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Microbial community analysis in a long-term membrane-less microbial electrolysis cell with hydrogen and methane production

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ABSTRACT

A single-chamber microbial electrolysis cell (MEC) aiming at hydrogen production with acetate as sole carbon source failed due to methanogenesis build-up despite the significant amount of 2-bromoethanesulfonate (BES) dosage, 50 mM. Specific batch experiments and a thorough microbial community analysis, pyrosequencing and qPCR, of cathode, anode and medium were performed to understand these observations. The experimental data rebuts different hypothesis and shows that methanogenesis at high BES concentration was likely due to the capacity of some *Archaea* (hydrogen-oxidizing genus *Methanobrevibacter*) to resist high BES concentration up to 200 mM. *Methanobrevibacter*, of the *Methanobacteriales* order, represented almost the 98% of the total *Archaea* in the cathode whereas *Geobacter* was highly abundant in the anode (72% of bacteria). Moreover, at higher BES concentration (up to 200 mM), methanogenesis activity decreased resulting in an increase of homoacetogenic activity, which challenged the performance of the MEC for H_2 production.

1. Introduction

The need of renewable energy resources is an important focus of current research. Hydrogen gas (H_2) is a preferred alternative energy source since it is a clean and renewable energy carrier, without an impact on the greenhouse gas emission during its energy generation step and a high combustion heat (120 kJ/g) when compared to other possible biofuels (CH_4, 50 kJ/g or ethanol, 26.8 kJ/g) [1]. However, nowadays, most H_2 is produced via steam reforming, a non-sustainable option. Among all the current biological H_2 production techniques, the utilization of bioelectrochemical systems is very attractive because high yields can be achieved. Dark fermentation would only produce a maximum amount of 4 mol of H_2 per mol of glucose while most of the electronic content in substrate, except for the growth requirements, could be recovered using bioelectrochemical systems [2].

H_2 production in bioelectrochemical systems is conducted in devices known as microbial electrolysis cells (MECs). MECs use the particular biochemical pathway of a group of bacteria named exoelectrogens, which are able to transfer the electrons gained in their metabolism out of the cell and use a solid as electron acceptor. These electrons flow from the anode to the cathode where they react with protons to produce H_2. These protons are generated in the anodic oxidation process and are transported from the anode to the cathode through the electrolyte. Hence, exoelectrogenic bacteria act as biocatalysts of the oxidation process enabling H_2 production. Fundamental thermodynamics indicate that the H_2 production process in an MEC is not spontaneous. Thus, an additional voltage must be applied depending on the organic substrate used. For example, 0.14 V (under standard biological conditions according to the Nernst equation) should be enough for H_2 production from acetate [3].

The truth is that, in practice, a higher applied voltage (0.5 to 1 V) is needed under lab conditions due to the high potential losses [4]. Among all the voltage losses of the cell, the losses over the membrane, when used, are very significant. Membranes provide a separation between anode and cathode, which prevents H_2 from being used by H_2 scavengers and avoids impurities in H_2 [5]. Thus, either membrane losses are reduced with the development of new materials or the membrane itself is suppressed (i.e. single-chamber systems). Call et al. [6] questioned for the first time the necessity of membranes in single-cell MECs since: i) MECs do not need oxygen as microbial fuel cells (MFCs) and hence oxygen leakage from the cathode to the anode is not possible; ii) H_2 has a very low solubility and should be scarcely used by microorganisms in the cell and therefore could be mostly recovered; iii) current densities should not be decreased because of the membrane absence, and iv) the possible H_2 losses are compensated by the reduced cost of the system in terms of materials and potential applied. Since this work, many MEC studies have been conducted with and without membrane.

Regarding membrane-less MECs, its success is challenged by the bacteria colonizing each electrode. The anode is mostly colonized by exoelectrogenic bacteria (usually from the genera *Geobacter* and

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Shewanella). *Geobacter* is the most dominant genera in acetate-fed MEC [7]. Nevertheless, MEC conditions (i.e. anaerobic environments with organic matter as electron donor) may enable the proliferation of methanogens. Methanogens competing against the exoelectrogens for the same substrate or using the H_2 produced in the cathode is one of the most important reported failures of MECs [8,9]. Thus, understanding and inhibiting methanogenesis in these systems are essential in view of its future implementation. The use of a chemical inhibitor, 2-bromoethanesulfonate (BES) has been proposed as a suitable option for methanogenesis prevention in different anaerobic research fields [10]. BES is a structural analog of Coenzyme M (CoM) and inhibits effectively the methyl-CoM reductase reaction [11–13]. As abovementioned, methanogenesis can be seen as a competition of bioelectrochemical H_2 production but also as an opportunity if methane (CH_4) production linked to H_2 production cannot be avoided. Producing CH_4 rather than H_2 gas may be the best option if a natural gas infrastructure is available. Methanogens can produce CH_4 from CO_2 and H_2 and also methanogens that can directly use electrons from the cathode to produce CH_4, i.e. electromethanogenesis, have been reported [14].

The objective of this study is to gain understanding on the biological processes occurring in the cathode of a membrane-less MEC aiming at H_2 production, through an engineering approach and a thorough microbial analysis. Several BES concentrations to limit methanogenesis are tested to elucidate the fate of H_2 under different scenarios.

