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Long-term continuous production of H_2 in a microbial electrolysis cell (MEC) treating saline wastewater

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ABSTRACT

A biofilm-based 4 L two chamber microbial electrolysis cell (MEC) was continuously fed with acetate under saline conditions (35 g/L NaCl) for more than 100 days. The MEC produced a biogas highly enriched in H_2 (≥90%). Both current (10.6 ± 0.2 A/m^2_{Anode} or 199.1 ± 4.0 A/m^3_{MEC}) and H_2 production (201.1 ± 7.5 $L_{H2}/m^2_{Cathode}\cdot d$ or 0.9 ± 0.0 $m^3_{H2}/m^3_{MEC}\cdot d$) rates were highly significant when considering the saline operating conditions. A microbial analysis revealed an important enrichment in the anodic biofilm with five main bacterial groups: 44% *Proteobacteria*, 32% *Bacteroidetes*, 18% *Firmicutes* and 5% *Spirochaetes* and 1% *Actinobacteria*. Of special interest is the emergence within the *Proteobacteria* phylum of the recently described halophilic anode-respiring bacteria *Geoalkalibacter* (unk. species), with a relative abundance up to 14%. These results provide for the first time a noteworthy alternative for the treatment of saline effluents and continuous production of H_2.

1. Introduction

A microbial electrolysis cell (MEC) is a well established microbial electrochemical technology (MET) used to produce high amounts of H_2 (Logan et al., 2008). A MEC profits from the activity of anode-respiring bacteria (ARB) embedded in anodic biofilms to break down the organic matter and harvest electrons that are chemically used at the abiotic chemical cathode to produce H_2. Nevertheless, the use of a biotic cathode is also possible as recently reported by Batlle-Vilanova et al. (2014) on the comparison of abiotic and biotic cathodes for microbial electrolysis of H_2. Usually, most of the MEC tests are performed using fresh wastewaters (*i.e.*: non saline) as anodic solutions that allow the successful development of electroactive biofilms from domestic wastewater (WW). Consequently, the application of such developed biofilms in MECs is mainly limited to the treatment of domestic WW. A successful treatment of other types of effluents such as saline WW which are significantly produced by the seafood, petroleum and leather industry will require the development of specific biofilms capable to work under such halophilic conditions. The enrichment of moderate halophilic biofilms composed of ARB could provide an alternative for the treatment of saline WWs which represent 5% of the WW generated worldwide and for which their treatment is usually limited by technical or economic constraints (Lefebvre and Moletta, 2006). However, another issue that emerges from an extensive overview of the literature on MECs (Logan et al., 2008) is the lack of studies working under saline conditions. Moreover, real WW treatment will require continuously operated MECs that will make necessary the development of a well attached-mature biofilm as an essential prerequisite for long-term operation of ARB biofilm-based METs.

Although the scalability of the MEC technology is of the main interest within the MET-related scientific community only a few attempts have been done to bring the MEC technology from the bench-via the pilot-to the full-scale size. In this context it is worth mentioning the works by Escapa et al. (2015), Gil-Carrera et al. (2013), Heidrich et al. (2013, 2014) who have very recently done significant attempts to scale-up the technology by operating 100 L size MECs fed with real domestic WW. Independently of the drawbacks encountered by these two engineering research groups, they have shown for the first time that the MEC technology is finally capable of converting low strength domestic WW to produce

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a gas stream highly enriched in hydrogen (~100%) and most importantly at very similar operational conditions as the ones of conventional WW treatment plants.

The aim of the present work was to operate a stable biofilm-based MEC in continuous mode for more than 100 days under saline conditions. A mature and moderate halophilic anodic ARB biofilm was first enriched from saline anoxic sediments under potentiostatic control during three acetate fed-batch cycles and then, operated under continuous mode. The long-term operation corroborated not only that a mature biofilm had well developed on the anode but also it confirmed the overall stability of the system. At the end of the experiment, the anode was removed and 1) the microbial composition by 16S rDNA sequencing and 2) biofilm attachment by scanning electron microscopy were analyzed. To the best of the authors' knowledge, this is the first work reporting such a long-term performance of a continuously operated MEC under saline conditions.

2. Materials and methods

2.1. General conditions

All chemicals were of analytical or biochemical grade and were purchased from Sigma-Aldrich and Merck. If not stated otherwise, all potentials provided in this manuscript refer to the SCE reference electrode (KCl 3.0 M, +240 mV vs. SHE, Materials Mates, La Guilletière 38700 Sarcenas, France). All media preparations were adjusted to pH 7.0. Bioelectrochemical experiments were conducted under potentiostatic control and incubations were performed at 37.0 °C.

