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Isolation and Identification of an Electricity Producing Microorganism from Lakeside Park in McPherson, KS

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ABSTRACT

Previous studies have suggested that Fe (III) reducing bacteria produce significant amounts of electrical current. The localization of cytochromes on the outside membrane of these bacterial cells allows for efficient electron transfer in the reduction process. In this study, soil samples from a recreational park in McPherson, Kansas were obtained and isolated for electricity producing bacteria using a dual-chambered fuel cell. The dual-chambered fuel cell was constructed using carbon fiber as the anaerobic chamber electrode and platinum as the aerobic chamber electrode. After a thirteen-day period, a positively identified bacterium *Klebsiella granulomatis* produced 0.638 C of total electricity. When actual electron yield (6.286×10^{-3} moles of electrons) was compared to potential electron yield (5.621×10^{-1} moles of electrons) available from oxidation of 1.5 grams of sodium acetate, the percent efficiency was determined to be 1.12%. While *Klebsiella granulomatis* proved to be an electrochemically active bacterium, its electricity producing percent efficiency was much lower compared to Fe (III) reducing bacteria as shown in current literature.

Keywords: *Microbial fuel cell, Bioremediation, electrode, Geobacter sulfurreducens, Klebsiella granulomatis*

INTRODUCTION

"Energy is the single greatest challenge facing humanity." This quote by Richard Smalley, a Nobel Prize winner in chemistry for the discovery of a new carbon allotrope, claims that the energy needs, not only in the U.S., but in the world, are increasing and will continue to increase until a solution is implemented (Logan and Regan, 2006). Since the demand for energy is increasing at a rapid pace, we are forced to consider alternative sources of energy (Logan and Regan, 2006).

Numerous alternatives have been explored, but microbial fuel cells (MFCs) have emerged as a scientifically possible and environmentally acceptable prospect (Logan and Regan, 2006).

Microbial fuel cells create electricity by the catalytic oxidation of organic compounds, offering a clean and renewable source of energy (Rabaey and Verstraete, 2005). In an aerobic environment, bacteria donate their electrons to oxygen, but when placed in an anaerobic environment, some bacteria can give their electrons to an electrode instead (Sader, 2006). It is this transfer of electrons from the microbe to the electrode that allows the production of electricity to be created and sustained (Dunaj, et al., 2012).

Dr. Bruce E. Logan, a professor at Pennsylvania State University, is one of the most prominent researchers when it comes to microbial fuel cells. His studies have focused on harnessing the metabolic activity of bacteria, specifically from the genus *Geobacter* (Logan and Regan, 2006).

Along With Logan, D.R. Lovley is another leading scientist on microbial fuel cells. Lovley discovered the stable electricity generating abilities of anaerobic *Geobacter sulfurreducens* (Bond and Lovley, 2003). Lovley has also provided sufficient evidence that a graphite electrode is one of the most prominent options for transferring electrons efficiently (Liu, 2012).

In addition to providing an alternate energy source, MFCs may also assist environmental protection by offering stability, providing wastewater treatment, and granting bioremediation, which is the process of removing pollutants by utilizing the bacterium's metabolic pathways (Lorenzo, 2008).

With bioremediation in mind, many scientists have begun experiments to create electricity out of wastewaters (Pant, et al., 2009) as well as comparing mixed cultures against pure cultures of bacteria.

Xin Wang,a microbiologist at the Harbin Institute of Technology in China, and his research group have validated that although pure-culture MFCs have previously been reported to have a shorter start-up period and a higher efficiency, they tend to grow slower (Wang, et al., 2008). They also provided data that mixed-culture MFCs may grow faster but, generally need more time to obtain a stable power output. There is an increasing interest in this area of microbial fuel cells.

In the present study, a soil sample from Lakeside Park in McPherson, KS has been obtained. A MFC was created utilizing the mixed-culture of bacteria from the soil sample and voltage levels were compared to the voltage levels a pure-culture of *Geobacter sulfurreducens* were able to generate (Malvankar et al., 2012).

