of protocols for the fabrication of hydrophobically modified chitosan scaffolds immobilizing NADH-dependent glucose oxidase, and chitosan/CNT composites for attachment of PQQ-enzymes. With respect to the fabrication of hydrophobically-modified chitosan scaffolds, we have demonstrated proof-of-principle data that shows that the power density can be significantly increased for mediator-based systems (Figure 1.2.9). This work has been published in the Journal of Materials Chemistry [11]. We have also fabricated three-dimensional chitosan/CNT scaffolds that provide a basis for enzymes that are capable of direct electron transport. The chitosan essentially acts as a binder of the carbon nanotubes and one is left with scaffold structures similar to that presented in Figure 1.2.9, but with carbon nanotubes lining the surface. These scaffolds are highly conductive and represent a new methodology to create
multidirectional and multidimensional 3D structures of electrochemically-active carbon nanotube surfaces [12].

We have also developed a liquid-phase prototype Bio-fuel Test Cell (Figure 1.2.10). This prototype, which we term the modular stack cell, was designed as a characterization tool. For example, we sent duplicate models of the modular stack cell to three cooperating laboratories in the U.S. that also specialize in enzyme fuel cell development. Each laboratory (Dr. Shelley Minteer at St. Louis University, Dr. Plamen Atanassov at the University of New Mexico, and Dr. Scott Barton at Michigan State University) was given the same protocol to execute (i.e., to develop a poly[methylene green] electrode film that oxidizes NADH), and the electrochemical data from all laboratories were consolidated and statistically analyzed for reproducibility. The results demonstrated that the modular stack cell provides a framework for comparative analysis of systems. The results have been published in the Journal of Electroanalysis [8, 17]. With this confidence, we have fabricated a full ethanol-based bio-fuel cell based on this design (Figure 1.2.11). Although we have achieved full operation (see power curves in Figure 1.2.11) with an air-breathing cathode, this work was not published.

![The modular stack cell](Figure 1.2.10)

![Operational biofuel cell, based on modular stack cell design](Figure 1.2.11)

Of special interest for an enzymatic power generation from ethanol as biofuel are NAD-dependent dehydrogenases, like alcohol dehydrogenase (ADH) or malate dehydrogenase (MDH), which lie within the Krebs cycle. This fundamental metabolic pathway involves eight enzymes for energy production through aerobic respiration. In order to exploit the entire cycle on a bioanode, and within the context of increased power generation, a polymer system that can immobilize and stabilize all eight enzymes in a three-dimensional matrix is needed. In support
of developing macroporous chitosan and chitosan-derivate scaffolds as advanced materials for fabrication of biofuel cell electrodes, as specified in 1), we have immobilized MDH within modified chitosan-polymer scaffolds placed upon a poly(methylene green) modified glassy carbon electrode. The current and power density for this MDH bioanode have been tested in half cell mode (Figure 1.2.12) and tested in a full biofuel cell. The half cell mode results represented a significant advance in the development of flow-through electrodes and confirmed the application of an enzyme immobilized in a modified chitosan polymer [14]. We have also used fluorescence to track the spatial distribution of enzyme immobilized in the hydrophobically-modified polymer (Figure 1.2.13) [5]. This work, which has been published, clearly demonstrated that the distribution of a fluorescently-tagged enzyme (the pink in Figure 1.2.13) distributes quite differently in various forms of the hydrophobically-modified chitosan polymer (i.e., native, butyl-modified, and ALA-modified).

