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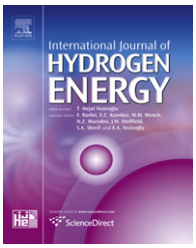

Optimization of culture conditions and electricity generation using *Geobacter sulfurreducens* in a dual-chambered microbial fuel-cell

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ARTICLE INFO

Article history:
Received 24 December 2009
Accepted 13 April 2010
Available online 14 May 2010

Keywords:
Microbial fuel-cell
Mediator-less
Geobacter sulfurreducens
Electricity generation
Power density
Carbon nanotubes

ABSTRACT

The promise of generating electricity from the oxidation of organic substances using metal-reducing bacteria is drawing attention as an alternate form of bio-technology with positive environmental implications. In this study, we examined various experimental factors to obtain the maximum power output in a dual-chamber mediator-less microbial fuel-cell (MFC) using *Geobacter sulfurreducens* and acetate as an electron donor in a semi-continuous mode. The *G. sulfurreducens* culture conditions were optimized in a nutrient buffer containing 20 mM of acetate and 50 mM of fumarate at pH 6.8 and 30 °C. For use in the MFC system, electrodes were made with carbon paper (area: 11.5 cm^2) and spaced 1.5 cm apart. Once the MFC was inoculated with the pre-cultured *G. sulfurreducens* in the anode chamber and while air was continuously sparged to the cathode chamber, the cells produced electricity stably over 60 days with the regular addition of 20 mM acetate, generating the maximum power density of 7 mW/m^2 with a 5000 Ω load. The current output was significantly increased, by 1.6 times after 20 days of incubation under the same experimental conditions, when the carbon-paper anode was coated with carbon nanotubes.

1. Introduction

Microbial electricity generation using a fuel-cell system (Microbial fuel-cell, MFC) has a great potential as a means of producing energy from organic wastes or carbohydrate substrates [1–3]. In the MFC system, an oxidation reaction occurs at the anode, and a reduction reaction at the cathode. Electrons and protons are generated from the oxidation reaction of organic substances (fuels) by metabolic activities of metal-reducing bacteria, and travel to the cathode, the former via an external circuit and the latter through electrolytes and a physical separator such as a salt bridge or a proton exchange membrane (PEM). These protons and electrons are subsequently consumed at the cathode, reducing oxygen (O_2) to water. Since most microorganisms are electrochemically inactive, artificial electron carriers (mediators) such as anthraquinone-2,6-disulfonate (AQDS), thionine and methyl viologen have been used to carry electrons from the inside of the microbial cells to the external electrode [1,2]. However, since most of the mediators are expensive and toxic, a mediator-type MFC for large-scale applications remains elusive [1].

Recently, a number of bacteria including *Geobacter sulfurreducens* [4], *Rhodoferax ferrireducens* [5] and *Shewanella putrefaciens* [6] have been isolated for their ability to use metal ions, including Fe(III), as terminal electron acceptors in the absence

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0360-3199/$ – see front matter
doi:10.1016/j.ijhydene.2010.04.061

of O_2. Iron-reducing bacteria can conserve energy for anaerobic growth by coupling the oxidation reaction of organic compounds such as glucose, lactate or acetate to the reduction of Fe(III). The attachment of these microorganisms to carbon electrodes results in direct electron transfer to the electrodes without the help of an exogenous mediator, producing electricity. Many studies have reported electric powers on the order of ~10 mW/m^2 when carbon-based electrodes were used [5–7].

Among these metal-reducing bacteria, *G. sulfurreducens* is receiving increased attention because its complete genome was recently sequenced, providing insights into its physiological, biochemical and genetic properties [8]. The genetic system, also available, would be useful to the manipulation of the metabolic genes and pathways in this strain [9]. Although the ability of *G. sulfurreducens* to transfer electrons to an electrode, via *c*-type cytochrome enzymes on outer cell surfaces and/or electrically conductive pili extended from those surfaces, has been noted several times [10,11], still there are very few studies on the cellular physiology of *G. sulfurreducens* under respiratory growth conditions or with regard to electricity generation with a carbon-based electrode serving as the sole electron acceptor. Furthermore, little is known about the long-term functioning of *G. sulfurreducens* that is necessary for practical applications.

In the present study, we investigated electricity generation from acetate as the sole electron donor using *G. sulfurreducens* in a mediator-less MFC equipped with carbon electrodes. The culture conditions, including the acetate concentration, pH and temperature, were optimized for the growth of *G. sulfurreducens* under fumarate respiration. MFC experiments were conducted under those optimal growth conditions, and the external load and anode-type electrodes (native or coated with carbon nanotubes (CNT)) were investigated to increase the electrical output. We operated the MFC in a semi-continuous mode for 65 days to assure the long-term stability of *G. sulfurreducens*, and attempted to evaluate its potential for electricity generation with acetate as a model substrate.