These results contribute not only to the ongoing comparison of pure-culture and mixed-culture bacterial fuel cells, but also to the primary aim of MFC research; to isolate electrogenic bacteria or

communities with high electrochemical activity (Zhang, et al., 2012).

MATERIALS AND METHODS

Microbial Fuel Cell Construction. A dual-chambered fuel cell was constructed and utilized. The fuel cell consisted of two 500 mL polycarbonate narrow mouth Nalgene® bottles that were connected by a ½ inch barbed bulk head fitting and a Nafion 212 proton-selective membrane (Figure 1). Each chamber was capped but entry was allowed through four ¼ inch barbed bulk head fittings, two on each chamber.

The anaerobic chamber, where inoculation occurred, contained an electrode made of 12 cm of exposed, unsized 25K carbon fiber (Fortafil Fibers, Inc.) connected to 3 inches of 12 ga braided copper wire by heat shrink tubing. Once the electrode was placed in the anaerobic chamber, 100% silicone sealant was used on the cap in order to ensure anaerobic conditions. The electrode used in the aerobic chamber, where inoculation did not occur, was 3 cm of platinum soldered to 12 ga copper wire which was surrounded by 1/32 inch Tygon® tubing. The platinum electrode was placed in the aerobic chamber through a ¼ inch hole drilled in the neck of the Nalgene® bottle. Silicone sealant was once again placed around the entry point of the platinum electrode to ensure bacterial growth did not occur in aerobic chamber.

Upon completion of construction, the anaerobic chamber was filled with 400 mL of fluid thioglycollate broth and 1/32 inch flexi tubing was fed through the other ¼ inch barbed bulk head fitting that connected a nitrogen tank to rid the oxygen present in the chamber (Figure 1). Nitrogen was gradually added until the fluid thioglycollate broth indicated that it was anoxic. The aerobic chamber was filled with 13.16 grams of ferricyanide and 400 mL of water. 1.5 grams of sodium acetate was then placed in the anaerobic chamber to serve as food for the microbes. The aerobic chamber also had 1/32 inch flexi tubing fed through the ¼ inch barbed bulk head fitting but it was connected to sterile air to ensure aerobic conditions. Both chambers were placed on Fisher Scientific Isotemp magnetic plate while stir bars were positioned in the chambers and were stirred at a constant rate of approximately 200 rpm.

