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Syntrophic interactions drive the hydrogen production from glucose at low temperature in microbial electrolysis cells

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HIGHLIGHTS

- H_2 production from glucose at 4 °C in MECs overcomes the dark-fermentation bottleneck.
- H_2 yield at 4 °C is comparable with that obtained at mesophilic temperatures in MECs.
- Combining pyrosequencing with CV reveal the syntrophic interactions in MECs at 4 °C.
- Psychrotolerant fermenters and exoelectrogens allowed current generation from glucose.
- Methanogenesis and homoacetogenesis were negligible in glucose-fed MECs at 4 °C.

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ABSTRACT

H_2 can be obtained from glucose by fermentation at mesophilic temperatures, but here we demonstrate that hydrogen can also be obtained from glucose at low temperatures using microbial electrolysis cells (MECs). H_2 was produced from glucose at 4 °C in single-chamber MECs at a yield of about 6 mol H_2 mol^{-1} glucose, and at rates of 0.25 ± 0.03–0.37 ± 0.04 m^3 H_2 m^{-3} d^{-1}. Pyrosequencing of 16S rRNA gene and electrochemical analyses showed that syntrophic interactions combining glucose fermentation with the oxidization of fermentation products by exoelectrogens was the predominant pathway for current production at a low temperature other than direct glucose oxidization by exoelectrogens. Another syntrophic interaction, methanogenesis and homoacetogenesis, which have been found in 25 °C reactors, were not detected in MECs at 4 °C. These results demonstrate the feasibility of H_2 production from abundant biomass of carbohydrates at low temperature in MECs.

1. Introduction

Microbial electrolysis cells (MECs) are a new method for electrochemically producing hydrogen using current generated by exoelectrogenic microorganisms. However, most MEC studies have examined systems at ambient temperatures using acetate as the fuel. It was recently shown that H_2 gas could also be produced in an MEC at relatively low temperatures (e.g. 4 and 9 °C) using acetate, making this technology a promising method for biohydrogen production even in very cold climates (Lu et al., 2011). However, it is important to consider the utilization of fuels other than acetate at low temperatures because most biomass available for biofuels production is primarily stored as fermentable carbohydrates such as glucose and cellulose. In previous bioelectrochemical system (BES) studies, including both MECs and microbial fuel cells (MFCs), there have been large differences in substrate metabolism and reactor performance in mesophilic environments using fermentable carbohydrates, such as glucose, compared to studies using acetate (Freguia et al., 2008; Lee et al., 2008). It is therefore important to better understand how fermentable substrates are degraded by microorganisms in MECs at low temperatures.

Glucose is a simple carbohydrate that can be converted to electrical current in BESs (Rabaey et al., 2003; Selembo et al., 2009). A few exoelectrogenic bacteria can directly oxidize glucose (e.g. *Rhodoferax ferrireducens*, *Klebsiella pneumoniae*, and *Aeromonas hydrophila*) and transfer electrons to electrodes (Logan, 2009). In mixed-culture systems, previous studies imply that syntrophic interaction between fermenters and exoelectrogens is the major route to metabolize glucose for current production under mesophilic conditions (Freguia et al., 2008; Zhang et al., 2011). Glucose is first oxidized to organic acids or H_2 by fermentation, followed by consumption of fermentation products by the exoelectrogens, which eliminates feedback inhibition of glucose fermentation

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(Freguia et al., 2008). However, fermentation leading to current generation is not invariably the dominant reaction in glucose-fed anode biofilms using mixed cultures, as some researchers have also found that Fe (III)-reducing bacteria, related to the *Aeromonas* genus, that could directly oxidize glucose were dominant in anode biofilms and therefore played an important role in generating current (Chung and Okahe, 2009; Park et al., 2008). Another important syntrophic process in glucose-fed BESs generally exists between fermenters and methanogens (Freguia et al., 2008) due to the competition of methanogens with exoelectrogens for fermentation products (acetate and formate or H_2) under anaerobic conditions. Community profiles from fermentable substrate-fed MFCs also suggest a third possible syntrophic interaction. Homoacetogenic bacteria can channel electrons from H_2 (produced by fermentation) to acetate that is re-oxidized by the exoelectrogens to produce electricity (Parameswaran et al., 2011). In MECs, the H_2 consumed by homoacetogens can come from the cathode.

All of these syntrophic interactions in BESs have previously been studied at mesophilic temperatures, and there have been no studies of microbial communities in systems fed carbohydrates at low temperatures (below 10 °C). The few studies conducted at low temperatures (4–15 °C) with MFCs have mainly been focused on a non-fermentable substrate (acetate) (Cheng et al., 2011; Patil et al., 2010). Since most fermentative bacteria and methanogens are generally considered to be inactive under psychrotolerant conditions (Scherer and Neuhaus, 2006), the dominant pathway of current production at low temperatures has yet to be established. It is not known whether current is produced by direct glucose oxidization by exoelectrogens, or through syntrophic process between fermentative and electrochemically active microorganisms. It may be that there are different exoelectrogenic microorganisms that function at lower, compared to higher, temperatures.

