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Synchrotron X-ray Based Investigation of Fe Environment in Porous Anode of *Shewanella oneidensis* Microbial Fuel Cell

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***Abstract*—**The iron environment in Fe-doped Vycor Anode was investigated with EXAFS using Brookhaven Synchrotron Light Source. The iron-reducing *Shewanella oneidensis* culture was grown in a microbial fuel cell under anaerobic respiration. The Fe bond length was found to decrease and correlate with the amount of biofilm growth on the Fe-doped Vycor Anode. The data suggests that Fe-doped Vycor Anode would be a good substrate to study the Shewanella oneidensis nanowire structure using EXAFS.

***Keywords*—**EXAFS, Fourier Transform, Microbial Fuel Cell, *Shewanella oneidensis.*

I. Introduction

MICROBIAL fuel cell technology is an important energy alternative. The technology uses electricigens to convert organic material into electricity, and shows great promise for efficient conversion of organic waste or renewable biomass to electricity. Due to improvements in efficiency and beneficial side effects, such as wastewater treatment, the technology is fast approaching commercialization. Usually microbes consume carbohydrates such as sugar in aerobic conditions to produce carbon dioxide and water. However, in the absence of oxygen, carbon dioxide, protons, and electrons are generated instead. The technology can generate a stable electrical power density of 80 mW/m^2 level for 10 days using organic compounds in wheat [1]. Peak power reaching 120 mW/m^2 was reported. The microbial species that convert organic compounds to electricity were shown to be dominated by Bacteroidetes (anode biofilm) and Firmicutes (suspended substrate). The maximum record of $1.8W/m^2$ was reported to be from a *Geobacter sulfurreducens* microbial fuel cell [2]. The reason has been attributed to the thick biofilm at the anode assisted by the nanowire-pilus network [3]. The thick *Geobacter sulfurreducens* biofilm c-type cytochrome OmcZ protein was crucial to the high current capability and recently OmcZ has been purified as well [4]. Using genetic mutation techniques, *Shewanella oneidensis* expressing the light sensitive proteorhodopsin protein in a microbial fuel cell with lactate and other organic compounds was reported to support electric current generation at 10 mA/m^2, given an illumination of 2 mW/m^2 from a green LED [5], [6]. It has been speculated that the incorporation of photosynthetic reaction centers into *Shewanella oneidensis* would increase the yield of light energy absorption and conversion to electricity. It was reported earlier that *Shewanella oneidensis* MR-1 can produce electrically conductive pilus under electron-acceptor limitation condition [7]. The investigation of nanowire pilus effect on the light sensitive *Shewanella oneidensis* microbial fuel cell for electricity production would be a pertinent issue in energy research.

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It has been demonstrated that the iron-reducing bacterium *Shewanella oneidensis* MR-1 is able to express outer membrane cytochromes such as MtrC and OmcA during anaerobic respiration which then transfers electrons directly to Fe(III) in a mineral. Atomic Force Microscopy data revealed that a single bacterium (2 by 0.5 μm) could express up to ten thousand cytochromes on its outer surface [8]. Crystalline iron oxides such as hematite have been shown as an effective respiration substrate and the MtrC and OmcA cytochromes function as terminal reductases [9]. OmcA cytochrome was found to be required for electron transfer efficiency and biofilm attachment with hematite as the terminal electron acceptor after MtrC cytochrome maximized the biofilm growth. Therefore electrode design would play a crucial role for the current recovery process. It was reported that a sputtered gold-copper anode increases a standard *Shewanella oneidensis* microbial fuel cell efficiency by 45% and the report attributed the reason to be the increase growth of the *Shewanella oneidensis* biofilm at the anode [10]. We have studied the growth of *Shewanella oneidensis* biofilm in Fe doped anodes that are fabricated from Corning Vycor porous glass (10-20nm pores) in a standard microbial fuel cell construction. The anode Fe property was studied with X-ray absorption fine structure measurement (EXAFS). The amount of metal absorbed into the Vycor glass, as well as the amount of metal incorporated in the pili, is very low (<0.0001 moles/gram of glass). This low concentration is partly required by the thinness of the interface from microbe to electrode. Our data was found to be consistent with the previously reported EXAFS data on porous iron oxide samples where Fe- O_1 has bond length of 195 pm and is shorter than the bond length of Fe- O_2 at 207 pm [11]. The details of our findings are reported in this publication.

