ISOLATION AND ANALYSIS OF NOVEL ELECTROCHEMICALLY ACTIVE BACTERIA FOR ENHANCED POWER GENERATION IN MICROBIAL FUEL CELLS

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Final Report

PROJECT SUMMARY

Bacteria capable of exocellular transfer of electrons to solid surfaces, called *exoelectrogens*, make it possible to obtain electricity from the breakdown of organic matter in microbial fuel cells (MFCs). We obtained several new exoelectrogenic bacteria during this project. We isolated *Rhodopseudomonas palustris* DX-1, and demonstrated for the first time that a pure culture could produce more power than the mixed culture it was derived from in a high-performance microbial fuel cell (MFC). In contrast, a common iron reducing strain (*Geobacter sulfurreducens*) produced less power than a mixed culture dominated by the same strain. Using a newly developed U-tube MFC, we isolated *Ochrobactrum anthropi* YZ-1, which had the remarkable characteristic that it was unable to respire using hydrous Fe(III) oxide but produced current in an MFC. This further demonstrated that not all exoelectrogens are capable of dissimilatory iron reduction. We obtained for the first time a bacterium (*Enterobacter cloaceae*) that was capable of simultaneous cellulose degradation and current generation. A strain of the denitrifying bacterium *Comamonas denitrificans*, isolated from an MFC also produced power showing the importance of some denitrifying bacteria for power generation. We analyzed the microbial communities that developed in MFCs and showed that various operational conditions and materials affect power generation and community structure. These findings demonstrate the importance of various microbial communities in the system for power generation in MFCs.

INTRODUCTION AND OBJECTIVES

Microbial fuel cells (MFCs) are an excellent technology for versatile power production with different substrates present in the natural environment, as bacteria can rapidly colonize the anode electrode and produce power. Known mechanisms used by bacteria to transfer electrons to the anode, derived in part from our understanding of microbial iron reduction, include self-produced chemical mediators and nanowires. The propose of this project was to: develop new methods to isolate bacteria and to identify new strains of bacteria capable of power generation; study the bacterial communities that produce current in these systems; and examine the mechanisms for power generation. One goal was to identify filamentous bacteria that produced power, but isolates that were obtained that produced reasonable amounts of power were not filamentous. We successfully developed new devices to enrich bacteria that might be non-culturable using standard agar plating techniques. With this device, we obtained several new bacteria that were shown for the first time to produce power in our MFCs. The findings related to this project are summarized below according to topics and published papers.

FINDINGS

Isolation of new types of exoelectrogenic bacteria using a novel type of U-tube MFC.

Exoelectrogenic bacteria can transfer electrons outside the cell to insoluble electron acceptors, such as metal oxides, or the anodes of MFCs. Very few exoelectrogens have been directly isolated from MFCs, and all have been obtained by techniques that potentially restrict the diversity of exoelectrogenic bacteria. A special U-tube-shaped MFC was therefore developed to enrich exoelectrogenic bacteria with isolation based on dilution-to-extinction methods. Using this device we obtained a pure culture which we identified as *Ochrobactrum anthropi* YZ-1 based on 16S rDNA sequencing and physiological and biochemical characterization. StrainYZ-1 was unable to respire using hydrous Fe(III) oxide but produced 89 mW/m^2 using acetate as the electron donor in the U-tube MFC. Further applications of this new U-tube MFC system will provide a method for obtaining additional exoelectrogenic microorganisms that do not necessarily require metal oxides for cell respiration. Using this device allows us to obtain new isolates

and to understand why different microbial assemblages and pure cultures can produce different levels of power *(Zuo et al., 2008, Appl. Environ. Microbiol.)*

