Bioelectrochemistry 117 (2017) 23–28

Contents lists available at ScienceDirect

Bioelectrochemistry

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

Thermophilic *Moorella thermoautotrophica*-immobilized cathode enhanced microbial electrosynthesis of acetate and formate from CO_2

Linpeng Yu [a], Yong Yuan [b], Jiahuan Tang [a], Shungui Zhou [a,*]

[a] Fujian Provincial Key Laboratory of Soil Environmental Health and Regulation, College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[b] School of Environmental Science and Engineering, Guangdong University of Technology, Guangzhou 510006, China

ARTICLE INFO

Article history:
Received 22 March 2017
Received in revised form 12 May 2017
Accepted 12 May 2017
Available online 13 May 2017

Keywords:
Microbial electrosynthesis
Moorella thermoautotrophica
Thermophilic
Bacterial immobilization

ABSTRACT

Microbial electrosynthesis (MES) is a promising technique that converts electricity and CO_2 to biofuels using microbes as the catalysts. However, most of previous MES are conducted at mesophilic temperatures and challenged by low performances. Here we report a significant electrosynthesis performance enhancement via immobilization of a thermophilic microbe to cathodes. A temperature-dependent electron uptake rate of *Moorella thermoautotrophica* was observed at a cathode potential of −0.4 V (vs. SHE), with a maximum current density of 63.47 mA m^{-2} at 55 °C. Moreover, electrosynthesis rates of formate and acetate at 55 °C were accelerated by 23.2 and 2.8 fold than those of 25 °C, respectively. Compared with natural biofilms, immobilization of *M. thermoautotrophica* with carbon nanoparticles to electrodes further enhanced acetate and formate production rates (by 14 and 7.9 fold), reaching 58.2 and 63.2 mmol m^{-2} day^{-1} at a coulombic efficiency of 65%, respectively. To our best knowledge, these are the highest electrosynthesis rates obtained thus far for pure cultures under the conditions of −0.4 V (vs. SHE) and 55 °C. This study, for the first time, demonstrates that embedding microbes to electrodes by carbon nanoparticles is a facile and efficient method of improving MES performance.

1. Introduction

Microbial electrosynthesis (MES) is a nascent technology that uses electricity as the reducing power to drive microbial CO_2 reduction for the production of extracellular organic compounds. It has been proposed as a potential strategy to convert solar energy into chemical fuels and can be termed an artificial photosynthesis [1]. Compared to plant photosynthesis, the main advantage of MES is that desired chemicals and fuels can be produced in a direct and sustainable way without the utilization of the land. Potential applications of MES would not only reduce the social demand on fossil fuel resources, but also alleviate the global greenhouse effect [2]. Thus, over last few years, increasing interest and efforts have been focused on developing MES into a versatile platform that can synthesize diverse fuels such as ethanol [3].

Microbial electrosynthesis depends on the catalytic activities of electroactive microorganisms on the electrode. To date, only a handful of pure cultures [4–6] and mixed microbial communities [7] have been demonstrated to perform electroautotrophic reactions. All of these electrosynthesis processes were narrowly conducted at mesophilic temperatures ranging from 20 to 37 °C [4–7]. Previously, Song et al. [8] suggested that *Moorella thermoacetica* possessed the best electrocatalytic CO_2 conversion activity at 55 °C [8]. Faraghiparapari et al. reported that the acetate production rates of *M. thermoacetica* and *M. thermoautotrophica* at 60 °C were ca. 2.6- and 3.0-fold higher than those of 37 °C, respectively [9]. Since elevated temperatures would inhibit the growth of most organics-consuming microbes and increase the diffusion coefficients of substrates, thermophilic MES is potentially superior to mesophilic systems in both the reaction activity and durability [10,11]. However, information on thermophilic MES remains surprisingly lacking.