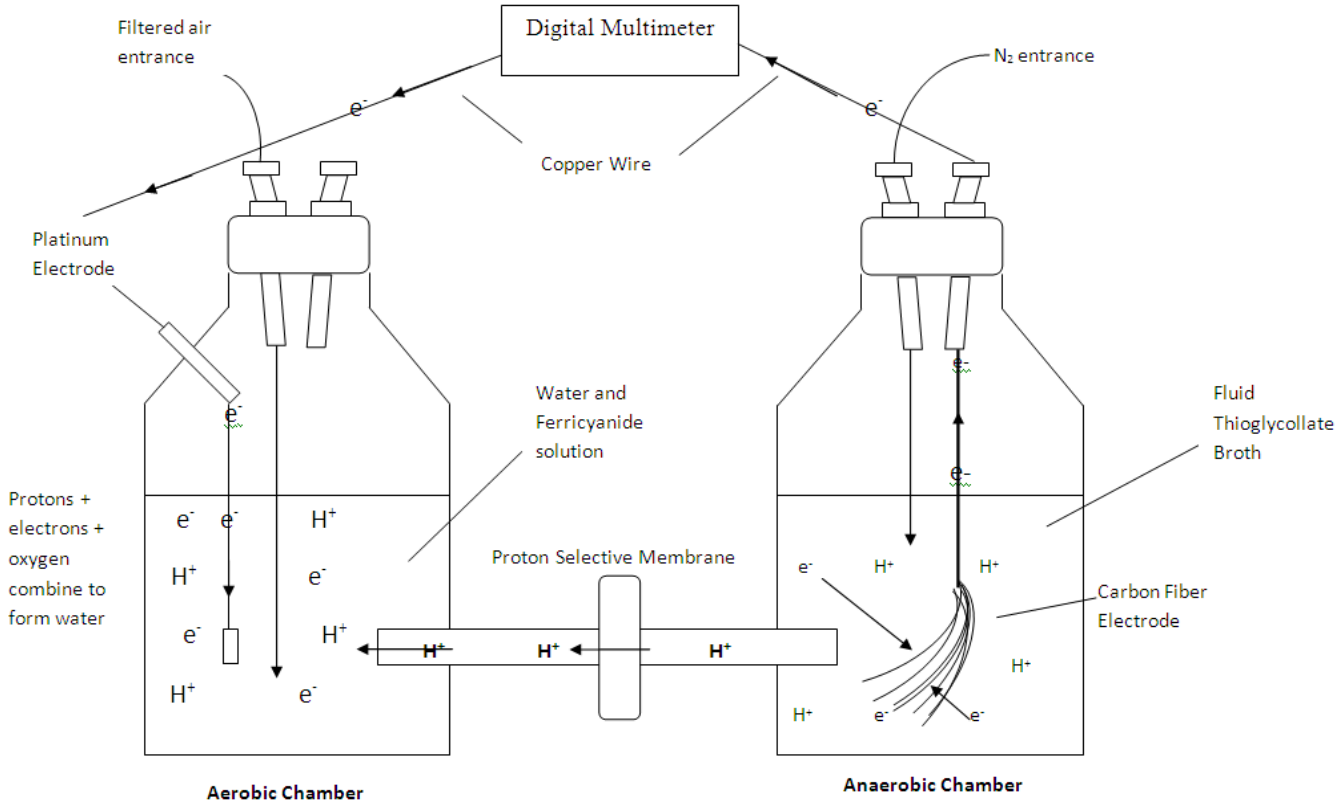

Figure 1. Diagram of the dual chambered fuel cell and the flow of electrons and protons.

Mud collection and Inoculation. 300 mL of mud was collected from the north pond in Lakeside Park located in McPherson, Kansas. The soil was placed

in a 500 mL BD sample dish and held in the laboratory for 2 weeks prior to inoculation. Once the fuel cell was completed and ready for inoculation, a pipette was used to obtain 1000 µL of the mud which was used to inoculate the anaerobic chamber. When the chamber was successfully inoculated, nitrogen was constantly mixed for approximately 3 hours or until the fluid thioglycollate broth indicated an anoxic environment by turning yellow. Air, which was filtered through a 0.02 µm filter, was constantly being pumped into the aerobic chamber by a Masterflex L/S Digital Standard peristalsis pump in order to guarantee an aerobic environment. The pump was held at a 10.5 mL/min rate.

Isolation and Identification. After the 10 days passed and the data was obtained, 500 mL of anaerobic agar was made and autoclaved at 121C for 15 minutes**.** Three petri dishes (100 mm diameter by 15 mm deep) were poured and once they gelled, the carbon fiber electrode was removed from the anaerobic chamber and gently touched to the surface of a Petri dish. The inoculated agar was held in the incubator at 25C for 3 days to ensure growth. Once growth was visible, a sterile inoculation loop was used to transfer bacteria from the initial Petri dish to another. Sterile streaking techniques were then used to isolate a colony of bacteria. The isolated colony was then sent to Molecular Epidemiology Incorporated for identification of the isolate.

Analysis. Fuel cell voltage and amperage levels were recorded on a daily basis (for 10 days) by utilizing a multimeter. The pH level of the anaerobic chamber was recorded at the beginning and end of the experiment. The results obtained from the mixed-culture of bacteria were then compared to current produced by *Geobacter sulfurreducens* by calculating the total electricity generated. The total electricity produced was determined by calculating the area under the curve of current (mA) vs. time.