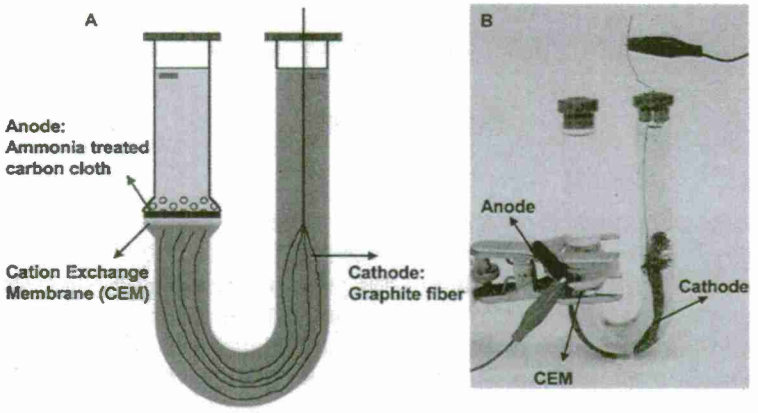

Figure 1. U-tube used for bacterial isolation: (A) Schematic showing electrodes and membrane; (B) photograph of a working device. (From Zuo et al. 2008).

Electricity generation by *Rhodopseudomonas palustris*

Bacteria able to generate electricity in microbial fuel cells (MFCs) are of great interest, but there are few strains capable of high power production in these systems. Here we report that the phototrophic purple non-sulfur bacterium *Rhodopseudomonas palustris* DX-1, isolated from an MFC, produced electricity at higher power densities (2720 mW/m^2) than mixed cultures in the same device. While *Rhodopseudomonas* species are known for their ability to generate hydrogen, they have not previously been shown to generate power in an MFC, and current was generated without the need for light or hydrogen production. Strain DX-1 utilizes a wide variety of substrates (volatile acids, yeast extract and thiosulfate) for power production in different metabolic modes, making it highly useful for studying power generation in MFCs and generating power from a range of simple and complex sources of organic matter. These results demonstrated that a phototrophic purple non-sulfur bacterium could efficiently generate electricity by direct electron transfer in MFCs, providing another model microorganism for MFC investigations. *(Xing et al., 2008, Environ. Sci. Technol.)*

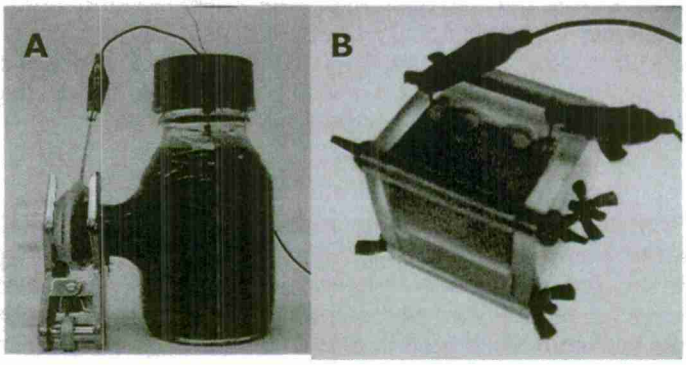

Figure 2. (A) Bottle and (B) cube-shaped air-cathode MFCs inoculated with *Rhodopseudomonas palustris* DX-1 that show the characteristic red color of these bacteria. (Pictures by D. Xing).

Comparison of electrode reduction activities of *Geobacter sulfurreducens* and an enriched consortium in an air-cathode microbial fuel cell.
An electricity generating bacterium, *Geobacter sulfurreducens* PCA, was inoculated into an air-cathode single-chamber MFC in order to determine the maximum electron transfer rate from bacteria to the anode. To create anodic reaction-limiting conditions, where electron transfer from bacteria to the anode is the rate-limiting step, anodes with electrogenic biofilms were reduced in size and tests conducted using anodes of six different sizes. The smallest anode (7 cm^2, or the 1.5 times larger than the cathode) achieved an anodic reaction-limiting condition as a result of a limited mass of bacteria on the electrode. Under these conditions, the limiting current density reached a maximum of 1530 mA/m^2, and power density reached a maximum of 461 mW/m^2. Per-biomass efficiency of the electron transfer rate was constant at 32 fmol $cell^{-1}$ d^{-1} (178 µmol g-$protein^{-1}$ min^{-1}), a rate comparable to that with solid iron as the electron acceptor but slower than rates achieved with fumarate or soluble iron. In comparison, an enriched electricity-generating consortium reached 374 µmol g-$protein^{-1}$ min^{-1} under the same conditions, suggesting that the consortium had a much greater capacity for electrode reduction. These results demonstrate that per-biomass electrode reducing rates (calculated by current density and biomass density on the anode) can be used to help make better comparisons of electrogenic activity in MFCs. (Ishii et al., 2008, *Appl. Environ. Microbiol.)*

