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Enhanced start-up of anaerobic facultatively autotrophic biocathodes in bioelectrochemical systems

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ABSTRACT

Biocathodes in bioelectrochemical systems (BESs) can be used to convert CO_2 into diverse organic compounds through a process called microbial electrosynthesis. Unfortunately, start-up of anaerobic biocathodes in BESs is a difficult and time consuming process. Here, a pre-enrichment method was developed to improve start-up of anaerobic facultatively autotrophic biocathodes capable of using cathodes as the electron donor (electrotrophs) and CO_2 as the electron acceptor. Anaerobic enrichment of bacteria from freshwater bog sediment samples was first performed in batch cultures fed with glucose and then used to inoculate BES cathode chambers set at −0.4 V (versus a standard hydrogen electrode; SHE). After two weeks of heterotrophic operation of BESs, CO_2 was provided as the sole electron acceptor and carbon source. Consumption of electrons from cathodes increased gradually and was sustained for about two months in concert with a significant decrease in cathode chamber headspace CO_2. The maximum current density consumed was -34 ± 4 mA/m^2. Biosynthesis resulted in organic compounds that included butanol, ethanol, acetate, propionate, butyrate, and hydrogen gas. Bacterial community analyses based on 16S rRNA gene clone libraries revealed *Trichococcus palustris* DSM 9172 (99% sequence identity) as the prevailing species in biocathode communities, followed by *Oscillibacter* sp. and *Clostridium* sp. Isolates from autotrophic cultivation were most closely related to *Clostridium propionicum* (99% sequence identity; ZZ16), *Clostridium celerecrescens* (98–99%; ZZ22, ZZ23), *Desulfotomaculum* sp. (97%; ZZ21), and *Tissierella* sp. (98%; ZZ25). This pre-enrichment procedure enables simplified start-up of anaerobic biocathodes for applications such as electrofuel production by facultatively autotrophic electrotrophs.

1. Introduction

Mankind faces increasing energy demands associated with accelerating global population growth and rising industrialization of emerging and developing countries. Capturing renewable energy from sun and wind through efficient photovoltaic devices or wind turbines is an elegant, carbon neutral way to help meet these increasing energy demands. However, the intermittent nature of these energy sources has to be addressed by finding adequate energy storage systems (Ibrahim et al., 2008; Lewis and Nocera, 2006; Zhang and Huang, 2012). Microbial electrosynthesis, a process through which microorganisms reduce inorganic compounds into stable, energy dense organic molecules in a bioelectrochemical system (BES), is one strategy to solve this storage problem (Nevin et al., 2010). Conversion of the greenhouse gas CO_2 into more highly reduced organic molecules such as transportation fuels that can be easily stored and distributed within the existing infrastructure is an attractive option (Li et al., 2012; Nevin et al., 2010).

Biocathodes that use microorganisms to catalyze reduction reactions are, in contrast to traditional chemically-catalyzed cathodes, self-renewable, and potentially less expensive (He and Angenent, 2006; Lovley, 2011). However, biocathode biofilms are difficult to establish, particularly under anaerobic conditions (Butler et al., 2010; Morita et al., 2011). Microorganisms that can directly or indirectly accept electrons from a cathode are referred to as electrotrophs, in contrast to exoelectrogens that are able to transfer electrons to an anode (Logan, 2009; Logan and Rabaey, 2012; Lovley, 2011). A variety of terminal electron acceptors can be used by electrotrophs, such as oxygen, nitrate, sulfate, iron, manganese, arsenate, fumarate, or carbon dioxide (Clauwaert et al., 2007; Cournet et al., 2010; Freguia et al., 2010; Gregory et al., 2004; Gregory and Lovley, 2005; Mao et al., 2010; Rabaey et al., 2008; Virdis et al., 2010). In addition to fuels, electrotrophs as cathode catalysts can be used to produce a variety of commodity chemicals via microbial electrosynthesis (Marshall et al., 2012).

