Applied Soil Ecology 109 (2017) 14–22

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

The power of soil microbes: Sustained power production in terrestrial microbial fuel cells under various temperature regimes

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ARTICLE INFO

Article history:
Received 15 March 2016
Received in revised form 30 September 2016
Accepted 1 October 2016
Available online xxx

Keywords:
Soil
Fuel cell
Power
Voltage
Energy
Bacteria
Archaea

ABSTRACT

Recent developments in the field of bioenergy advance the feasibility for energy sources in remote locations with limited infrastructure requirements. Though most research efforts have focused on advancing power output in the marine environment, there is potential to generate power from terrestrial sources. The diversity of native soil biota serves as the inoculum at the electrode surface. In this study, we investigated how microbial fuel cells (MFCs) perform according to a range of temperature regimes, with specific inquiries regarding the level of power output generated at a range of temperatures representative of field conditions and the types of microbes which colonize the electrode surface. Our findings show that there was a notable lag in the increase in power output for all active terrestrial microbial fuel cells (tMFCs) and that the tMFCs incubating at 35 °C produced five times the power density than the tMFCs incubating at 5 °C. As expected, soil microbial activity, as measured through soil respiration, was proportional to both the incubation temperature of the tMFCs and the measured power output. Oppositely, microbial abundance did not increase concurrently with temperature or power output, as demonstrated by archaeal abundance observed to be consistently highest at 25 °C. Amplicon sequencing of the 16S rRNA gene revealed differences in community composition between the cathode and anode, with different communities emerging at different temperature profiles.

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1. Introduction

Alternative sources of power are in high demand, especially as the technology of mobile devices advances and power requirements lessen. Microorganisms, bacteria in particular, generate power in microbial fuel cells (MFCs) by oxidizing complex carbon substrates and reducing alternate electron acceptors such as iron and nitrate. Common sources of inoculum for MFCs include ocean sediment (Bond et al., 2002; Tender et al., 2002), wastewater effluent (Logan et al., 2006; Min et al., 2008; Lyon et al., 2010; Michie et al., 2011), and soil (Dunaj et al., 2012; Deng et al., 2014). Though power output does not rival traditional batteries, the sustainable nature of MFCs enables them to be excellent candidates to charge batteries for equipment in limited access locations (Shantaram et al., 2005; Cheng et al., 2006; Lovley, 2008).

The soil microbiome serves as a promising source of electrogenic bacteria to inoculate the anode surface. Examples of electrogenic bacteria include *Geobacter sulfurreducens* (Bond and Lovely, 2003; Jiang et al., 2010), *Rhodoferax ferrireducens* (Chaudhuri and Lovely, 2003), *Shewanella putrefaciens* (Kim et al., 1999), *Clostridium* spp. (Rismani-Yazdi et al., 2007), and *Bradyrhizobium* spp. (Zhang et al., 2012). An electric current is generated when these bacteria oxidize organic matter and reduce metals and subsequently transfer electrons through mechanisms such as nanowires, soluble electron shuttles (e.g. riboflavin), or cytochromes (Debabov, 2008; Lovley, 2008). Electron acceptance by the anode is a critical step in power generation, and it is suspected that archaea, namely methanogens, could in fact steal electrons from the anode to conduct their own metabolic processes (Chae et al., 2010). When this occurs, fewer electrons are transferred to the anode, severely limiting power generation. Therefore, it is critical to understand microbial community composition at the anode surface to better predict power generation for a range of realistic field conditions. While the bacteria listed above have demonstrated contributions to power generation in tMFCs, there

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http://dx.doi.org/10.1016/j.apsoil.2016.10.001
0929-1393/Published by Elsevier B.V.

are likely other viable electrogenic microorganisms that have yet to be identified, especially from inoculum originating from highly diverse environments. Therefore, a better understanding of the microbial communities that colonize the electrode surfaces, and likely contribute to the production of energy should provide more opportunities in making this technology viable in the near future.

In this study, our objectives were to measure power output from tMFCs composed entirely of soil and determine how power output varied according to incubation temperature. We characterized microbial community activity, abundance, and composition to reveal how microbial communities differed as a result of temperature, time (twice following tMFC assembly), and electrode type. Incubation temperatures were selected within the range of field data of surface soils from central New Hampshire, ranging from −8 °C in the winter to 45 °C in the summer (Barbato et al., 2016). The results from our study could be used to inform tMFC performance under dynamic environmental conditions and provide implications regarding the overall impact of temperature on tMFC performance.

2. Materials and methods

2.1. Soil properties

Soil for this study was collected from a field site at the Cold Regions Research and Engineering Laboratory (Hanover, NH) in June of 2013. The mean annual temperature and precipitation from this area are 7.8 °C and 102 cm, respectively. The soil was a sandy loam composed of 67.2 ± 0.6% sand, 26.3 ± 0.4% silt, and 6.5 ± 0.2% clay, with a cation exchange capacity of 10.87 ± 0.09 milliequivalents per 100 g of soil.

