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Substrate and electrode potential affect electrotrophic activity of inverted bioanodes

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ABSTRACT

Electricity-consuming microbial communities can serve as biocathodic catalysts in microbial electrochemical technologies. Initiating their functionality, however, remains a challenge. One promising approach is the polarity inversion of bioanodes. The objective of this study was to examine the impact of bioanode substrate and electrode potentials on inverted electrotrophic activity. Bioanodes derived from domestic wastewater were operated at −0.15 V or +0.15 V (vs. standard hydrogen electrode) with either acetate or formate as the sole carbon source. After this enrichment phase, cathodic linear sweep voltammetry and polarization revealed that formate-enriched cultures consumed almost 20 times the current (−3.0 ± 0.78 mA; −100 ± 26 A/m^3) than those established with acetate (−0.16 ± 0.09 mA; −5.2 ± 2.9 A/m^3). The enrichment electrode potential had an appreciable impact for formate, but not acetate, adapted cultures, with the +0.15 V enrichment generating twice the cathodic current of the −0.15 V enrichment. The total charge consumed during cathodic polarization was comparable to the charge released during subsequent anodic polarization for the formate-adapted cultures, suggesting that these communities accumulated charge or generated reduced products that could be rapidly oxidized. These findings imply that it may be possible to optimize electrotrophic activity through specific bioanodic enrichment procedures.

1. Introduction

Microbial communities capable of consuming electricity (electrotrophs, biocathodes) can provide multiple functions in microbial electrochemical technologies (METs) [1,2]. These biological communities have the unique ability to accept electrons either directly or indirectly (via soluble mediators) from electrodes [1]. They transfer these electrons to suitable electron acceptors, forming reduced products. In microbial fuel cells (MFCs), these organisms reduce oxygen to form water or nitrate to produce nitrogen gas [2–6]. In microbial electrolysis cells (MECs), biocathodes generate H_2 or CH_4 gas [7,8]. Electrotrophs can also synthesize high-value commodity chemicals and biofuel precursors through microbial electrosynthesis [9,10]. Biocathodic microorganisms are self-regenerating biocatalysts and therefore offer a promising alternative to costly, foul-prone chemical catalysts such as platinum and create potential for a wider range of cathode functions.

Initiating and optimizing electrotrophic activity presents several challenges for which many strategies have been examined. Direct cathodic enrichment typically requires extended periods of time (months) [4,7] and often results in limited biofilm coverage compared to electricity-generating exoelectrogenic biofilms [6,9,11,12]. Large surface area electrodes and surface modifications have increased biofilm coverage in some, but not all cases [13–16]. Supplying different carbon sources during cathodic polarization also impacts current initiation [15–17]. Supplementing a polarized cathode with acetate, for example, reduced startup time by half and increased maximum current generation rates in comparison to bicarbonate enrichment, likely due to acetate facilitating heterotrophic growth [15]. Establishing microbial communities with more negative electrode potentials can theoretically provide more energy to electrotrophs and decrease startup times. This was not observed for H_2-generating biocathodes, however, possibly due to the interference of abiotic H_2 evolution [15].

One alternative to cathodic polarization enrichment is polarity inversion of electricity-generating bioanodes into electrotrophic biocathodes [5,18,19]. Anode-to-cathode inversion employs the same microbial biofilm to initially oxidize electron donors at one potential (more positive than the electron donor) and subsequently catalyze reduction reactions at a second potential (more negative than the electron donor). Rozendal et al. developed a H_2-generating biocathode through a multistep acclimation to acetate and H_2 gas [8]. Acetate-adapted bioanodes immersed in sediments resulted in CH_4-generating biocathodes when the polarity was switched [19]. Across these studies, different bioanode enrichment methods have been used, but a systematic understanding of how bioanode operating conditions affect electrotrophic activity is lacking. Two factors

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that are known to impact bioanode development are substrate and electrode potential. Substrates ranging from simple carbon-containing compounds to complex wastewaters generate community structure and activity differences [20–23]. Similar observations have been made when different anode potentials are used [24,25]. Anode potentials can also influence the way cells "connect" to an electrode via different redox active components, such as cytochromes [24,26].

The objective of this study was to examine the influence of bioanode enrichment phase substrates and electrode potentials on inverted electrotrophic activity. We hypothesized that varying these two factors would cause differences in electron uptake because of their ability to influence anode microbial community structure and activity. Two substrates, acetate and formate, were selected to enrich bioanodes. Acetate is well known to produce high-current generating bioanodes, thick biofilms, and has previously resulted in successful bioanode inversion [8,19,27]. Formate was selected because it has previously been shown to generate more diverse bioanode communities than acetate and many methanogenic and formate-utilizing microorganisms are associated with CO_2 fixation [22,25,28]. These two substrates were examined in conjunction with two fixed bioanode potentials, −0.15 V and +0.15 V [vs. the standard hydrogen electrode (SHE)], to vary the available energy to microbial communities and to induce different electron transfer carriers and pathways to the electrode. The polarity of these enriched bioanodes was subsequently inverted and the ability to consume electrical current investigated. Using a suite of electrochemical tests, we discovered that bioanode enrichment substrate and electrode potential can have an appreciable impact on electrotrophic activity.