Approaches developed for establishment of anaerobic biocathodes include the use of set potentials at cathodes and the

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addition of hydrogen and/or organic compounds (Gregory et al., 2004; Jeremiasse et al., 2012; Villano et al., 2011). One of the first approaches used was electrical inversion of exoelectrogenic bioanodes fed with hydrogen to obtain hydrogen-evolving biocathodes based on the reversibility of hydrogenases (Rozendal et al., 2008). This approach required the use of non-renewable ferricyanide as catholyte and ferrocyanide as anolyte. A different inversion method was used to obtain biocathodes for oxygen reduction based on inverting the polarity of a BES repeatedly by using a potentiostat and by alternating exposure of electrode biofilm to oxygen and the addition of acetate (Cheng et al., 2010). A similar method was used to establish a methanogenic biocathode by switching periodically the polarity of anode and cathode using a stack of rotatable conductive disks that were one-half submerged in wastewater and one-half exposed to headspace gas (Cheng et al., 2011). More recently, a sediment microbial fuel cell (sMFC) electrode inversion technique was developed to facilitate enrichment of anaerobic exoelectrogenic bacteria from sediments that subsequently selects for electrotrophic bacteria by inversion of the sMFC anode into a biocathode (Pisciotta et al., 2012). These previous methods for the establishment of anaerobic biocathodes are complicated and time consuming multi-step-procedures.

A specialized method was developed here to accelerate start-up of anaerobic facultatively autotrophic biocathodes based on heterotrophic pre-enrichment. Acetogenic bacteria are able to reduce CO_2 with H_2 into acetate and other organic compounds by utilizing the reductive acetyl-CoA (Wood-Ljungdahl) pathway and have recently been shown to convert CO_2 into organic compounds in BES by replacing hydrogen with a cathode as the energy and electron source (Berg, 2011; Drake et al., 2008; Nevin et al., 2010). Pure culture studies with selected acetogens including *Sporomusa* spp. and *Clostridium* spp. have demonstrated electrotrophic activity (Nevin et al., 2011, 2010; Song et al., 2011). It was therefore hypothesized that enrichment of these and related microorganisms in BESs would select for new facultatively autotrophic electrotrophs for biochemical or biofuel synthesis. Based on facultative autotrophy of acetogens, a pre-enrichment procedure was developed where bacteria were first enriched heterotrophically on glucose (Berg, 2011; Drake et al., 2008). After heterotrophic pre-enrichment and acclimation of the enriched culture to a BES, carbon dioxide was provided as the sole electron acceptor and carbon source to select for microorganisms able to switch from heterotrophic to autotrophic metabolism (Fig. 1). This specialized method could provide a simplified way to isolate biofuel or biochemical producing electrotrophs from various inoculum sources. This procedure may also provide a general approach by which microorganisms with specific metabolic abilities can be enriched using various electron donors and/or substrates for development of specialized anaerobic biocathodes (Fig. S1).

2. Materials and methods

2.1. Pre-enrichment

The inoculum was sediment from a bog (Black Moshannon Park, Philipsburg, PA) collected in May 2011 from the subsurface and transferred into plastic containers completely filled to avoid any gas headspace. Samples were stored at 4 °C in the dark and processed the next day. Approximately 54 g (~20 mL) sediment slurry samples were transferred into three sterile 150-mL serum bottles using a sterile spatula under anoxic conditions in an anaerobic glove box (Coy, Laboratory Products, Grass Lake, MI) with N_2:H_2 (95:5, v/v) atmosphere. Anaerobic basal medium (80 mL) was added into each culture bottle which contained 1.5 g/L KH_2PO_4, 2.9 g/L K_2HPO_4, 0.5 g/L NH_4Cl, 0.18 g/L $MgCl_2{\cdot}6\ H_2O$, 0.09 g/L $CaCl_2{\cdot}2\ H_2O$, 0.3 g/L $Na_2S{\cdot}9H_2O$, 8 g/L $NaHCO_3$ supplemented with 10 mL/L minerals solution (SL10) (Atlas, 2010). In addition, 2 mL of Wolfe's vitamins solution (Wolin et al., 1963), 2 mL glucose solution (from 1 M stock), and 2 mL sodium-2-bromoethane-sulfonate (from 500 mM stock solution, to inhibit growth of methanogens) (Chae et al., 2010; Zehnder and Brock, 1980; Zinder et al., 1984) were injected into each serum bottle sealed with a butyl rubber stopper (Chemglass, Vineland, NJ) and an aluminum crimp top. Subsequently, the headspace was degassed for 10 min with a sterile N_2:CO_2-gas-mix (80:20, v/v). Final pH was adjusted to 7.3. Enrichment cultures were incubated in the dark at 30 °C and agitated on a shaker (at 125 rpm). After a 7-day incubation, 20 mL of the enrichment culture were used to inoculate new serum bottles containing 80 mL fresh anaerobic basal medium. To each serum bottle 2 mL vitamins, 2 mL glucose, and 1 mL sodium-2-bromoethane-sulfonate were supplemented from stock solutions mentioned above. Cultures were incubated under the same conditions for another week.