2.2. Assembly and monitoring of tMFCs

Control and treatment tMFCs were assembled using the MudWatt technology kits and protocol (Keego Technologies, LLC, Menlo Park, CA). The internal diameter and external height of each vessel was 9 and 10 cm, respectively. After soil was collected from the field, it was air-dried, sieved to 2 mm, and stored at 23 °C until used for the incubation study. To prepare for the incubation study, soil was then rehydrated with deionized water to −18 kPa (25% gravimetric water content) to achieve optimal moisture conditions for microbial growth (Moyano et al., 2013). All soil mass calculations for the construction of the tMFCs were based on wet soil mass of 25% water content. In brief, we added 166.7 g of wet soil of 25% moisture content to the vessel lightly compacted it, placed the anode, added 413.3 g of wet soil of 25% moisture content on top of the anode lightly compacted it, and then placed the cathode. The anode and cathode felts had approximately three cm of soil separating them in each vessel. For each incubation temperature, a total of 24 MFCs were assembled. Twelve of these tMFCs had their anodes and cathodes connected to a circuit board and were treated as experimental active tMFCs. The remaining twelve were disconnected throughout incubation and were treated as experimental inactive controls. Each treatment and temperature was assembled in quadruplicate. After assembly, the tMFCs were incubated in the dark at 5 °C, 25 °C, or 35 °C to capture a broad temperature range representative of military-relevant sub-arctic, temperate grassland/ambient, and desert regions, respectively, with limited resources.

Voltage was manually measured with a multimeter (Gardner Bender, Milwaukee, WI). To determine the internal resistance of the tMFCs, the maximum power output was measured over a range of external resistances (Logan and Regan, 2006; Lyon et al., 2010). Specifically, a power sweep was performed on four randomly selected active tMFCs using the following resistors: 47 Ω, 100 Ω, 220 Ω, 470 Ω, 1000 Ω. After each resistor was connected to the unit for 30 min, the voltage was recorded and power output (P) was calculated using voltage (V) and resistance (R) as in Eq. (1). Power density was calculated as a function of the power output and the surface area of the anode (66.5 cm^2).

$$P = \frac{V^2}{R} \quad (1)$$

2.3. Sampling the tMFCs

Our aim was to capture microbial community succession on the electrode surfaces; therefore, we investigated microbial metrics from the tMFCs twice during this study. For comparison purposes, it would have beneficial if these destructive sampling events occurred at the same time for each set of incubation temperatures. However, destructive sampling at the same time was not achieved because rates of power density increased differentially in the tMFCs as a function of the incubation temperature. Furthermore, rapid increases in power density occurred unexpectedly during the study. Interestingly, a period of lag occurred, followed by periods of power increase and periods of stabilization. Therefore, we sought to destructively sample once when power density was increasing and then again once power density stabilized. Sampling during an episode of increasing power output provided insight into which microbes were present on the electrodes as power output increased rapidly. Additionally, sampling during a period of stabilization revealed microbial successional patterns as power output stabilized.

Specifically, the tMFCs incubating at 5 °C were sampled after 304 and 858 h. The tMFCs incubating at 25 °C were sampled after 64 and 352 h and the tMFCs incubating at 35 °C were sampled after 136 and 352 h. We recognize that since we first sampled the 25 °C tMFCs during the start of a rapid increase in power density and the 35 °C tMFCs near the end of a period of rapid increase in power density, direct temporal comparisons would likely be limited. However, despite the fact that the tMFCs were sampled at different times, temperature comparisons could still be made. After the two sampling episodes, four active tMFCs per temperature tested remained for further power output measurements, and measurements ceased when we observed that the units were drying out, commensurate with decreasing voltage.

At each destructive sampling event, each cathode was carefully removed. Then, bulk soil under the cathode, but above the anode was removed with a sterile scoop and placed in a sterile whirlpak bag. Each anode was then carefully removed, and the bulk soil under the anode was removed and placed in a sterile whirlpak bag. The soils were well mixed and subsamples were collected for subsequent analyses. Bulk soils in quadruplicate as well as corresponding electrodes, were aseptically sampled twice for each incubation temperature and then stored at −20 °C for molecular analysis. Water content was determined gravimetrically with a 10 g subsample of the bulk soil that was dried at 105 °C for 24 h. To determine the percent of carbon by loss on ignition (LOI), the dried samples were then ground, added to crucibles, and baked in a muffle furnace at 360 °C for four hours (Schulte and Hoskins, 2009). Soil pH was measured twice using 10 g of dry soil in a 1:1 liquid mixture of either water or 0.01 M $CaCl_2$ solution, separately.

2.4. Soil activity and abundance

Aerobic respiration of the anode and cathode soils was determined by measuring CO_2 efflux after a 24 h incubation of 2 g of the bulk soils in a sealed 22 mL vial using a Hewlett-Packard