Additional work has been executed wherein polarity sensitive fluorescent probes have been attached to enzyme and immobilized within native and modified chitosan polymer [6]. This work has been designed to quantitatively characterize the chemical microenvironment immediately surrounding the enzyme when in the immobilized state. Specifically, polar sensitive probes were used to correlate the relative hydrophobicity of the chemical microenvironment. The results are shown in Figure 1.2.14 wherein acrylodan labeled cMDH was suspended in the presence of aqueous native and modified chitosan (A), immobilized in native and modified chitosan scaffolds (B), immobilized in native and modified chitosan dehydrated scaffolds (B), and immobilized in native and modified chitosan rehydrated scaffolds (C). Figure 1.2.14 compares emission profiles of acrylodan-tagged enzymes in aqueous solution against those immobilized within the polymers. In Figure 1.2.14(A), the emission peaks of acrylodan-cMDH suspended in aqueous solutions of native and modified chitosan polymer did not vary relative to each other, suggesting that the various polymers (as 0.5 (w/w)% solutions) provided identical chemical microenvironments (in terms of polarity – i.e., neither were relatively more or less hydrophobic). This assumes that the chemical environment surrounding
the tagged enzyme is dominated by water and not the chitosan polymer. In contrast, when the tagged enzymes were immobilized within the same polymers, the emission maxima not only occurred at lower wavelengths, but they also varied significantly across the three polymers (Figure 1.2.14(B)). This result suggests that when in the immobilized state the various modified polymers provided altered chemical microenvironments, thus corroborating two separate theories: (1) that modified chitosan polymers can provide altered chemical microenvironment, and (2) that the altered chemical microenvironments are most pronounced after the polymer has been precipitated (e.g., through freezing or drying processes) into its final structure. The latter result supports our previous suggestion that the enzymes are not interacting with or being affected by the amphiphilic regions of the polymer, until the freezing or precipitating process. The overall blue shift observed in the dried polymer can be partially attributed to their dehydration during the freeze-drying process. The removal of water molecules surrounding the immobilized enzyme will obviously lead to a reduction in polarity. By contrast, re-hydration should reintroduce water molecules into the chemical environment and thereby increase the relative polarity. To verify this, the scaffold films were rehydrated in identical buffer solution. Although the rehydrated films showed a trend for relatively more polar chemical microenvironments, the same trends in blue shift was observed across all forms of the polymer (Figure 1.2.14(C)). This confirms that the chitosan polymer does impact the chemical microenvironment of the enzyme when in the immobilized state.
For the purposes of correlation, malate dehydrogenase was immobilized within the three target polymers: native chitosan, butyl-modified and alpha linoleic acid a poly(methylene green) coated glassy carbon electrode. The electrocatalytic activity of each polymer was then measured in half cell mode using amperometry. The amperometric current of NADH oxidation on poly(methylene green) was measured at an applied potential of +300 mV. The catalytic oxidation of NADH occurs in the chosen potential range (selected to be higher than the half-wave potential to maximize electrocatalytic reaction). Electrochemical currents, measured at the same potentials, can be used as apparent catalytic activity. The results, normalized to 1.0, are shown in Figure 1.2.15. Normalizing the activity to the highest output, the butyl-modified chitosan gave a 10-fold increase in current density and the ALA-modified a 4-fold increase when compared against the unmodified native chitosan. This suggests that the activity of the immobilized enzyme follows the measurement of the chemical microenvironment in terms of polarity.

In most recent work we were able to use fluorescent tags to individual enzymes to verify the presence of enzyme aggregation in the immobilized state [6]. Specifically, we combined three techniques; (1) light scattering, (2) Förster Resonance Energy Transfer (FRET), and (3) traditional native PAGE gels to measure the aggregation of a single enzyme in solution and immobilized within three-dimensional chitosan scaffolds (Figure 1.2.16). The analysis has been applied to a range of chitosan polymers (of varying amphiphilicity) previously used to correlate enzyme activity to the relative polarity of the chemical microenvironment [18]. Based on the FRET analysis, the average distance between proteins in aqueous solutions (chitosan: \( r = 51.3 \pm 0.9 \text{ nm} \); C4-chitosan: \( r = 53.3 \pm 0.2 \text{ nm} \)) correlate to the aggregation state of a monomer/dimer mix. When the protein is immobilized within a chitosan scaffold, a decrease in the average separation (\( r = 45.9 \pm 0.1 \text{ nm} \)) suggests an increase in the aggregation due to immobilization. Further, the hydrophobic modification of the polymer results in a further decrease in protein separation (\( r = 41.8 \pm 0.3 \text{ nm} \)). This indicates that the immobilization process is inducing aggregation and may be a method for producing enzyme complexes that mimic metabolic pathways.

