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Isolation and Identification of an Electricity Producing Microorganism Obtained from the Quivira National Wildlife Refuge

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ABSTRACT

A strictly anaerobic, Gram-positive, spore forming, short rod-shaped bacterium was isolated from Kansas marshland and shown to be capable of mediator-free electricity generation. A dual-chambered fuel cell was constructed using carbon fiber as the electrode material. After a ten day period, the isolated bacteria produced a peak current level of 13.68 microamperes. Though a current was produced, the studied bacteria yielded much lower current levels than other bacteria prominent in fuel cell studies.

Keywords: *microbial fuel cell, electricity generation, carbon fiber electrode, Quivira National Wildlife Refuge*

INTRODUCTION

With the global fossil fuel consumption rate steadily increasing, scientists have begun to seek alternative means of electricity generation. In 1962, J.B. Davis and H.F. Yarbough Jr. began exploratory experimentation with *Nocardia* and *Escherichia coli* bacteria studying their electricity generating capabilities with various hydrocarbon food sources. Anaerobic *E. coli* experiments with platinum sheets serving as electrodes proved positive and thus led to a greater scientific interest in the harvesting of microbial generated electricity (Davis, Yarbough Jr. 1962).

Microbial fuel cells capitalize on the natural metabolism of bacteria. Bacteria in an anaerobic chamber donate electrons to an electrode rather than to oxygen, because it is not present. Other electron acceptors are excluded to allow a greater chance of electron transfer to the electrode. The electrons are transferred from the electrode through wires and are harvested as electricity.

Researcher D.R. Lovley is presently one of the most prominent scientists in the field of microbial electricity generation. His studies have focused primarily on the electron transfer ability of bacteria in the *Geobacteraceae* family (Bond, Lovley 2003; Bond et al. 2002). Lovley's articles have documented his breakthrough discovery of the mediator-free, stable electricity generating capabilities of anaerobic *Geobacter sulfurreducens* More importantly, his experiments have produced exceptionally efficient electron transfer results to the graphite electrodes (Bond, Lovley 2003).

H. Liu and B.E. Logan have focused their experiments on the use of waste water as a food source for microbial energy generation (Liu et al. 2004). Their studies have shown a positive production as well as the bacteria's ability to remove approximately 78% of organic matter from the water (Ehrenman 2004). Logan now plans to focus on increasing power production, reducing costs, and making the technology available to the public.

Though a typical fuel cell may produce only enough energy to power a small stopwatch, advances are continually being made. As an alternative to fossil fuel consumption, microbial energy production offers stability, less environmental harm, and removal of organic matter from waste water.

The goal of this research project was to prove the availability of an electricity producing microorganism in Kansas marshland, thus strengthening the future significance of microbial generated electricity. It focused on an area of the United States that had not previously studied (Bond, Lovley 2003; Liu et al. 2004). This study reports that Quivira National Wildlife Refuge harbors a microorganism capable of mediator-free electron transfer to a carbon fiber electrode and demonstrates its electricity generating capabilities.

MATERIALS AND METHODS

Media and growth conditions. Soil was collected from marshland at Quivira National Wildlife Refuge, 25 miles SE of Great Bend, Kansas. Fluid Thioglycollate broth (Difco) was used as growth medium for the bacteria. A 400 mL volume of growth medium was mixed according to package instructions, boiled and autoclaved in 40 10 mL screw top test tubes at 121C for 15 minutes. Tubes were inoculated with soil and incubated at 24.7C for 20 days. A 375 mL volume of growth medium agar was then prepared according to the package and 12 Petri dishes were poured with the agar. The dishes were streaked with the soil bacteria and placed in a GasPak chamber to ensure an anaerobic environment. The GasPak chamber was incubated at 24.7C for 10 days. A pure culture was obtained by selecting isolated colonies and re-streaking repeatedly. Well-isolated, non-spreading colonies were selected for re-streaking based on the assumption that immotile bacteria would best

