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Autotrophic hydrogen-producing biofilm growth sustained by a cathode as the sole electron and energy source

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ABSTRACT

It is still unclear whether autotrophic microbial biocathode biofilms are able to self-regenerate under purely cathodic conditions without any external electron or organic carbon sources. Here we report on the successful development and long-term operation of an autotrophic biocathode whereby an electroactive biofilm was able to grow and sustain itself with CO_2 as a sole carbon source and using the cathode as electron source, with H_2 as sole product. From a small inoculum of 15 mg_{COD} (in 250 mL), containing 30.3% Archaea, the bioelectrochemical system operating at −0.5 V vs. SHE enabled an estimated biofilm growth of 300 mg as COD over a period of 276 days. A dramatic change in the microbial population was observed during this period with Archaea disappearing completely (<0.1% of population). The predominant phyla enriched were Proteobacteria (57.3%), Firmicutes (12.4%), Bacteroidetes (11.6%) and Actinobacteria (1.1%). Up to 9.2 L H_2 m^{-2} day^{-1} (1.88 A m^{-2}) was achieved when the cathode potential was decreased to −0.75 V vs. SHE. This study demonstrates that purely autotrophic biofilm growth coupled to proton reduction to hydrogen alone can be sustained with a cathode as the sole electron source, while avoiding the development of H_2-consuming microorganisms such as methanogens and acetogens.

1. Introduction

The efficient and sustainable production of hydrogen in a microbial electrolysis cell (MEC) is a potential strategy for green energy production from renewable resources, water and biomass, which has gained recognition in the last few years [1–3]. Conventionally, expensive materials such as platinum were used as cathode catalyst for hydrogen production in MEC, but were shown to be not economically viable [4]. Nickel and stainless steels have also been investigated due to their stability, reduced cost, and low overpotential, and comparative efficiency with platinum catalyst for hydrogen evolution [3–6]. Microbial biocathodes have been developed as promising alternatives, due to their low cost (for both electrode material and catalyst) and low overpotential. Immobilized hydrogenases that catalyze the reversible reaction $2H^+ + 2e^- \leftrightarrow H_2$ on carbon electrodes were first shown to successfully catalyze hydrogen production [7–12]. However, enzymatic biocathodes are not self-regenerating, are unstable and lose their catalytic activity over time. Therefore, whole cells of pure cultures of hydrogenase-possessing microorganisms, such as *Desulfovibrio* sp., have been immobilized with methyl viologen as a redox mediator, and were reportedly successful in catalyzing hydrogen production [13,14]. Nevertheless, from an economical and technical point of view, mediator-less mixed culture microbial biocathodes would be much more desirable in respect to the stability of operation of the BES [15]. In this perspective, hydrogen-producing microbial biocathodes based on naturally selected mixed cultures were investigated and showed bioelectrocatalytic activity for enhanced hydrogen production [15–19]. In all these studies, electrochemically active biofilms were grown on anodes with acetate and/or hydrogen as electron donor and then, the polarity of the electrode was reversed to biocathode mode for hydrogen production [15–17,19]. Alternatively, the inocula used in biocathodes were pre-enriched as bioanodes with acetate as electron source [18]. It is not clear from the reported results whether these biofilms are able to self-regenerate under purely cathode conditions without any organic carbon sources. The challenge is to generate a self-maintaining biocathode, with living microorganisms which are able to survive, conserve energy and grow for continuous hydrogen production, preferably under autotrophic conditions using carbon dioxide as the sole carbon source.

Anode processes and anodic extracellular electron transfer (EET) mechanisms have been extensively investigated in the last decade. Two different EET pathways have been identified [20]. The first one involves the direct contact of electron transfer chain components of the microbes with the electrode surface and is referred to as direct electron transfer (DET). The second mechanism is called mediated electron

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transfer and involves electron shuttles serving as electron carriers thanks to their ability to reversibly be oxidized and reduced between the microbes and the electrodes. However, information on the reverse process, i.e. electron flow from electrodes (cathodes) to microorganisms, is rather limited. Until now, the exact cathodic electron transfer pathways are still unknown. Many potential mechanisms have been proposed [21–24] and are believed to be similar to the processes occurring at bioanodes, but operating at different redox potentials [23]. It has been highlighted that DET from electrodes to microorganisms would hold most promise for microbial biocathode applications [25]. In the context of hydrogen-producing microbial biocathodes, evidence was found for direct electron transfer from a polarized graphite cathode (−0.9 V vs. SHE) to the hydrogenase-possessing microorganism *Desulfovibrio paquesii*, which catalyzes H_2 production from H^+ [26].

While anodic microorganisms consume part of the electrons available in the organic substrate for growth and maintenance with an electrode serving as the sole electron acceptor, cell growth and energy conservation with an electrode serving as the sole electron donor remains to be shown. Contradictory opinions exist on whether effective growth can be sustained at biocathodes. It was suggested that, due to their redox potentials, the electron transfer chain components within the cells may not necessarily lead to energy conservation for the microorganisms [23] and that a lithoheterotrophic metabolism would be required for cathodic growth [27,28]. However, cathodic growth is theoretically possible when the final electron acceptors are reduced in the cytoplasm or within the inner membrane [22]. Lovley argued that protons will be consumed for the production of the reduced end-product. Therefore, if the reduction of these electron acceptors occurs within the cytoplasm, this will result in a proton gradient across the inner membrane leading to ATP formation [22]. In the context of bioproduction, autotrophic growth solely using electrical current has been very challenging and only pre-grown biofilms (using e.g. acetate or hydrogen as electron donor) have been obtained thus far [15,29–33].