In this study, glucose was used as an electron donor in MECs in order to determine if (i) H_2 could be successfully produced from a fermentable substrate at a low temperature (4 °C) and (ii) if the primary pathway of glucose conversion to current was direct or indirectly through fermentation processes. To accomplish these goals, we examined the formation of the intermediates during electrohydrogenesis of glucose in concert with hydrogen gas production. Cyclic voltammetry (CV) was employed to evaluate the electrochemical features of the anode biofilms, allowing identification of the midpoint potential and inference of possible extracellular electron transfer mechanisms. Dominant microbial populations in the biofilms of the MECs under psychrotolerant conditions were investigated using two independent molecular biology methods: (i) The highly parallel 454 GS-FLX pyrosequencing based on 16S rRNA gene with 100 times the throughput of a traditional Sanger sequencing used in low throughput methods (e.g. clone libraries and denaturing gradient gel electrophoresis [DGGE]) and (ii) the tranditional 16S rRNA gene clone libraries.

2. Methods

2.1. MEC construction and operation

Single-chamber, membraneless MECs (liquid volume 26 mL) were constructed as previously described (Lu et al., 2009) with graphite brush anodes (2.5 cm diameter × 2.5 cm length; fiber type: T700–12 K, Toray, Inc.) and carbon cloth air cathodes (7 cm^2, type B, E-TEK, Inc.) containing 0.5 mg cm^{-2} Pt catalyst. MEC anodes were initially enriched in similarly constructed microbial fuel cells (MFCs) with cathodes being exposed to air. MFC reactors were frequently inoculated (intervals of 2–3 days) with a 50:50 mixture of the two effluents from a room temperature glucose-fed MFC reactor (23 ± 2 °C, operating about 6 months) and an acetate-fed psychrotolerant MEC (Lu et al., 2011) (operating more than 12 months at 4 °C), with 11.1 mM (2 g L^{-1}) glucose as the fuel. Acclimation was conducted in fed-batch mode at 4 °C (three reactors) except two MFCs were enriched at 25 °C (as controls) for community structure comparison to psychrotolerant electroactive biofims. Anodes were considered fully enriched when a reproducible maximum voltage (over 1 kΩ resistor) was obtained, and then they were transfer to MECs for H_2 production.

All MECs were fed 11.1 mM (2 g L^{-1}) glucose in a 50 mM nutrient phosphate buffer solution (NPBS) (Na_2HPO_4, 4.58 g L^{-1}; $NaH_2PO_4{\cdot}H_2O$, 2.45 g L^{-1}; NH_4Cl, 0.31 g L^{-1}; KCl, 0.13 g L^{-1}; NH_4Cl, 0.31 g L^{-1}; trace minerals and vitamins) and operated at 4 °C except control reactors (25 °C). Prior to being fed into reactors, the solution was sparged with nitrogen gas (99.999%) for 15 min to remove oxygen, then a fixed voltage of 0.6 V or 0.8 V was applied to the MECs by a programmable power source (3645A, Array, Inc.).

Current was determined by measuring the voltage over a high-precision resistor (10 Ω) using a multimeter/data acquisition system (model 2700 with 7702 module, Keithley, Inc.) at 10 min intervals. A reference electrode (Ag/AgCl, 0.197 V vs. SHE; RE-5B, BASi, Inc.) was inserted into the chamber to measure the anode and cathode potentials. When the current decreased below 0.3 mA, the reactors were refilled with fresh medium. MECs were operated continuously over a period of about two months.

2.2. Analyses and calculations

The gas produced by the MECs was collected in a gas bag (0.1 L Cali-5-Bond, Calibrate, Inc.), and the total volume was measured using a glass syringe. Gas composition was analyzed using a gas chromatograph (4890D, Agilent, Inc.). Liquid samples from MECs were immediately filtered through 0.22 μm pore-diameter cellulose acetate filters, and analyzed for volatile fatty acids (VFAs), ethanol and chemical oxygen demand (COD). The concentrations of VFAs (including acetic, propionic, butyric and valeric acid) and ethanol were analyzed using another gas chromatograph (7890A, Agilent, Inc.). The COD was measured according to standard methods (APHA, 1998).

All electrochemical experiments were conducted using a multichannel potentiostat (WMPG-1000S, WonATech Co., Ltd). If not stated otherwise, all potentials provided refer to a Ag/AgCl reference electrode (0.197 V vs. a standard hydrogen electrode, SHE). Cyclic voltammetry (CV) was conducted at a scan rate of 1 or 5 mV s^{-1}, in the potential range from −0.8 to 0.2 V using a three-electrode arrangement in anaerobic conditions. Linear sweep voltammetry (LSV) was performed to determine the power densities (normalized to cathode surface area) of MFCs from −0.8 to −0.05 V using a scan rate of 0.1 mV s^{-1}.Hydrogen production rate Q (m^3 H_2 m^{-3} day^{-1}), volumetric current density I_V (A m^{-3}), hydrogen yield Y_{H_2} (mol H_2 mol^{-1} glucose) and Coulombic efficiency (C_E) calculated on the basis of COD, cathodic hydrogen recovery (r_{cat}), overall hydrogen recovery ($R_{H_2} = C_E r_{cat}$) and energy recovery relative to the electrical input (η_E) were used to evaluate the performance of MECs according to previously described (Logan et al., 2008).

2.3. Bacterial community analysis

At the end of MECs operation, the graphite fibers were cut from the anodes of psychrotolerant MECs and those in controls (25 °C reactor), and were fragmented using sterile scissors. Graphite fibers were sampled equably from three different sections of each brush and were combined for DNA extraction. Total genomic DNA was extracted using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. The bacterial 16S rRNA-gene clone libraries were con-