2. Materials and methods

2.1. Reactor description and operation

A large single-chamber membrane-less MEC (1300 mL) was used to carry out the experiments. A carbon fiber brush (PANEX®33 160 K, ZOLTEK) [15] previously inoculated in an MFC, as previously described [16], was used as anode (0.8 m^2). The cathode (0.034 m^2) was made with carbon cloth coated with carbon powder and platinum suspension on the side facing the anode [17]. Both electrodes were arranged concentrically with the cathode in the outer perimeter, so that all ends of the anode were at the same distance from the cathode. The reactor operated in batch mode, with constant agitation and an applied potential of 1.2 V between anode and cathode (HQ Power, PS-23023). The medium used (pH 7.3) was a 100 mM phosphate buffer with acetate (12 mM) as substrate and the following components in 1 L of deionized water: NH_4Cl (0.41 g), mineral media (5 mL), 1 mL of 4 $g \cdot L^{-1}$ $FeCl_2$ stock solution, and 0.5 mL of 37.2 $g \cdot L^{-1}$ $Na_2S \cdot 9H_2O$ stock solution. The mineral medium had the composition previously described in Parameswaran et al. [18]. MEC was operated with the addition of BES (Sigma-Aldrich, USA). BES ($Br–CH_2CH_2–SO_3Na$) is a sulfonate composed of a sulfite group (SO_3^{2-}) and an ethyl group ($C_2H_5^-$) with one H substituted by a bromine (Br) atom. The MEC was inoculated and operated for 4 months using 50 mM of BES concentration, in accordance with common practice [19]. Afterwards, the medium was replaced twice adding increasing BES concentrations. First, 100 mM of BES concentration was maintained for 15 days (one batch cycle) and finally fresh medium with 200 mM of BES was added and operated for 20 additional days (one interrupted batch cycle). Other details about the equipment and monitoring system are described in Ruiz et al. [20].

2.2. Electrochemical calculations

Coulombic efficiency (CE) was calculated as in Eq. (1).

$$CE = \frac{\text{Coulombs recovered as current intensity}}{\text{Coulombs in substrate}} = \frac{\int_{t_0}^{t_F} Idt}{F \cdot b_{Ac} \cdot V_L \cdot \Delta c \cdot M^{-1}} \quad (1)$$

where t_0 and t_F are the initial and final time of an experiment, Δc is the change in acetate concentration during the experiment (g acetate $\cdot L^{-1}$ cell), M is the molecular weight of acetate (59 $g \cdot mol^{-1}$), b_{Ac} is the number of e^- transferred per mole of acetate (8 mol $e^- \cdot mol^{-1}$ acetate), F is the Faraday's constant (96,485 $C \cdot mol^{-1}$ e^-), I is the current intensity and V_L is the volume of liquid in the reactor (L).

Cathodic gas recovery (r_{CAT}) was calculated as in Eq. (2).

$$r_{CAT} = \frac{\text{Coulombs in } H_2}{\text{Coulombs recovered as current intensity}} = \frac{V_{F,H_2} \cdot 2 \cdot F \cdot V_m^{-1}}{\int_{t_0}^{t_F} Idt} \quad (2)$$

where V_m is the molar gas volume (24.03 $L \cdot mol^{-1}$) at 20 °C and $V_{F,H2}$ is the volume of H_2 at the end of the cycle.

2.3. Chemical analyses

Acetate was analyzed by gas chromatography (Agilent Technologies, 7820-A) using a flame ionization detector (FID) with helium as carrier gas. H_2 and CH_4 production was analyzed by the same gas chromatograph using a thermal conductivity detector with argon as carrier gas.

CH_4 relative composition was calculated as the ratio of CH_4 with respect to the total amount of CH_4 and H_2 (Eq. (3)).

$$\text{Relative composition } CH_4 = \frac{\text{Volume } CH_4}{\text{Volume } H_2 + \text{Volume } CH_4} \quad (3)$$

This ratio did not consider CO_2, but only H_2 and CH_4. CO_2 could not be quantified simultaneously to CH_4 and H_2 because of using argon as carrier gas in the GC. Individual CO_2 analysis with helium as carrier gas was done sporadically and CO_2 concentration was always around 5%.

BES concentration was measured in medium samples that were serially diluted, filtered (0.22 μm) and analyzed with ion chromatography Dionex ICS-2000 (RFIC) with an Ultimate 3000 Autosampler Column Compartment, a column IonPac AS18 and a pre-column IonPac AG18 (ThermoScientific, USA).

2.4. DNA extraction

Samples were obtained from the anode and the cathode at the end of each experimental period with different BES concentrations. The anode graphite fibers were rinsed with 1 mL of sterile MilliQ water to remove residues from the growth medium or residues from biofilm and then were cut and combined for DNA extraction. The same process was applied for the cathode carbon cloth. The medium was also sampled at the end of the period with 50 mM of BES. For this, 1.3 L of the MEC medium was centrifuged at 10000 *g* (Beckmann Coulter TM, Avanti J20XP; USA) to remove supernatant. Total DNA was extracted from approximately 0.15 g of samples using a PowerBiofilm DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. Quality and quantity of the DNA were measured using a NanoDrop® spectrophotometer (ThermoScientific). Moreover, DNA was visualized under UV in a 0.7% gel electrophoresis with TBE 0.5× (Tris-Borate 50 mM; EDTA 0.1 mM; pH 7.5–8).

2.5. Quantitative real-time PCR

Quantitative hydrolysis probes based real-time PCR (qPCR) was used to quantify total *Archaea*, the hydrogenotrophic methanogen order *Methanobacteriales* (MBT) and the exoelectrogen proteobacteria *Geobacter* as a member of the Fe(III)-reducing *Geobacteraceae* family.

qPCR was performed with a Lightcycler 480 instrument (LC480; Roche) using the corresponding primers and probes previously described [21–23] (Table 1). Each reaction mixture of 20 μL was prepared using the LightCycler 480 Probe Master kit (Roche Diagnostics), primers for *Archaea*