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Current generation in microbial electrolysis cells with addition of amorphous ferric hydroxide, Tween 80, or DNA

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ARTICLE INFO

Article history:
Received 7 June 2012
Received in revised form
24 August 2012
Accepted 25 August 2012
Available online 25 September 2012

Keywords:
Amorphous ferric hydroxide
Tween 80
DNA
Microbial electrolysis cells

ABSTRACT

Iron-oxide nanoparticles and the Tween 80 have previously been shown to improve power generation in microbial fuel cells (MFCs), presumably by improving electron transfer from the bacteria to the anode. We examined whether several chemicals would affect current production in single-chamber microbial electrolysis cells (MECs), where hydrogen gas is produced at the cathode, using mixed cultures and *Geobacter sulfurreducens*. Tween 80 did not increase the current. $Fe(OH)_3$ addition increased the maximum current density of both the mixed cultures (from 6.1 ± 0.9 A/m^2 to 8.8 ± 0.3 A/m^2) and pure cultures (from 4.8 ± 0.5 A/m^2 to 7.4 ± 1.1 A/m^2). Improved current production was sustained even after iron was no longer added to the medium. It was demonstrated that increased current resulted from improved cathode performance. Analysis using electrochemical impedance spectroscopy (EIS) showed that the iron primarily reduced the diffusion resistances of the cathodes, and scanning electron microscopy (SEM) images showed the formation of highly porous structures on the cathode. The addition of DNA also did not improve MEC or MFC performance. These results demonstrated that among these treatments only $Fe(OH)_3$ addition was a viable method for enhancing current densities in MECs, primarily by improving cathode performance.

1. Introduction

A bioelectrochemical system (BES) is a device in which organic matter is oxidized by microorganisms using the anode as an electron acceptor, with electrons transferred through an external circuit to the cathode for final reduction of various chemical species. In microbial fuel cells (MFCs), oxygen is usually reduced on the cathode, although other terminal electron acceptors have been used [1,2]. In a microbial electrolysis cell (MEC), oxygen is omitted from the cathode chamber and hydrogen evolution occurs when a sufficient additional voltage is added [2,3]. In both types of BESs, improvements in system performance are needed to enable the commercialization of these processes for wastewater treatment and other applications such as biofuel production [4–6].

Electron transfer rates from microorganisms on the anode, and to chemicals at the cathode, are critical for BES performance [7,8]. Several methods have been examined to improve performance, including adding materials such as carbon nanotubes and graphene to the electrode surfaces [9–12]. An alternative approach is the addition of chemicals that affect bacteria on the anode and improve current densities. For example, the power density of an air-cathode, single-chamber

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0360-3199/$ – see front matter
http://dx.doi.org/10.1016/j.ijhydene.2012.08.119

MFC was increased by 8.7 times following the addition of 80 mg/L of Tween 80, a non-ionic surfactant [7]. The increase was presumed to be a result of increased permeability of microbial cell membranes, because this surfactant is known to change the cell membrane structure by forming trans-membrane channels [13,14]. Conductive and semi-conductive iron-oxide nanoparticles such as hematite, magnetite, or ferrihydrite have also been shown to increase current production of MFCs by over 30 times [15]. It was believed that this increase resulted from the nanoparticles improving transfer of electrons from the microorganisms to the anode, but the analysis was insufficient to determine which electrode was responsible for the change in performance [15]. In both cases, tests were conducted using MFCs, where oxygen transfer into the reactor can play a role in reactor performance and biological activity [16–19].

Other factors are known to affect the stability and electrical conductivity of biofilms. For example, it has been shown that extracellular DNA (eDNA) is important in biofilm formation, and that removal of DNA (using DNase) affects the initial establishment of the biofilm [20]. eDNA enhances the strength of both pure- and mixed-culture microbial biofilms by enhancing cell-to-cell interconnections [21,22]. There is also evidence that eDNA is electrically conductive, with electron transfer occurring due to charge transport along the DNA strands [23,24]. In BESs, electrons are thought to be able to move through the biofilm by electron transfer between a series of redox proteins (electron superexchange) [25], although others have indicated that it is due to a metallic-like conductivity of the biofilm [26]. One criticism of the electron superexchange mechanism is insufficient proximity of cytochromes to enable electron transfer [27]. We therefore wondered if eDNA might be important for electron conductivity of exoelectrogenic biofilms, and whether it was needed for biofilm integrity and stable current generation. To date, there has been no examination of a possible role for eDNA in BESs.