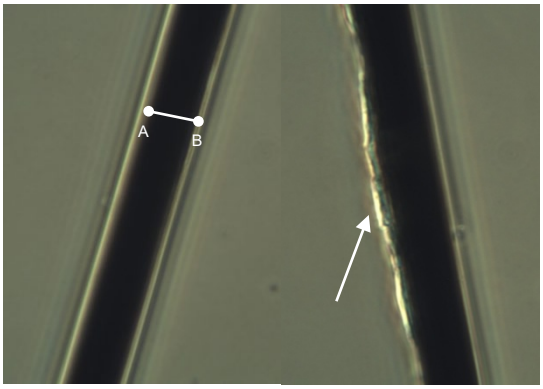

Figure 2. A single strand of carbon fiber used as an electrode with no colonization present is shown on the left. The image on the right shows that bacteria successfully colonized on the electrode. The width of the fiber, from point A to B, is 8 µm.

RESULTS

Bacteria isolation and identification. Molecular Epidemiology Incorporated identified the bacteria from the microbial fuel cell as *Klebsiella granulomatis.* However, *Klebsiella pneumonia* and *Klebsiella variicola* also proved to be genetically similar microorganisms. All three possibilities are members of the genus, *Klebsiella. Klebsiella* bacteria are gram-negative, rod shaped, oxidase negative, catalase positive, and facultative anaerobic bacteria.

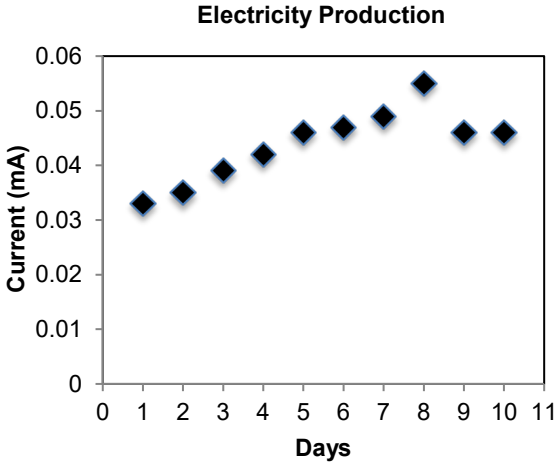

Figure 3. Total current production over 10 days was 0.638 C. The current peaked on day 8 at 0.055 mA. See results section for explanation.

Electrode Observations. With further observation of the carbon fiber electrode, it was discovered that *Klebsiella granulomatis, Klebsiella pneumonia, or Klebsiella variicola* did colonize on the surface (Figure 2). The width of the bacteria present on the single strand of carbon fiber was found to be 2 µm in size.

Electricity generation. The microbial fuel cell produced a current for the duration of the experiment (Figure 3). The initial increase in current is most likely due to the rapid growth of bacteria after inoculation as well as the addition of acetate, which was provided to stimulate and provide nutrition for the bacteria.

As time passed, the current continually increased until it reaches a peak on day 8 of 0.055 mA. The peak may signify the carrying capacity of the bacteria on the electrode or the carrying capacity of the media in the chamber. The reason the current plateau expressed in Figure 3 follows the peak is most likely because the food supply is sufficient. If a depletion of food were to occur, a gradual decrease of current would be expected until the end of the experiment.

In order to confirm or invalidate the reasoning behind the current plateau, further experimentation is necessary.

The total amount of electricity produced by *Klebsiella granulomatis, Klebsiella pneumonia, or Klebsiella variicola* was 0.399 C in 10 days.

DISCUSSION

The present study demonstrates that bacteria from Lakeside Park in McPherson, Kansas have the ability to metabolize acetate in the anodic chamber in order to produce an electrical current.

Bacteria. *Klebsiella granulomatis* can be compared to previously studied bacteria used in microbial fuel cells. *Geobacter sulfurreducens, Rhodoferax ferrireducens,* and *Shewanella putrefaciens* are the leading electricity generating bacteria used in microbial fuel cells (Prasad et al., 2006; Bond and Lovley, 2003; Malvankar et al., 2012). *K. granulomatis* is gram-negative, rod shaped and facultative anaerobic. Among the prominent bacteria used in fuel cells, *R. ferrireducens* and *S. putrefaciens* are facultative anaerobes while *G. sulfurreducens* is strictly anaerobic.

G. sulfurreducens, S. putrefaciens and *R. ferrireducens* are all rod-shaped, non spore-forming and gram-negative bacteria. All bacteria, including *K. granulomatis*, can be found in a sedimentary environment (Dunaj et al., 2012).