2. Materials and methods

2.1. Microorganism and culture conditions

G. sulfurreducens (ATCC 51573) was obtained from the American Type Culture Collection (ATCC). The growth medium (pH 6.8) contained the following (per liter): 1.5 g NH_4Cl, 0.6 g NaH_2PO_4, 0.1 g KCl, 2.5 g $NaHCO_3$, as well as metal (10 mL) and vitamin (10 mL) solutions [10]. Acetate and fumarate were separately supplied, to 20 mM and 50 mM, as the electron donor and acceptor, respectively, if not otherwise specified.

Batch cultivation was performed using a serum bottle of 150 mL (working volume, 50 mL) at 30 °C (unless stated otherwise). After inoculation, the bottle was flushed with a N_2-CO_2 gas mixture (4:1, v/v) for 30 min for developing anaerobic condition and sealed with a 12 mm-thick butyl rubber septum and aluminum cap. The inoculum was cultivated in the same bottle and transferred anaerobically in the late-exponential phase by a sterile hypodermic disposable syringe. Three different independent experiments were carried out, and the values were averaged.

2.2. Construction and operation of MFC

Two-chambered MFCs were constructed from two (anode and cathode) acrylic rectangular chambers (working volume: 20 mL each), physically separated by a PEM (Nafion 117®, DuPont Co., USA) with a projected area of 12 cm^2. Each chamber contained a plain porous carbon-paper electrode (without wet proofing; TGPH-090, E-Tek, USA) of 11.5 cm^2 surface area, if not otherwise specified. The electrode surface area (geometric area) was assumed to be twice that of one side of the projected surface. The anode and cathode electrodes were spaced 1.5 cm apart. The CNT-coated carbon electrode was prepared by electrophoretic deposition [12]. CNT were refluxed with concentrated acid for 10 h, then washed with MilliQ water, and dried. Six mg of CNT was dispersed in 60 mL of absolute ethanol by ultrasonication. The cleaned plain carbon paper was used as a cathode, and a stainless-steel plate was used as an anode. DC voltage was applied between the carbon paper and the stainless-steel plate (as a counter electrode) holding the CNT suspension. The distance between the container and the carbon paper was 2 cm; constant voltage in the range of 50–100 V was applied between them for 3–5 min. A magnetic stirrer was used during the deposition to minimize particle sedimentation. CNT deposition occurred at one side of the carbon paper and was observed by scanning electron microscopy (SEM) (data not shown). New electrodes were soaked in 1 N HCl for 30 min and in 1 N NaOH for 30 min, and stored in MilliQ water before use. The Nafion membrane was pretreated, rendered in the H^+ form, by soaking it in 0.5 M H_2SO_4 for 30 min, after which it was stored in MilliQ water prior to use. For all of the fuel-cell experiments, new electrodes and Nafion membranes were utilized to prevent possible metal and organic contamination due to repeated use. The detailed experimental conditions and procedures have been given previously [13].

The MFC system was sterilized by immersing it in 0.4% (w/v) NaClO and washed carefully in MilliQ water before use. The anode chamber (in which cells were used to donate electrons to the electrode) was filled with the same medium that was used for cell growth, except for the concentrations of NH_4Cl (0.2 g/L) and $NaHCO_3$ (2 g/L) (fuel-cell growth medium, hereafter). The cells of *G. sulfurreducens* were harvested during the late-exponential phase in a serum bottle culture, washed twice with the fuel-cell growth medium, and placed in the anode chamber to the optical density (OD) of 0.1 at 660 nm. A concentrated stock solution of acetate was prepared in the fuel-cell growth medium, and added separately to the chamber, to 20 mM, as the sole electron donor. The cathode chamber (in which O_2 was used as the electron acceptor for the electrode) was filled with an anaerobic salts buffer (NaH_2PO_4, 0.6 g/L; KCl, 0.1 g/L; NaCl, 2.9 g/L, and Tris–HCl, 4.78 g/L) [10]. The anode and cathode chambers were continuously aerated with argon (Ar) gas through an O_2 trap (Supelco Co., USA) and air, respectively. The gases were filtered through 0.20 μm-pore membranes positioned in front of the MFC, and their flow rates were maintained at 7 mL/min. All of the stock solutions were flushed carefully with