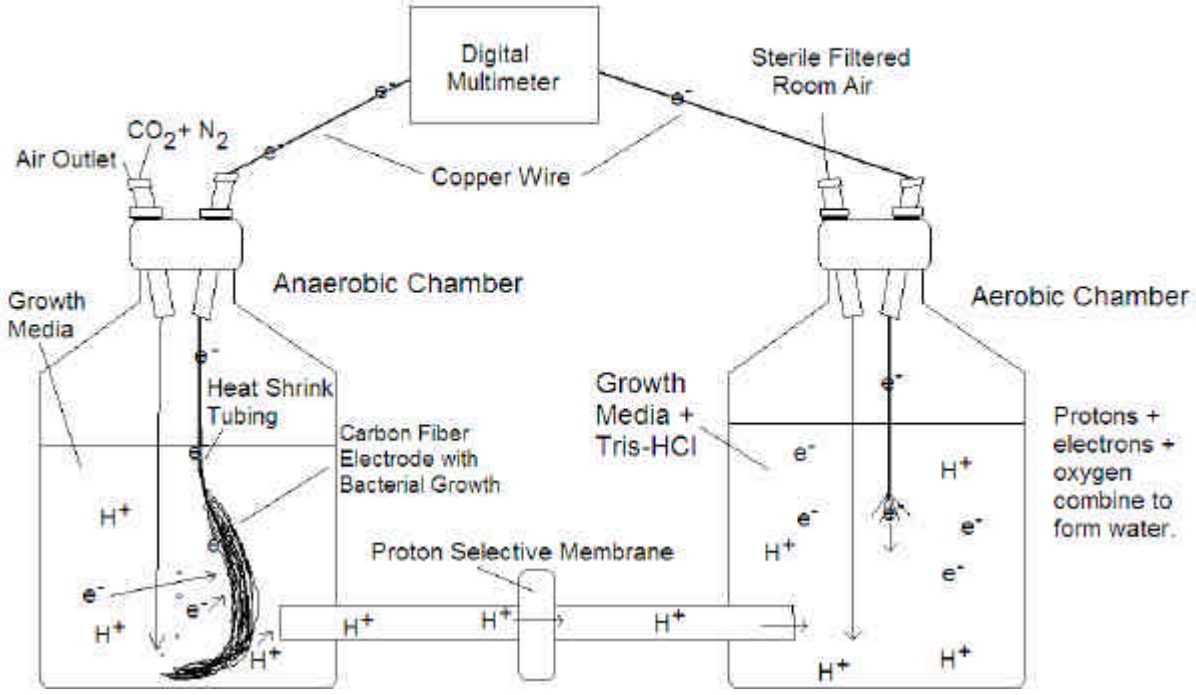

Figure 1. Diagram of the duel chambered fuel cell and the flow of electrons and protons.

colonize the surface of an electrode.

Electrodes and electrode chambers. The dual-chambered fuel cell consisted of two 500 mL polycarbonate narrow mouth square bottles (Nalgene) (Figure 1). The chambers were connected by a ½ inch barbed bulk head fitting and a Nafion 117 proton-selective membrane. Each chamber was capped and entry was allowed through two ¼ inch barbed bulk head fittings, located on each cap.

The anaerobic chamber, where bacterial growth occurred, contained 10 cm of exposed, unsized 25K carbon fiber (Fortafil Fibers, Inc.) connected to 2 inches of 12 ga braided copper wire by heat shrink tubing. Total electrode surface area was approximately 470 cm^2. Approximately 1 inch of copper wire was exposed in the aerobic chamber to transport electrons.

After construction, each chamber was filled with 450 mL of Fluid Thioglycollate broth and autoclaved at 121C for 30 minutes to sterilize. To serve as a buffer, 4 g of Tris-HCl was then added to the aerobic chamber. The chamber containing the carbon fiber electrode was continually sparged with a 20% CO_2 + 80% N_2 gas mixture to ensure anaerobic conditions. Air was filter-sterilized through a 0.02µm pore size filter and bubbled into the aerobic chamber. Both chambers were continually mixed with magnetic stir bars at approximately 200 rpm.

The anaerobic chamber was then inoculated with 1.5 mL of fluid Thioglycollate broth culture of the isolated strain via a sampling port.

Analysis. Fuel cell resistance and voltage levels were recorded on a daily basis for a twelve-day period. Amperage was then calculated using these measurements and based on the formula: $A = V / ?$. The pH level for each chamber was recorded at the end of the experiment. To test the conductivity of the cell, resistance levels were recorded with direct multimeter electrode contact with the chamber medias at the end of the experiment. Resistance was also measured after connecting both wires together and attaching the multimeter to the carbon fiber electrode and the exposed wire at the aerobic chamber end.

RESULTS

Bacteria isolation and identification. Strictly anaerobic, peroxidase negative, spore forming, Gram-positive, short rod-shaped bacteria were isolated to a pure culture. The species was not identified.

Electricity generation. The fuel cell produced a current for the duration of the experiment (Figure 2). The initial current increase likely represents population growth of bacteria after inoculation. The current continues to increase and peaks near Day 6. This point may reflect 1) the carrying capacity of

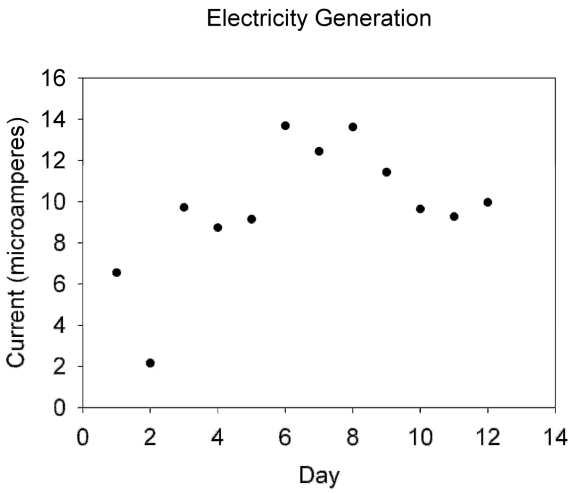

Figure 2. Current production levels of isolated bacteria over a ten day period. The current peaked on Day 6 at 13.68 µA. Day 8 represents an estimated value. See Results section for explanation.