In order to better understand the effects of different types of chemicals on the performance of BESs, we examined the effects of amorphous ferric hydroxide [$Fe(OH)_3$], Tween 80, and eDNA addition on current generation in MECs. By using MECs, we were able to avoid any additional effects of oxygen intrusion into the reactors on current generation. High current densities in BESs have been correlated to the presence of different *Geobacter* species [28,29]. Therefore, we tested both a pure culture of *Geobacter sulfurreducens* and mixed cultures (wastewater inoculum). The improved performance of the iron oxide-supplemented systems was further investigated using electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Reactor construction and operation

Single chambered mini-MECs were constructed as described previously [30] using 5 mL clear glass serum bottles (Wheaton). The anode was a graphite plate with a thickness of 0.32 cm and dimensions of 1.5 cm × 1 cm (Grade GM-10; GraphiteStore.com, Inc.). Stainless steel (SS) mesh (Type 304, Mesh size 60 × 60; McMaster-Carr) was cut to the same projected area, and used as the cathode. Additional tests to determine the effects of $Fe(OH)_3$ on hydrogen generation were conducted using larger single-chamber MECs made with 100 mL, wide-mouth bottles (PYREX® Media Bottles, Graduated, Corning®) sealed with butyl rubber stoppers. These larger reactors were used in order to produce more gas for analysis than that produced by mini MECs. The same anode and cathode materials were used, with electrode dimensions of 4.5 cm × 2 cm. A gas bag (250 mL) and gastight tubing were used to collect bio-gas through a syringe needle inserted through the stopper. Reactors used for *G. sulfurreducens* cultures were sparged with anaerobic gas (CO_2/N_2, [20/80]) and autoclaved. Reference electrodes (Ag/AgCl; BASi) were used in some experiments to record anode and cathode potentials by inserting the electrode (0.57-cm diameter) through a hole cut in the butyl stopper (0.79-cm diameter), with the tip placed between the anode and cathode. All electrode potential values were reported here versus Ag/AgCl [+200 mV versus standard hydrogen electrode (SHE)]. MECs were connected to a programmable power supply (model 3645A; Circuit 128 Specialists, Inc.) with a circuit containing a 10 Ω resistor connected in series for recording the voltage produced by each reactor. All tests were operated at an applied potential of $E_{AP} = 0.7$ V [6,31].

Further tests on the effects of eDNA were examined in MFCs to obtain a more comprehensive examination of the effect of this chemical on current generation, as no data is reported of a possible role so far for eDNA in BESs. Single chamber, air-cathode MFCs were constructed using cube-shaped blocks of Lexan with a single cylindrical chamber (14 mL, 7 cm^2 cross sectional area) as previously described [32]. The anode was a graphite plate treated with ammonia gas at 700 °C for 1 h [33], connected to titanium wire, and then placed at one end of the cylindrical chamber. Two different anode sizes were used: 1 cm × 1 cm, and 0.7 cm × 1 cm. The air cathode (projected surface area of 7 cm^2) was made of carbon cloth (30 wt.% wetproof, Fuelcellearth, #CC640WP30), and had a catalyst loading of 0.5 mg-Pt/cm^2on the water side, and four PTFE diffusion layers on the air side [34]. The electrode spacing was 2 cm from the front of the graphite plate anode to the surface of the cathode. The terminals of the MFCs were connected to resistor boxes using copper wires, with the voltage across the resistor recorded using a multimeter (model 2700; Keithley Instruments, Inc.). Reactors were operated in fed-batch mode, with medium replacement after the current dropped below 0.15 mA. All tests were conducted in duplicate reactors in a temperature controlled room (30 °C).

2.2. Microorganisms and media

Cell suspensions of *G. sulfurreducens* were prepared from a stock culture as previously described [31], in an acetic acid growth medium using fumarate (22.5 g/L) as the electron acceptor. MECs were inoculated with *G. sulfurreducens* in the late exponential growth phase using 2.5 mL of the culture and 2.5 mL of growth medium. The mixed culture inoculum for MECs and MFCs was effluent from MFCs previously inoculated