Simultaneous cellulose degradation and electricity production by *Enterobacter cloacae* in an MFC.
Electricity can be directly generated by bacteria in MFCs from many different biodegradable substrates. When cellulose is used as the substrate, electricity generation requires a microbial community with both cellulolytic and exoelectrogenic activity. Cellulose degradation with electricity production by a pure culture has not been previously demonstrated without addition of an exogenous mediator. Using a specially designed U-tube MFC, we enriched a consortium of exoelectrogenic bacteria capable of using cellulose as the sole electron donor. After 19 dilution-to-extinction serial transfers of the consortium, 16S rRNA gene-based community analysis using denaturing gradient gel electrophoresis and band sequencing revealed that the dominant bacterium was *Enterobacter cloacae*. An isolate designated *E. cloacae* FR from this enrichment was found to be 100% identical to the type strain *Enterobacter cloacae* 13047 based on a partial 16S rRNA sequence. In polarization tests using the U-tube MFC and cellulose as a substrate, strain FR produced 4.9 mW/m^2 compared to 5.4 mW/m^2 for strain 13047. These results demonstrate for the first time that it is possible to generate electricity from cellulose using a single bacterial strain without the need for exogenous mediators. *(Rezaei et al.,2009, Appl. Environ. Microbiol.)*

Change in microbial communities in acetate- and glucose-fed microbial fuel cells in the presence of light.
Power densities produced by MFCs in natural systems are changed by exposure to light through the enrichment of photosynthetic microorganisms. When MFCs with brush anodes were exposed to light (4000 lx), power densities increased by 8-10% for glucose-fed reactors, and 34% for acetate-fed reactors. Denaturing gradient gel electrophoresis (DGGE) profiles based on the 16S rRNA gene showed that exposure to high light levels changed the microbial communities on the anodes. Based on 16S rRNA gene clone libraries of light exposed systems the anode communities using glucose were also significantly different than those fed acetate. Dominant bacteria that are known exoelectrogens were identified in the anode biofilm, including a purple nonsulfur (PNS) photosynthetic bacterium, *Rhodopseudomonas palustris,* and a dissimilatory iron-reducing bacterium, *Geobacter sulfurreducens.* Pure culture tests confirmed that PNS photosynthetic bacteria increased power production when exposed to high light intensities (4000 lx). These results demonstrated that power production and community composition are affected by light conditions as well as electron donors in single-chamber air-cathode MFCs. *(Xing et al., 2009. Biosens Bioelec.)*

Isolation of the exoelectrogenic denitrifying bacterium *Comamonas denitrificans* based on dilution-to-extinction of the microbial community.
The anode biofilm in a microbial fuel cell (MFC) is composed of diverse populations of bacteria, many of whose capacities for electricity generation are unknown. To identify functional populations in these exoelectrogenic communities, a culture-dependent approach based on dilution-to-extinction was combined with culture-independent community analysis. We analyzed the diversity and dynamics of microbial communities in MFCs with different anode surfaces using denaturing gradient gel

electrophoresis (DGGE) based on the 16S rRNA gene. Phylogenetic analyses showed that the bacteria enriched in all reactors belonged primarily to five phylogenetic groups: *Firmicutes*, *Actinobacteria*, *α-Proteobacteria*, *β-Proteobacteria*, and *γ-Proteobacteria*. Dilution-to-extinction experiments further demonstrated that *Comamonas denitrificans* and *Clostridium aminobutyricum* were dominant members of the community. A pure culture isolated from an anode biofilm after dilution-to-extinction was identified as *Comamonas denitrificans* DX-4 based on 16S rRNA sequence and physiological and biochemical characterizations. Strain DX-4 was unable to respire using hydrous Fe(III) oxide but produced 35 mW/m^2 using acetate as the electron donor in an MFC. Power generation by the facultative *C. denitrificans* depends on oxygen and MFC configuration, suggesting that a switch of metabolic pathway occurs for extracellular electron transfer by this denitrifying bacterium. *(Xing etal., Appl. Microbiol. Biotechnol. Submitted.)*