Electrical current. With the data that was obtained from the present experiment, *K. granulomatis* is proven capable of generating electricity but did not prove to contain the capability of generating an electrical current that exceeds other published findings (Park et al., 2001; Chaudhuri and Lovley, 2003; Bond and Lovley, 2003). *K.* granulomatis had a peak of 0.055 mA while fuel cells powered by *G.* sulfurreducens produced 0.40 mA (Bond and Lovley, 2003) and *R. ferrireducens* was able to create a current of 0.20 mA (Chaudhuri and Lovley, 2003).

The rate of electricity production by a fuel cell also depends on the surface area of the electrode. The total electrode surface area of the carbon fiber electrode utilized in the present experiment was approximately 500 cm^2. In Dr. Lovley's experiment using *Geobacter sulfurreducens,* the electrode surface area was approximately 1,200 cm^2 (Bond and Lovley, 2003). This substantial difference in electrode surface area is more evidence why the fuel cell with *Klebsiella granulomatis* did not generate as much electricity as *Geobacter sulfurreducens* and other Fe (III) reducing organisms.

The use of acetate as a substrate in the anodic chamber for the present study was to create an efficient fuel cell that gave the bacteria from Lakeside Park the best environment to generate electricity. Both the present study and studies performed by Lovley, Bond, and Logan utilize acetate as a substrate in the anaerobic chamber.

Acetate is a simple substrate used to induce electroactive bacteria (Bond and Lovley, 2003). Acetate is the end product of several metabolic pathways for higher order carbon sources (Pant et al., 2009). Using a single-chambered MFC, Chae et al. compared the performance of four different substrates and found acetate-fed fuel cells to show the highest power output (Chae et al., 2009).

The ability to produce an efficient MFC is vital to utilizing electrochemical bacteria as an alternative energy source. The efficiency of the present fuel cell can be found applying the equation: *efficiency=moles of electrons moving through circuit/ potential number of electrons from 1.5 grams of sodium acetate.* 6.286×10^{-3} moles of electrons moved through the circuit and 0.56211 moles of electrons were generated from 1.5 grams of sodium acetate. Therefore, the fuel cell had an efficiency level of 1.12%.

One possible explanation for such a low efficiency is that there were planktonic bacteria not attached to the electrode in the anaerobic chamber. Because the freely moving bacteria metabolized the acetate at the same time the bacteria attached to the electrode did, the level of acetate available for the bacteria on the electrode was decreased. If one were to remove the electrode once bacteria were present and place it in another anaerobic chamber that has not been inoculated, the efficiency would be increased. Placing the electrode in a chamber with no planktonic bacteria would allow the acetate to be metabolized solely by the bacteria on the electrode thus increasing the production of current by increasing the number of electrons moving through the circuit.

Another aspect necessary to consider while interpreting the results of this study is that the leading electrical bacteria are all Fe (III)-reducing in nature. They have the ability to grow in a medium with organic compound as the donor and Fe (III) as the electron acceptor. Having this ability to reduce Fe (III) allows the bacteria to localize the majority of its membrane-bound cytochromes and electrons on its outer membrane. The electroactive membrane-bound cytochromes present on the outer membrane are responsible for such an efficient transfer of electrons between microorganism and electrode (Prasad et al., 2006).

At this point, it is unknown whether *K. granulomatis* has the capability to reduce Fe(III) and localize its membrane-bound cytochromes to its outer membrane. For this reason, further experimentation utilizing *K. granulomatis* in a MFC is necessary to fully evaluate the electricity generating ability of *K. granulomatis*.

In order for microbial fuel cells to take over and become the alternative energy source, several things will have to be accomplished. We will have to develop a fuel cell that is capable of generating sufficient and efficient energy as well as developing a fuel cell that is affordable and socially acceptable. The present study provides evidence that it is not

feasible to utilize just any strain of bacteria for a fuel cell. In depth research needs to be executed and characteristics of possible electricity generating bacteria must be investigated before we will develop an alternative energy source.

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