II. Materials and Methods

The EXAFS and XANES data were collected at beamline X10C of National Synchrotron Light Source at Brookhaven national Laboratory. Rhodium coated cylindrical mirror is used to focus the beam on the sample. A double crystal Si(220) mono-chromator was used for energy selection. Ion chambers were used to measure beam intensity before the hutch, I_0 (before sample), and transmission intensity. A 7-element Si drift detector was used to measure fluorescence intensity. Piezoelectric driver using A/C feedback system locks the beam. Beam size on the sample was 10 mm x 2 mm. Beamline and data collection was controlled by microvax II computer running on VMS. *Shewanella oneidensis* MR-1 culture was grown in a microbial fuel cell configuration with Fe-doped Vycor anode in overpressure carbon dioxide purging anaerobic condition, and the LB nutrient solution, purchased from Sigma Aldrich, was used. Standard procedures for decontamination by autoclaving were used.

The details of the Fe doping process in Vycor was reported earlier [12]-[14]. Briefly, Code 7930 porous glass was obtained from Corning Glass Works. Pieces of 1”x 1”x 2 mm were continuously extracted with distilled water for 24 hours and then dried at 600C in a vacuum oven ($<10^{-4}$ Torr). The dried samples were calcined in air at 5500C for at least 24 hours and stored at this temperature until needed. Precursor material for impregnation $Fe(CO)_5$, is obtained from Sigma Aldrich. Note that $Fe(CO)_5$ is volatile at room temperature. When clean Porous Vycor Glass (PVG) expose to this vapor, $Fe(CO)_5$ absorb into the pores. Photolysis of $Fe(CO)_5$ decomposes precursor into CO_2 and FeO. To remove any un reacted material samples were heated to 650C for 24 hours. That oxidizes most of the iron.

The data analysis was done with EXAFSPAK and WIN-XAS packages. A constant phase shift of 40 nm was added for calibration, a standard EXAFS analysis procedure.

III. Results and Discussion

A typical EXAFS of Fe-dope Vycor Anode sample is shown in Fig. 1. Recently, Fe Mossbauer spectroscopy has been used to study the redox cycling of Fe(II) and Fe(III) in magnetite by Fe-metabolizing bacteria in microbial fuel cell [15]. Fig. 2 contains the Fe Mossbauer Spectroscopy data of the Fe-doped Vycor sample shown in Fig. 1. The Mossbauer signal contribution came from the 4-nm Fe_2O_3 nanoparticles with no FeO generation in the doping process. The Fourier transform of the Fig. 1 EXAFS data is shown in Fig. 3.

The Fe-doped Vycor Anode property in two biofilm growth stages inside the microbial fuel cell under anaerobic respiration was investigated. The anaerobic condition was maintained by overpressure carbon dioxide purging. The continuous monitoring of the microbial fuel cell current generation was also used as a consistency checking mechanism. The FFT results are displayed in Figs. 4 and 5.

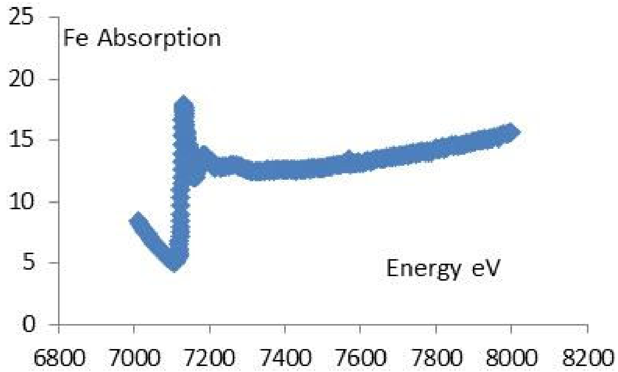

Fig. 1 The EXAFS of Fe-doped Vycor data (no *Shewanella oneidensis* growth)