Acetate is the most common electrosynthesis product, which is a useful intermediate for further industrial conversion. The efficiencies of acetate MES by pure or mixed microbial cultures have been extensively reviewed by Tremblay et al. [12]. Among these pure cultures, *Sporomusa ovata* was the most efficient acetogenic electroautotroph with an acetate production rate up to 56.40 mmol m^{-2} day^{-1} [12]. Other organic compounds such as methane, acetone and butyrate can also be synthesized simultaneously [13–15]. However, most reported MES are still challenged by low performances that are insufficient for industrial up-scaling and commercialization. One limiting factor of the MES performance is probably related to the low electrode biomass. To increase the quantity of electroactive bacteria on the electrode, one routine method is to acclimate the MES system so that a stable electrode biofilm is established. For example, Marshall et al. acclimated a mixed culture biofilm for 150 d and obtained a maximum acetate production rate of 1.3 mmol day^{-1} at a cathodic potential of −0.59 V (vs. SHE)

* Corresponding author.
E-mail address: sgzhou@fafu.edu.cn (S. Zhou).

http://dx.doi.org/10.1016/j.bioelechem.2017.05.001
1567-5394/

[16]. However, the acclimation of cathodic biofilms is rather time-consuming, taking several days or even several months to form thick and multiple layers of biofilms. In this regard, many attempts have been made to immobilize microorganisms directly to electrode surfaces. Various matrices such as polyvinyl alcohol [17], pectin [18], latex [19] and hydrogel [20] have been used to entrap microorganisms on the electrodes. However, these immobilization matrices are electrical insulators, which might completely inhibit microbial extracellular electron transfer (EET). Although carbon nanoparticles were previously used to entrap microbes on the anodes and enhanced the anodic electricity generation [21–23], it is still unclear whether direct immobilization of microbes on cathodes can improve their electron uptake and MES performance.

Here, we explored for the first time how would immobilization of *M. thermoautotrophica* on the electrodes influence its electron uptake activity and MES performance. Different temperatures were selected to investigate the effects of temperature on MES. Cyclic voltammetry (CV) and scanning electron microscopy (SEM) were used to determine the electrochemical activity of *M. thermoautotrophica* biofilms and their morphologies, respectively.

2. Materials and methods

2.1. Microorganism and culture conditions

Moorella thermoautotrophica (DSM 7417) was purchased from the Deutsche Sam-mlung Mikroorganismen und Zellkulturen (DSMZ). The strain was routinely cultured in the DSMZ Medium 135 at 55 °C.

2.2. Bioreactor construction and operation

Dual-chambered glass bioelectrochemical cells were constructed using the same reactors as previously described except that each cathodic chamber was equipped with three parallel carbon cloth (a total normalized surface area of 147.0 cm^2) electrodes [24]. For the experiments of MES performance enhancement, a single carbon cloth electrode (a normalized surface area of 12.5 cm^2) was used to fabricate the bacteria-immobilized cathode. Carbon felt (3 cm × 5 cm × 0.5 cm) and saturated calomel reference electrodes (SCE, +0.241 V vs. a standard hydrogen electrode (SHE)) were used as the anode and reference electrode, respectively. The anodic and cathodic chambers were separated by proton exchange membranes (PEM, Dupont, USA). The reactors were connected to a multi-potentiostat (CHI1040, Chenhua Co., Ltd., Shanghai, China) with cathodic potentials poised at −0.4 V (vs. SHE). This applied potential can provide sufficient energy for MES without significant production of hydrogen [4]. All of the potentials reported in this work were relative to SHE unless otherwise noted. A control treatment was set simultaneously using open-circuit reactors that were disconnected to the multi-potentiostat. The incubation temperatures were maintained at 25, 37 and 55 °C by three incubators, respectively. Three replicates were conducted for each temperature. The catholyte was 120 ml of DSMZ Medium 135 (pH = 7.0) omitting yeast extract, fructose, resazurin and cysteine. Such a medium contained no organics and the cathodes served as a sole electron donor. CO_2 was mainly provided in the form of sodium bicarbonate (10.0 g/l). The anodic chamber was filled with 120 ml of 0.1 M potassium phosphate buffer solution (PBS, pH 7.0 with 0.1 M KCl). Prior to the experiments, all the reactors and electrolytes were autoclaved and purged with N_2-CO_2 (80:20) for 1 h and then sealed with rubber stoppers. Aliquots (160 ml) of *M. thermoautotrophica* culture medium (OD_{600} = 0.4) at the log phase were centrifuged (8000 rpm) and washed for three times, and then transferred into a single cathode chamber. Currents were recorded by the multi-potentiostat every 60 s. After the electrosynthesis experiments, cyclic voltammetry (CV) was conducted using the intact reactors in a potential range of −0.7 to +0.4 V (vs. SHE) at a scan rate of 10 mV/s. CV was also performed in the absence of an external carbon source using deoxygenated PBS buffers (0.1 M, pH 7.0 with 0.1 M KCl) as the catholytes under a N_2 condition.