2.2. BES construction and operation

Two-chamber H-type BESs were constructed from two 250-mL media bottles each joined together with a glass tube (2.5 cm diameter, 4.5 cm length; 325 mL total volume of each chamber, Penn State glass workshop, University Park, PA). The two chambers were separated by a pretreated cation exchange membrane (Nafion 117, DuPont, Newark, DE) as previously described (Oh and Logan, 2006; Rezaei et al., 2008). Electrodes were carbon fiber rods (7.5 cm length, 0.6 cm diameter; 0.00147 m^2 surface area; McMaster-Carr), polished using sandpaper (grit type 400) and treated in 1 M HCl over night as described before (Call and Logan, 2011). Titanium wire (0.06 cm diameter; McMaster-Carr) was cut to 12-cm length and polished with sandpaper (aluminum oxide; 3M, St. Paul, MN) to remove any oxide layer, and attached to the carbon rod (Call and Logan, 2011). The wire of the electrodes was threaded through rubber stoppers and autoclaved along with reactors filled with DI water, and capped loosely with lids. Reference electrodes (Ag/AgCl, RE-5B, Bioanalytical Systems, Inc.; E(Ag/AgCl) = E(SHE) −0.211 V) (Tokash and Logan, 2011) were checked for accuracy, sterilized with 70% (v/v) ethanol in a laminar airflow cabinet, and threaded through sterile stoppers attached with electrodes determined for being the working electrode (cathode). Autoclaved and emptied two-chamber reactors were then closed with stoppers, attached with the respective electrodes for the cathode and anode chambers, and transferred into a COY anaerobic chamber. Then, 200 mL of anaerobically prepared sterile basal medium (see above), supplemented with 2 mL/L vitamin solution and 2 mL/L sodium-2-bromoethane-sulfonate solution (1 mM final concentration), was added into each BES chamber. Both chambers were inoculated by addition of 50 mL pre-enriched culture (working volume = 250 mL; headspace = 75 mL) (Fig. 1). The headspace of all reactor chambers was purged with sterile N_2:CO_2-gas-mix (80:20, v/v) for 15 min to provide CO_2. For chronoamperometric and chronocoulometric measurements the BESs were connected to a potentiostat (model MPG2; Bio-Logic – Science Instruments, Claix, France) by applying a cathode potential of −0.400 V (versus SHE). BESs were operated in the dark at a constant temperature of 30 ± 1 °C. During the BES start-up period, 1.5 mL glucose (6 mM final concentration) was added every second day to the cathode compartments based on fast glucose consumption rates within two days. Addition of glucose was stopped on day 14 of BESs operation when uptake of current started. The pH was checked and adjusted if needed using phosphate buffers. Current densities were calculated as $J = I/A_{cat}$, where J is the current density, I is current, A_{Cat} is cathode surface area. Power densities were calculated using $P_{cat} = IE_{cell}/A_{\mathrm{cat}}$, where P_{Cat} is current density and E_{Cell} is the cell potential. Statistical calculations are based on $\alpha = 0.05$. All potential values are reported versus SHE.