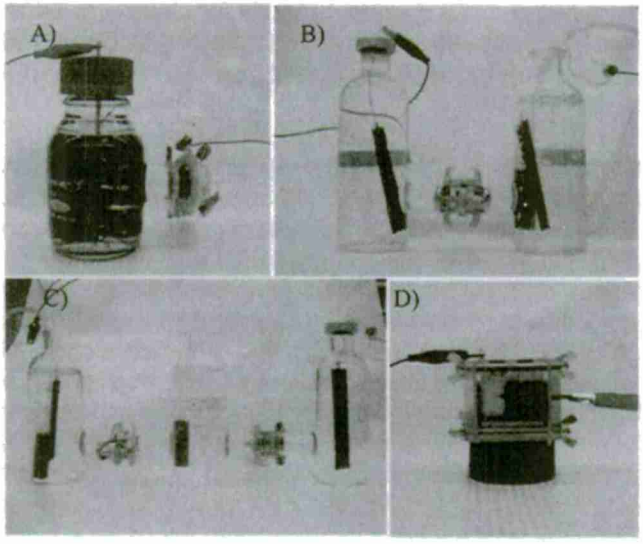

Figure 3. MFC reactor configurations (A) 1-bottle, (B) 2-bottle, (C) 3-bottle, and (D) cubic.

Power production in MFCs inoculated with *Shewanella oneidensis* MR-1 or mixed cultures. Power densities and oxidation-reduction potentials (ORPs) of a pure culture of *S. oneidensis* MR-1 were compared to mixed cultures (wastewater inoculum) in cube shaped, 1-, 2-, and 3-bottle batch-fed MFC reactor configurations. The reactor architecture altered the relative power output by the two inocula, with the mixed culture producing 68 to 480% more power than strain MR-1 in the four MFCs. The mixed culture produced the maximum power density of 858 m^{-2} in the cubic MFC, while MR-1 produced 148 mW m^{-2}. The higher power by the mixed culture was primarily a result of lower internal resistances than those produced by the pure culture. Power was a direct function of ohmic resistance for the mixed culture, but not for strain MR-1. ORP varied with reactor configuration and inoculum, and it was always negative during maximum power production but it did not vary in proportion to power output. The ORP varied primarily at the end of the cycle when substrate was depleted, with a change from a reductive environment during maximum power production (~ -175 mV for mixed and ~ -210 mV for MR-1 in cubic MFCs), to an oxidative environment at the end of the batch cycle (~ 250 mV for mixed and ~ 300 mV for MR-1). Mixed cultures produced more power than MR-1 MFCs even though their redox potential was less negative. These results demonstrate that variability in power densities produced by pure and mixed cultures depends on the specific MFC architecture, and that redox potentials vary substantially at the end of the fed-batch cycle in MFCs. *(Watson and Logan, Biotechnol. Bioengin. Submitted.)*

Exoelectrogenic bacteria that power microbial fuel cells (Review paper).
I was invited to provide a review of recent advances in the study of bacteria capable of power generation in MFCs. In only a few years there was a large increase in reports of microorganisms that can generate electrical current in MFCs. While many new strains were identified, few strains individually were found to produce power densities as high as those of mixed communities. Enriched anodic biofilms of exoelectrogenic bacteria have generated power densities as high as 6.9 W/m^2 (projected anode area), and therefore are approaching theoretical limits. To understand bacterial versatility in mechanisms used for current generation, I explored in this paper the reasons for exocellular electron transfer that included cellular respiration and possibly cell-cell communication. *(Logan, 2009, Nature Rev. Microbiol.)*