bacteria on the electrode or 2) the carrying capacity of the entire chamber. In the former, a current plateau would likely follow this peak if bacteria food levels were adequate. In the latter, a depletion of food would occur and cause a decline in current. Figure 2 shows a gradual current decrease after Day 8 until the end of the experiment. If the study would have continued past Day 12, current levels would have likely continued to decrease. Future experiments are necessary to confirm this and disprove the option of a current plateau.

Amperage and resistance for Day 8 were estimated based on the observation of an abnormally high resistance level and were altered based on the conclusion that switching multimeter wires on the chambers resulted in an approximate doubling of resistance levels.

Electrode observations. Microscopic examination revealed bacterial colonization on the surface of individual carbon fiber strands (Figure 3). Unattached bacteria near the electrode surface were observed as immotile.

Other results. The final pH of the anaerobic chamber was 6.49. The aerobic chamber's final pH was 8.54. The less acidic nature of the aerobic chamber reflects the Tris-HCl buffer added at the beginning of the experiment. Electrical resistance of the growth medias after the experiment was 250 kiloOhms. Resistance of the wires and electrode was 200 ohms. This likely reflects the heat shrink tubing connection.

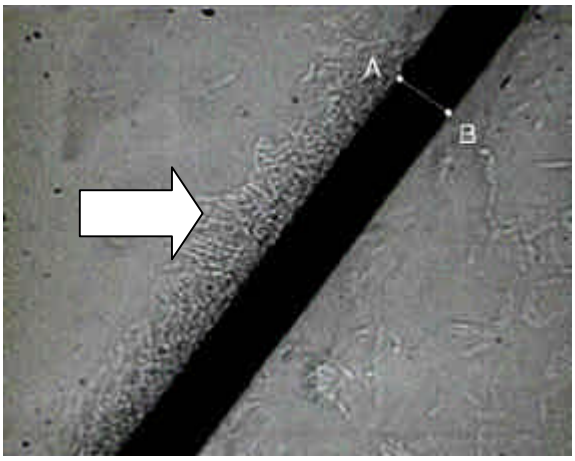

Figure 3. Colonization of bacteria on the surface of an individual carbon fiber strand of the fuel cell electrode used in study. The width of the fiber, from point A to B, is 6 µm.

DISCUSSION

This study shows that bacteria from a Kansas marshland can successfully generate an electrical current via the direct transfer of electrons to the surface of a carbon fiber electrode.

Bacteria. The isolated bacterium can be compared to previously studied bacteria used in microbial fuel cells. *Clostridium butyricum*, *G. sufurreducens*, *Rhodoferax ferrireducens*, and the studied bacteria are all rod-shaped. The isolated bacteria and *C. butyricum* are spore-forming and Gram-positive while both *R. ferrireducens* and *G. sufurreducens* are not spore-forming and Gram-negative. Motile bacteria are *C. butyricum* and *R. ferrireducens*. *R. ferrireducens* is the only facultative anaerobe. Each bacteria can be found in a sediment environment (Buchanan, Gibbons 1974; Finneran et al. 2003; Caccavo et al. 1994).

Electrical current. The current generated in this fuel cell can be compared to previous findings. This fuel cell produced a peak of 0.0137 mA at 5570 ohms. Fuel cells powered by *G. sulfurreducens* produced 0.40 mA of current at a resistance of 500 ohms (Bond, Lovley 2003). The estimated current of this study's fuel cell at 500 ohms is 0.1524 mA. Studies of *C. butyricum* showed it was able to create a current of 0.22 mA at 1000 ohms (Park et al. 2001). *R. ferrireduccens* generated 0.20 mA at 1000 ohms of resistance (Chaudhuri, Lovley 2003). The estimated current for this study at 1000 ohms is 0.0762 mA.

This experiment resulted in a fuel cell capable of microbial electricity generation but generation not surpassing other published findings.

Electrodes. The carbon fiber allowed the colonization of bacteria and the transportation of

electrons. Various other graphitic forms of carbon have been used in previous studies because of their high conductivity; however, no other publications where found using carbon fiber as the electrode material. Graphite foam, felt, blocks, rods, and discs have been tested. Future experiments could be conducted to evaluate the efficiency of the carbon fiber electrode by comparing fuel cells of different electrode types using previously studied bacteria, such as *G. sulfurreducens* or *R. ferrireducens*. Positive aspects of using carbon fiber as an electrode include excellent flexibility and conformity, large surface area for microbial colonization, and good electrical conductivity.

ACKNOWLEDGEMENTS

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