![Figure 1.2.15 Electrochemical activity as normalized current density of immobilized MDH in different chitosan polymers. (c): C4-Chitosan film; (b): Chitosan film; (a): ALA-Chitosan film](image)
Figure 1.2.16 (A) Calculated radial separation from the emission scans in pt A (calculations detailed in text) [inset] = FRET efficiency calculated from scans in pt A. (note: the data highlighted in the boxes represents the initial dispersal of the protein within the buffer); (B) Calculated radial separation as a function of time over the span of 3.5 hrs in 5 min increments (not all data shown) for a solution of 50% Alexa®555 tagged cMDH (30 M) and 50% Alexa®647 tagged cMDH (30 M) in a 600 M glutaraldehyde, 50 mM TRIS buffer (pH7.4) solution. The solid bars represent the average radial separation in A – aqueous chitosan, B – aqueous C4 – chitosan, C – freeze dried chitosan scaffolds, and D – freeze dried C4 – chitosan scaffolds.

The poly(methylene green) mediator has been considered one of the best mediators for a NAD+-dependent biocatalytic process and thus of great interest to the enzymatic bio-fuel cell operation. However, the interfacial property of this mediator polymer film on biocatalytic electrodes is not well understood to date. We have used electrochemical microgravimetric imaging ellipsometry (EmIE) to study the interfacial property of this mediator compound on Pt or glassy carbon electrode surfaces. The EmIE technique allows us to correlate changes of mass, charge, and ellipsometric angle measurements in a synchronized manner to derive information on chemical and electrochemical behavior of this polymer mediator film (Figure 1.2.7). This technique can be applied to both steady state and transient dynamic regimes.

As a result of such studies, we have produced some interesting results based on the EmIE approach in the study of poly(methylene green) mediator [7, 19]. Highlights are as follows:
Figure 1.2.17 shows that the transient change of mass and current do not coincide in the synchronized measurements. However, the ellipsometric measurements show that the ellipsometric angle $\Delta$ is in sync with transient mass changes at all times, indicating that the mass change is actually detectable and the corresponding change in such a surface film thickness is also measurable by the ellipsometric method. The electrochemical redox reaction, on the other hand, occurs at a different time scale and the corresponding current change is also in sync with the ellipsometric angle change in $\Psi$, which has a split peak: one corresponds to the mass (thus film thickness) change and the other to the chemical change in the redox process. It is therefore interesting to observe this transient behavior of the film development, where the mass change is related to adsorption, while the redox reaction does not have a direct correlation with mass measured [19].

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**Figure 1.2.18 Simulation of film thickness changes with deposition conditions**
We also showed that with proper estimates of the parameters in the optical model, we can simulate the film thickness changes with deposition conditions (Figure 1.2.18). This is a powerful tool to allow real-time \textit{in situ} observation with quantitative characterization [20].

In the effort to continue to develop PQQ-dependent enzymatic systems for bio-anode application\textsuperscript{1}; we found the reconstitution of the PQQ-dependent enzymes such as glucose dehydrogenase (GDH) on glassy carbon electrodes with chitosan-carbon nanotube matrix have not been effective in making electronic conduction to promote the enzymatic electrocatalytic kinetics [13]. The turnover rate for the reconstituted GDH electrodes has been low. We are searching for other mediators that can provide better relay units for charge transfer.

In the effort to develop \textit{in situ} interfacial characterization techniques to assess the practicality of using self-assembled monolayers (SAMs) for enzyme immobilization, we have found that the surface conditions of the glassy carbon or gold electrode are very sensitive to contamination and thus the SAM formation is not reproducible and difficult to control (Svoboda and Liaw, unpublished results). In short, both the mediator-cofactor reconstitution and SAM-based immobilization approaches have produced limited success in improving enzymatic electrocatalysis processes. We consider these techniques to possess limited potential for significant improvement of bio-fuel cell performance at the present time.

\textit{Summary of additional work accomplished during this period.} Work towards the development and characterization of chitosan co-block polymers that immobilize multiple enzymes that can more fully oxidize complex energy fuels begins with the development of a technique to lay down and characterize micron thin films. This work is in its initial stages and now under the direction of a graduate student. To date, a thin-film fabrication technique has been developed. Specifically, the thin-film scaffolds were prepared using a modified technique based on the procedure outlined elsewhere [5]. The system application is shown in Figure 1.2.19.