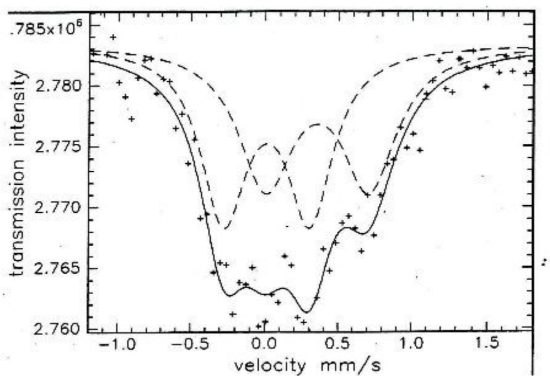

Fig. 2 Fe Mossbauer Spectroscopy of the sample in Fig. 1. The FeO component was not detected in the data fit.

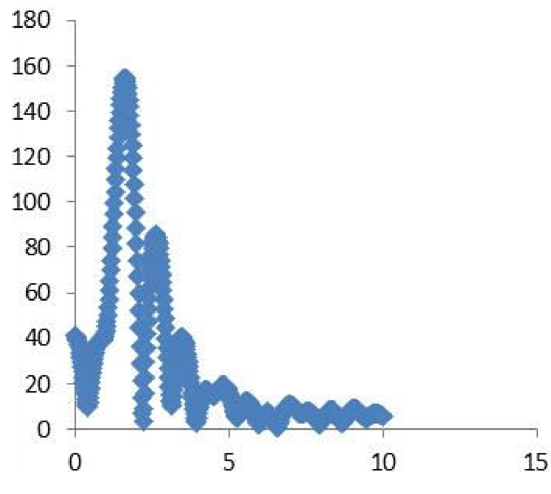

Fig. 3 The Fourier Transform data using the k-cubed--weighted Fig. 1 EXAFS data (Y-axis) The x-axis is in Angstrom (100 pm). The first peak was at 196 nm using a phase shift of 40 nm

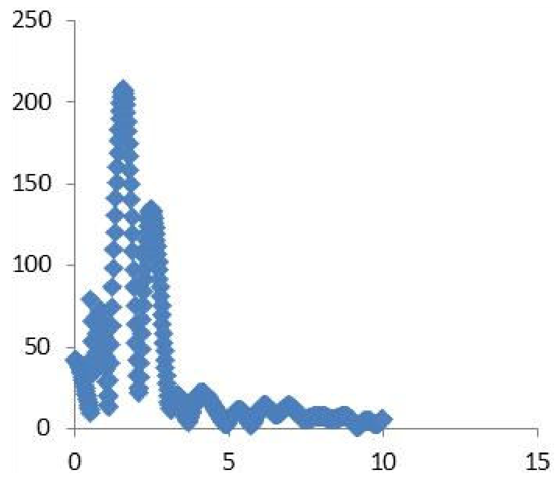

Fig. 4 The Fourier Transform data using the k-cubed--weighted EXAFS data of Fe-doped Vycor Anode after 1 day of *Shewanella oneidensis* growth inside the microbial fuel cell under anaerobic respiration (Y-axis). The x-axis is in Angstrom (100 pm). The first peak was at 192 nm using a phase shift of 40 nm

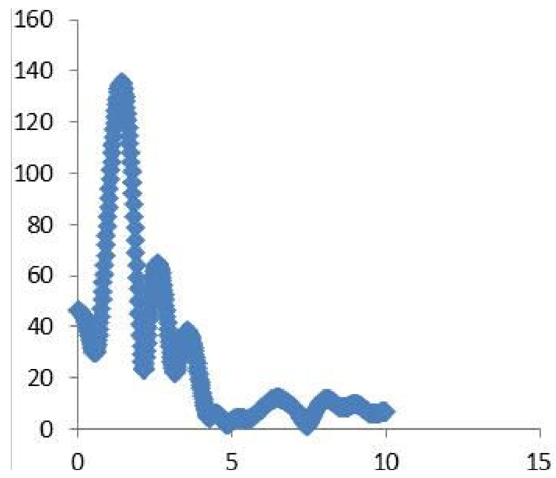

Fig. 5 The Fourier Transform data using the k-cubed--weighted EXAFS data of Fe-doped Vycor Anode after 4 days of *Shewanella oneidensis* growth inside the microbial fuel cell under anaerobic respiration (Y-axis) The x-axis is in Angstrom (100 pm) The first peak was at 176 nm using a phase shift of 40 nm