Hydrogen generation by *Rhodopseudomonas palustris* DX-1 in the presence of ammonium via electrohydogenesis.
Electrohydrogenesis is a new process for producing hydrogen gas in microbial electrolysis cells (MECs). Using mixed cultures results in a product gas that is contaminated with methane, but methanogensis can be avoided using pure cultures. The exoelectrogenic PPNS bacterium, *Rhodopseudomonas palustris* DX-1, was examined here for hydrogen production by electrohydrogenesis due to its ability to produce high current densities in a microbial fuel cell. Photosynthetic purple nonsulfur (PPNS) have previously been used to directly produce H_2 using nitrogenases with energy from organic compounds and light, but hydrogen gas production by wild-type strains is inhibited by ammonium, nitrogen gas, or oxygen. It was shown here that hydrogen gas can be produced by using strain DX-1 in an MEC, even in the presence of ammonium, at a maximum rate of 6.44 m^3/m^3 d (531 A/m^3) and an overall yield of 3.85 mol H_2/mol-acetate in an MEC pre-sparged with Ar gas (applied voltage of E_{ap}=0.8 V). A lower production rate of 4.0 $m^3/m^3 \cdot d$ (341 A/m^3), and a similar high yield (3.6 mol H_2/mol acetate) were obtained when the MEC was pre-sparged with N_2 gas. The energy recovery was 211% based on electricity, and 98% based on energy in both electricity and light (Ar; E_{ap} = 0.8 V). Overall energy efficiency based on substrate, electricity, and light were similar (50–55%) for the MECs with the two different gases (E_{ap} = 0.6–0.8 V). These results demonstrate that high rates of hydrogen production can be accomplished using *R. palustris* DX-1 via electrohydogenesis even in the presence of solutions containing ammonium, that the headspace gas affects hydrogen production rates and yields, and that overall energy yields are higher than those so far reported for direct hydrogen production using other R. palustris strains. *(Xing et al., Environ. Sci. Technol., Submitted.)*

PUBLICATIONS

Zuo, Y., D. Xing, J.M. Regan, and B.E. Logan. 2008. Isolation of the exoelectrogenic bacterium *Ochrobactrum anthropi* YZ-1 by using a U-tube microbial fuel cell. *Appl. Environ. Microbiol.* 74(10):3130-3137.

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Xing, D., S. Cheng, J.M. Regan and B.E. Logan. 2009. Change in microbial communities in acetate- and glucose-fed microbial fuel cells in the presence of light. *Biosens Bioelec.* doi:10.1016/j.bios.2009.06.013.

Xing, D., S. Cheng, J.M. Regan, and B.E. Logan. 2009. Isolation of the exoelectrogenic denitrifying bacterium *Comamonas denitrificans* based on dilution-to-extinction of the microbial community. *Appl. Microbiol. Biotechnol.* Submitted.

Xing, D., S. Cheng and B.E. Logan. 2009. Hydrogen generation by *Rhodopseudomonas palustris* DX-1 in the presence of ammonium via electrohydogenesis. Submitted.

Watson, V.J., and B.E. Logan. 2009. Power production in MFCs inoculated with *Shewanella oneidensis* MR-1 or mixed cultures. *Biotechnol. Bioengin.* Submitted.

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14. ABSTRACT
Using a U-tube MFC approach developed earlier as a part of this project, we isolated a pure culture classified Enterobacter cloacae based on 16S rDNA sequencing and physiological and biochemical characterization. This strain was remarkable because it was able to produce electricity in a microbial fuel cell from the degradation of cellulose. This is the first time that a bacterium has been shown to be able to accomplish both of these tasks. We examined the communities that developed in MFCs based on electrode surface material and the effects of light on the community. We have also shown that Rhodopseudomonas palustris strain DX-1, a strain previously isolated as a part of this project, was capable of being used in a microbial electrolysis cell for the biologically-driven electrochemical production of hydrogen gas.

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