\textsuperscript{1} These enzymes can be bound to either chitosan-CNT or conductive polymers, such as polypyrrole, as novel immobilization approaches that can realize direct electron transfer.
Figure 1.2.19  Schematic of film deposition with inlaid microscope image from thickness measurement of air-dried film (top left). Deacetylated or butyl-modified chitosan solutions were pipetted on the obtuse side of the intersection of the two glass slides held at an angle of 30 ± 1°. A meniscus forms under the leading slide as it pushes against the solution droplet and across the lower glass substrate at a constant velocity, leaving a thin layer of solution behind. Films formed as the solution was air-dried.

This work characterizes the method of spread coating to form polymeric films with controlled thickness. The thickness of spread-coated films made from both deacetylated and butyl-modified chitosan was correlated to deposition rate and solution micellar structure, and demonstrated how differences in the underlying micellar structure can, to a certain degree, impact the final film thickness. At intermediate deposition rates, the thickness of chitosan films was predictable and well controlled. Furthermore, it was shown that hydrophobic modification of the chitosan extended the range of deposition rates (from 5-16 cm/hr to 6-30 cm/hr) that allowed for which a linear relationship between film thickness and deposition rate were found. Hydrophobic modification also extended the range of thickness achieved from 0.06-0.10 μm to 0.04-0.14 μm.

These features are accredited to the domination of intramolecular forces at lower concentrations of hydrophobically modified chitosan solutions as opposed to equal concentration of the deacetylated chitosan solutions, as supported by the viscosity and fluorescence experiments. Although both deacetylated and butyl-modified chitosan solutions were found to have inter- and intramolecular interactions and hydrophobic domains able to incorporate a fluorophore, deacetylated chitosan is much more interconnected via intermolecular interactions at higher concentrations. This work has been submitted to Langmuir and is currently under review.

In the effort of continuing to apply in situ characterization techniques based on spectroscopic imaging ellipsometry with microgravimetric and electrochemical techniques to study the enzyme-electrode interaction on the electrode surface and the associated dynamic behavior, aiming to understanding the charge transfer process with direct measurements and correlations, We have improved the capability for such characterization. We have upgraded our imaging ellipsometry to a spectroscopic model with a high intensity Xenon arc lamp as white light source,
which can provide a wavelength range from 360 nm to 1,000 nm with a monochromator that consists of 46 interference filters to provide ± 6 nm of bandwidth resolution. The spectroscopic model will provide us additional control on the wavelength control for the incident light besides incident angle for more information gathering on the surface and film properties. We are currently using this capability to study the poly(methylene green) mediator properties (Chiu and Liaw). We anticipate receiving more quantitative results on the film characterization.

In addition to these efforts outlined above, through the IC Postdoctoral Fellow Research Program, we have investigated other alternative approaches to harnessing chemical energy in bio-fuels for fuel cell operation [21]. One such investigation has led us to achieve a sustainable current generation with ammonia oxidation, with assistance from conductive polymers such as polypyrrole [21]. Figure 1.2.20 shows the current production as a function of ammonia concentration in the presence of polypyrrole and hydrogen peroxide at different concentrations in the solution. Without ammonia, the current production was not obvious, even with the presence of peroxide. Therefore, it is a clear indication that the current was produced by the oxidation of ammonia, which was promoted by peroxide in the interaction with polypyrrole.

In a separate study of glucose oxidation, we also discovered a unique pathway to promote charge transfer of partial oxidation of glucose to produce current with the help of mediators such as methyl viologen [15]. In this study, high current density and power density were observed with glucose oxidation in alkaline solutions with the presence of a mediator.

1.2.2.3 References


1.2.2.4 Papers and Presentations Resulting from Efforts (since 2005)

Papers


Presentations


2010  Cooney, M. J. Modification and Characterization of Chemical Microenvironments for Enzyme Immobilization. 240th American Chemical Society, August 22-26, Boston, MA.


2008 **Cooney**, M. J.  *Microporous chitosan scaffolds as a material for fabrication of enzyme catalyzed flow through electrodes*.  213th ECS meeting, May 18-23, Phoenix Arizona.


2006 Cooney, M.J., Svoboda, V., Rippolz, C., and Liaw, B.Y. Design considerations and characterization of enzymatic ethanol fuel cells. 209th ECS Meeting, May 7-12, Denver, CO.


2006 Cooney, M.J., Svoboda, V., Rippolz, C., and Liaw, B.Y. Design considerations and characterization of enzymatic ethanol fuel cells. 209th ECS Meeting, May 7-12, Denver, CO.