The reduction of Fe bond length from 192 nm to 176 nm would be consistent with the emergence of Fe-O_1 during the biofilm growth of the iron-reducing *Shewanella oneidensis*, given an initial condition of Fe-O_2 in the Vycor; as supported by the Mossbauer data shown in Fig. 2 and FFT result shown in Fig. 3. Another investigation region was selected to be closer to the surface of the nutrient fluid and carbon dioxide purging gas. The result is shown in Fig. 6.

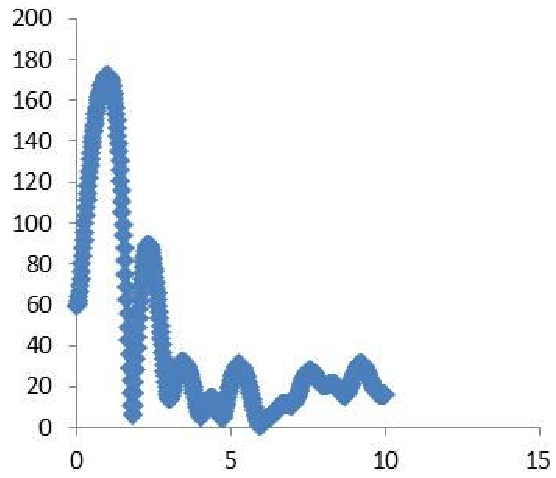

Fig. 6 The Fourier Transform data using the k-cubed--weighted EXAFS data of Fe-doped Vycor Anode after 4 days of *Shewanella oneidensis* growth inside the microbial fuel cell under anaerobic respiration (Y-axis). The investigation region was selected to be closer to the surface of the nutrient and carbon dioxide purging gas. The x-axis is in Angstrom (100 pm). The first peak was at 130 nm using a phase shift of 40 nm.

A general picture has emerged where the iron-reducing bacterium *Shewanella oneidensis* MR-1 was able to shorten the Fe bond length in the Fe-doped Vycor Anode under anaerobic respiration. The observed short bond length of 130 pm for the Vycor Anode region near the gas-fluid interface would be an interesting future project. It was reported that *Shewanella oneidensis* exhibits electron hopping mechanism [16], and recently the ability of *Shewanella oneidensis* using its decaheme outer membrane cytochromes to facilitate anode adhesion instead of developing pili like the Geobacter genus was confirmed [17]. A 2015 high resolution study on *Shewanella oneidensis* type IV pilin, a conductive nanowire candidate, revealed an unusual position of the disulfide bridge and a straight α-helical section [18]. The bacteria nanowire structure is an important geometry in the development of high efficient anode in the microbial fuel cell technology [19]. It was also reported that charge localizing sites generated by *Shewanella oneidensis* could be less than 1 nm [20]. Knowing that nanoparticle favors short bond length, the observed 130 pm bond length could be related to particle size of less than the typical 4-nm Fe_2 O_3 particle in Fe-dope Vycor. Whether the Fe in the cytochromes MtrC and OmcA of the *Shewanella oneidensis* would break down the 4-nm sized nanoparticles near the carbon dioxide gas and nutrient fluid interface region during anaerobic respiration is an interesting future project.

IV. Conclusion

The iron environment in Fe-doped Vycor Anode was investigated with EXAFS using Brookhaven Synchrotron Light Source. The decreasing Fe bond length was found to correlate with iron-reducing *Shewanella oneidensis* biofilm growth in anaerobic respiration inside a microbial fuel cell. The data suggests that Fe-doped Vycor Anode would be a good substrate to study the bacteria nanowire structure using EXAFS. The ability of *Shewanella oneidensis* to reduce the 4-nm size iron oxide nanoparticles in the Fe-doped Vycor Anode to smaller physical sizes is an interesting future project.

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