2.3. Preparation of M. thermoautotrophica-immobilized biocathodes

Immobilization of *M. thermoautotrophica* on the carbon cloth electrode was conducted as suggested by Yuan et al. [18]. Briefly, 4.0 g of carbon nanoparticles (CNP, 300 nm in diameter, Vulcan XC-72R) was mixed with 5 ml of sterilized water in a 100-ml centrifuge tube. Meanwhile, bacterial cultures (160 ml) at the log phase were harvested by centrifugation. The pellets were washed and resuspended in 5 ml of deoxygenated and sterile cathodic medium. The bacterial suspension was then transferred to the mixture and stirred vigorously under N_2 atmosphere. After adding 5 ml of Teflon emulsion (Teflon PTFE30, 116 DuPont, USA), this mixture was stirred again for at least 3 min and was allowed to stick together. Such a mixture was pasted homogeneously onto a carbon cloth electrode (a thickness of 0.8 cm and a normalized surface area of 12.5 cm^2). Following the preparation, the reactor lid (5.0 cm in diameter) of the cathodic chamber (containing 120 ml of deoxygenated DSMZ Medium 135) was opened under N_2 atmosphere. Then the as-prepared *M. thermoautotrophica*-immobilized electrodes (CNP/bacteria electrodes for short) were inserted vertically into the cathodic chamber with whole electrode surfaces immersed in the medium. A titanium wire (0.5 mm in diameter) that was mechanically connected with the CNP/bacteria cathode was pierced through a rubber stopper on the lid. The cathodic chamber was finally covered with the lid to maintain the anaerobic environment. The CNP control electrode was fabricated similarly as mentioned above except that the bacterial suspension was replaced with 5 ml of deoxygenated and sterile water. For the CNP control reactor, the same amount of *M. thermoautotrophica* was inoculated into the cathode chamber without immobilization. Both the CNP/bacteria reactors and CNP control reactors were run in duplicate at a cathodic potential of −0.4 V (vs. SHE) at 55 °C.

2.4. Analytic techniques

Acetate and formate in the catholyte were measured by high performance liquid chromatography (HPLC, Shimadzu Co., Ltd., LC-16 system equipped with a symmetry C_{18} column (5 μm, 4.6 × 250 mm^2, Waters, USA)). The mobile phase of HPLC was 18 mM KH_2PO_4 at pH 2.5 adjusted with H_3PO_4. The flow rate was 0.5 ml/min and the detection wavelength was 213 nm. Calibration curves were generated using a series of standard acetate and formate solutions. Hydrogen (200 μl sampled with a gastight syringe) in the headspace of cathodic chambers was detected by gas chromatograph (GC) with a reducing compound photometer (Peak Performer 1; Peak Laboratories LLC., USA). The carrier gas was ultrapure nitrogen (99.999%). The temperatures of mercury bed and GC column (Unibeads 1S and MS 13×) were 210 and 100 °C, respectively. Multi-point hydrogen calibration curve was obtained by using a series of external hydrogen standard samples. Sample preparations for determining the total proteins of the biofilms and scanning electron microscopy (SEM, S-4800 FESEM, Hitachi Inc., Japan) were performed as previously described [25,26].

The half-cell reactions of CO_2 reduction to acetate and formate can be expressed as follows [27,28]:

$$2CO_2 + 7H^+ + 8e^- \rightarrow CH_3COO^- + 2H_2O \quad E_0' = -0.29\ V(\text{vs. SHE}) \qquad (1)$$

$$CO_2 + H^+ + 2e^- \rightarrow HCOO^- \quad E_0' = -0.42\ V(\text{vs. SHE}) \qquad (2)$$