2. Materials and methods

2.1. Phase I — anode enrichment

Electrochemical reactors were constructed from polycarbonate with an internal cylindrical chamber (30 mL volume) as previously described [29]. Anodes were graphite brushes [cleaned by heat-treatment at 450 °C for 30 min [30]] placed opposite air-exposed, platinum-coated carbon cloth cathodes [29]. A stoppered, crimped anaerobic tube was attached to the top of the reactor to capture and analyze gases and an Ag/AgCl reference electrode [+200 mV vs. SHE] was positioned between the two electrodes. All potentials reported herein are in reference to the SHE. Reactors were filled with a 30 mM bicarbonate-buffered freshwater medium as previously described [27]. The effect of different carbon sources during anode enrichment were examined at equivalent chemical oxygen demand (COD) concentrations (0.85 g-COD/L) with 1 g/L sodium acetate (12 mM) and 3.32 g/L sodium formate (49 mM). Reactors were inoculated with raw wastewater influent from a treatment plant that receives primarily domestic wastewater (Metropolitan Syracuse Wastewater Treatment Plant, Syracuse, NY) and operated in fed-batch using a 50:50 (v/v) inoculum-medium every 24 to 48 h until current production was reproducible (about 21 days with initial cycles lasting longer due to anode biofilm formation). The reactors and medium were not deoxygenated prior to the start of each cycle. The anodes [working electrodes (WE)] were poised with a multichannel potentiostat (VMP3, BioLogic) at either $E_{WE} = -0.15$ V or +0.15 V. All reactors were operated in duplicate at a constant temperature of 30 °C.

2.2. Phase II — anode-to-cathode inversion

The initial anode-to-cathode inversion tests were performed in the single-chamber systems using a three-electrode setup. The enriched bioanodes served as the WE and the platinized cathodes as the counter electrodes (CE). The medium was identical to Phase I, except the organic carbon sources (acetate and formate) were absent and the liquid purged with an anaerobic gas mix (20% CO_2/80% N_2) prior to the start of each cycle. To test biocathodic activity, slow-scan linear sweep voltammetry (LSV) was conducted from −0.3 V to −0.8 V at a scan rate of 0.03 mV/s. Biocathodic electron consumption and discharge were examined using two approaches. First, following a −0.6 V WE cathodic polarization (CP) for 1 h, cyclic voltammetry (CV) was performed from −0.4 V to +0.5 V at a rate of 1 mV/s for two cycles. Second, the WE was fixed for 2 h at −0.6 V and then the WE was fixed to a potential [anodic polarization (AP)] of +0.4 V for 2 h. Prior to this second set of tests, reactors were expanded to a double-chamber configuration by moving the CEs to a second polycarbonate chamber (2.5 cm long) and separating the chambers with an anionic exchange membrane (AEM; Membranes International). This was done to minimize the introduction of O_2 as a terminal electron acceptor and instead promote biocathodic reduction of CO_2. At the start and end of the CP and AP cycles, the reactor headspace was analyzed for CH_4 and H_2 by collecting samples (50 μL) from the 1.6 mL headspace. Gases were measured with a Clarus 500 gas chromatograph using a RT-Msieve 5 A (30 m, 0.53 mmID, 50 μm df; Restek) column with oven and injection temperatures of 100 °C, split of 40 mL/m, and 5 psi ultra-purified helium carrier gas. The minimum detection limits for both CH_4 and H_2 were 1.4×10^{-8} mol.

3. Results

3.1. Bioanode enrichment

During bioanode enrichment, maximum currents approached 4.8 ± 1.4 mA [160 ± 47 A/m^3 (normalized to liquid volume)] for all reactors except the acetate-supplied reactors poised at +0.15 V, which reached 19 ± 5 mA (620 ± 160 A/m^3) (Fig. 1). Current stabilized over a period of approximately 21 days (14 batch cycles). These results are consistent with previous reports of acetate-fed bioanodes generating higher currents than formate-fed systems [20,22,25]. There was no clear trend between electrode potential and current. The +0.15 V anode resulted in higher current than the −0.15 V anode for acetate, but the reverse was observed for the formate cultures. The currents generated by the formate-fed reactors were almost twice that previously reported in similar reactors at the same electrode potentials, which may reflect the higher concentration used in this study (3.3 g/L) versus the previous one (1 g/L) [25]. The higher current

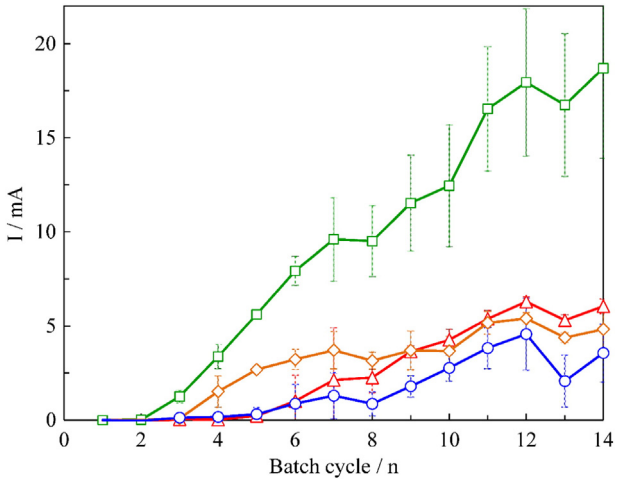

Fig. 1. Current generation as a function of batch cycle during bioanode startup. Two substrates (acetate, formate) and two electrode potentials (−0.15 V, +0.15 V vs. SHE) were tested. Approximately 21 days elapsed during the fourteen cycles shown. Error bars represent standard deviation of replicates. □Acetate +0.15 V, △Formate −0.15 V, ◇Acetate −0.15 V, ○Formate +0.15 V.