Here we report the generation of a microbial biocathode with living cells, which are able to self-regenerate and grow using the cathode as the sole electron donor and CO_2 as the only carbon source, for long-term sustained hydrogen production. This study also highlights a new H_2-producing electroactive microorganism enrichment method using CO_2 while avoiding archaea and methanogen development as well as any other H_2-consuming species (e.g. acetogens). This study shows that autotrophic electroactive biofilm growth can be sustained using a biocathode as the sole electron source for proton reduction to hydrogen.

2. Materials and methods

2.1. Graphite plate electrode preparation

Graphite plates (50 mm × 19.3 mm × 4 mm; Morgan AM&T, Sydney, NSW, Australia) were used as cathode electrodes. They were specifically modified by making 1.4 mm deep grooves on each side, in order to obtain 8 easily detachable 8.5 mm^2 squares per electrode, for further analysis. The total surface area of the modified plates was calculated to be 29 cm^2. The graphite electrodes were washed with 1 M hydrochloric acid, 1 M sodium hydroxide (24 h in each), and deionized water in succession to remove organic and metal contamination.

Graphite plates were pierced with a 0.5 mm thick Ti wire that acted as a current collector. The electrical connection was reinforced by means of conductive carbon paint that was left to dry for 3 days. Epoxy glue was also applied on the immersed surface of the Ti wire to avoid any contribution of the Ti wire to the electrochemical performance.

All electrodes were pre-treated in a N_2 plasma for 20 min before being introduced in the reactor, in order to remove surface contamination and render the surface hydrophilic [34].

2.2. Source of microorganisms

A mixed microbial consortia from natural environments (stormwater pond sediments, located on the University of Queensland St Lucia campus, latitude −27.500373, longitude 153.015062) and engineered anaerobic systems (from the Luggage Point Waste Water Treatment Plant anaerobic digester, Brisbane, Australia) were combined and added to a final concentration of 60 $mg_{COD}\ L^{-1}$ in the reactor. This amount of inoculum was very small compared to previous biocathode studies in order to easily monitor the actual growth of the microorganisms.

2.3. Electrochemical experiments

All bioelectrochemical experiments were carried out under strictly anaerobic conditions, at ambient temperature (ca. 24 ± 1 °C), in three-electrode/two-chamber systems. The whole experimental setup was kept under dark conditions to avoid phototrophic activity. Glass bottles were specifically designed, with a cathode chamber volume of approximately 300 mL. A 1 cm diameter, 15 cm long glass tube was inserted through the bottle top and served as the anode chamber, with platinum wire as counter electrode (purity 99.95%, temper hard, 0.50 mm diameter × 50 mm long, Advent Research Material Ltd, Oxford, England). The chambers were separated by a cation exchange membrane (Ultrex CM17000, Membranes International Inc., NJ, USA). Two ports were equipped with rubber septa to take samples from both the liquid phase and the headspace. A custom-made KCl saturated Ag/AgCl reference electrode was inserted into the bottle in proximity of the cathode. All potentials are reported here versus Standard Hydrogen Electrode (SHE).

Two duplicate BESs reactors were operated in fed-batch mode. After each liquid sample was taken out, on average every 7 days, exactly the same volume of fresh catholyte medium was added to keep the volume constant in the cathode chamber. A multi-channel potentiostat (CH Instruments, Austin, TX, USA) was used for all experiments. During the first 276 days, the cathode was poised chronoamperometrically at −0.5 V and then decreased to −0.75 V. Periodically, cathodes were subjected to cyclic voltammetry (CV) for the identification of any catalytic effects. The scan range was from 0 to −1.0 V, and the scan rate was 1 mV/s. The total charge (Coulomb) consumed was calculated by integrating the area under the current versus time curve (i–t curve). Two graphite plate electrodes were immersed in the cathode chamber and run in parallel on one potentiostat channel. A gas bag (Flexfoil plus, Air-Met Scientific Pty Ltd, QLD, Australia), specified for collection of CO_2, H_2 and CH_4, was connected to the reactor to measure gas composition and production rate and avoid pressure increase within the cathode chamber. An identical, but abiotic control reactor without an inoculum addition was also run in the same conditions.

The cathode chamber was filled with 250 mL of a medium containing: 0.2 g L^{-1} NH_4Cl, 0.04 g L^{-1} $MgCl_2 \cdot 6H_2O$, 0.015 g L^{-1} $CaCl_2$, 6 g L^{-1} Na_2HPO_4, 3 g L^{-1} KH_2PO_4 and 1 mL L^{-1} of a mixed trace element solution. $NaHCO_3$ to a final concentration of 0.5 g L^{-1} was added periodically as sole carbon source. The trace element solution contained 1.5 g L^{-1} $FeCl_3 \cdot 6H_20$, 0.15 g L^{-1} H_3BO_3, 0.03 g L^{-1} $CuSO_4 \cdot 5H_2O$, 0.18 g L^{-1} KI, 0.12 g L^{-1} $MnCl_2 \cdot 4H_2O$, 0.06 g L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 0.12 g L^{-1} $ZnSO_4 \cdot 7H_2O$, 0.15 g L^{-1} $CoCl_2 \cdot 6H_2O$, 0.023 g L^{-1} $NiCl_2 \cdot 6H_2O$ and 10 g L^{-1} EDTA. The medium was prepared under anaerobic conditions (flushed with 100% N_2) and introduced into the cathode compartment inside an anaerobic chamber. During the electrochemical experiments (chronoamperometry and cyclic voltammetry), the catholyte medium pH was controlled to 7 by dosing 1 M HCl as needed. The anolyte used contained 44 mg L^{-1} Na_2HPO_4 and 25 mg L^{-1} KH_2PO_4.

Results obtained on the duplicate reactor (data shown in supplementary information) were always in close accordance with the results showed below.