2.2. Metabolite and biogas analysis

The model substrate was acetate and its concentration was determined by liquid injection into a gas chromatograph (Clarus 580 GC, PerkinElmer). Biogas composition (CH_4, CO_2, H_2 and N_2) was determined using a gas chromatograph (Clarus 580, Perkin Elmer) coupled to thermal catharometer detector, as described elsewhere (Quéméneur et al., 2012). The biogas was periodically analyzed for its H_2S content using a second gas chromatograph (Clarus 480, Perkin Elmer) equipped with an RtUBond-SiOH column and a molecular sieve (RtMolsieve). Operating conditions were as follows: the carrier gas was helium at a pressure of 250.0 kPa under a flow rate of 4.0 mL min^{-1}; temperature of the injector and the detector was fixed at 150.0 °C. Throughout the manuscript the average gas composition is reported. During Phase II the gas was daily sampled. During Phase III the gas was sampled before increasing/changing the substrate concentration. Averages and standard deviations are calculated taking into account two samples at each point. More details regarding the follow-up of gas production are given in Fig. S5 and S6.

2.3. Origin of inoculum

Usually, MEC studies make use of domestic wastewater (*i.e.*, NaCl concentrations $\leq$ 1.0 g/L) as source of microorganisms. With the objective to operate the MEC under moderate saline conditions (e.g., 35.0 g/L NaCl), saline sediments (10.0% w/v) were used as inoculum (pH of 7.8 ± 0.2, 36.1 ± 3.5 g of volatile solids per gram of sediments and a value of conductivity of 93.6 ± 12.1 mS/cm). Sediments were collected in a lagoon receiving wastewaters from a salt factory (Salins de Saint Martin, Gruissan, France: 43° 5′ 34.23″, 3° 4′ 57.47″) and stored at room temperature as reported elsewhere (Pierra et al., 2015).

2.4. Growth medium during fed-batch and continuous operation

A specific saline growth medium (35.0 g/L NaCl), namely modified-Starkey medium and previously developed for the selection of fermentative bacteria, was used throughout the study to promote the growth of electroactive biofilm on the electrode surface (Rafrafi et al., 2013). This medium was used since it has been previously utilized in our research group for successful development of electroactive biofilms under saline conditions (Carmona-Martínez et al., 2013).

Modified Starkey medium contained the following (per liter of tap water): 35.0 g NaCl, 0.5 g K_2HPO_4, 2.0 g NH_4Cl, 7.6 g MES buffer, 0.2 g Yeast Extract, 1 mL of oligo-elements solution (Carmona-Martínez et al., 2013) and different COD concentrations as fed acetate (0.64–6.42 g COD/L) of acetic acid as electron donor (see also Table S2). Although the model substrate used here was acetate, real saline WW is characterized for its high content of organic matter (Lefebvre and Moletta, 2006). The treatment of such WW in a MEC might follow conventional degradation steps: hydrolysis, acidogenesis, acetogenesis and finally, electron harvesting by halophilic ARB. During fed-batch chronoamperometric biofilm growth, the medium was supplemented with 50.0 mM of 2-(N-morpholino) ethanesulfonic acid (MES) to buffer the pH at around 7.0. For continuous MEC operation the medium inlet was adjusted at pH 7.0 with 1 M NaOH. Either during fed-batch or continuous operation, pH of the effluent slightly increased and remained relatively neutral (7.4 ± 0.2).

2.5. Bench scale 4L microbial electrolysis cell (MEC) configuration

A scheme of the unstirred up-flow microbial electrolysis cell (MEC) is presented in Fig. 1. The MEC body was a polycarbonate cylinder constituted of several parts (from outside to inside): (1) a water jacket (20.0 cm diameter and 30.0 cm height) used to keep a constant temperature of 37.0 °C (see cross-section and top view in Fig. 1); (2) the MEC body (16.0 cm diameter and 30.0 cm height) that contained both anode and cathode, had a total volume of 6.0 L and a final working volume of 4.0 L (*i.e.*, after placing electrodes). As anode material, a flexible 1 cm thick piece of graphite felt (12.0 cm diameter and 20.0 cm height) with a projected surface area of 754.0 cm^2 (grade RVG 4000, Mersen, France) was used. The graphite felt was slightly compressed and cylinder-shaped by a welded grid of titanium as electron collector, with two wires piercing the upper part of the MEC body to allow the connection with the potentiostat. An anionic exchange membrane (AEM) was placed between anode and cathode, as shown in Fig. S1 (FAA-PK, FuMA-Tech GmbH, Germany). The presence of a membrane in a MEC avoids H_2 recycling at the anode. Furthermore, the use of a AEM usually leads to a better electrochemical performance in comparison to other ion exchange membranes (Rozendal et al., 2008). As cathode material (188.0 cm^2 of catholyte exposed surface area), an empty cylindrical stainless steel 254SMO tube with an external diameter of 2.1 cm was located at the center of the MEC (Flowell, France). Through the cathode, a 2.0 mm diameter hole was drilled at the top of the electrode to collect the biogas. The volume of biogas was daily measured by displacement of water in a graduated column. Additionally, a SCE reference electrode was used to monitor a constant applied potential of +200.0 mV (+444.0 mV vs. SHE) at the anode.

2.6. MEC start-up and operation: phase I, II and III

At all times the anode potential was constantly fixed at +200.0 mV vs. SCE (+444.0 mV vs. SHE) instead of adding a specific voltage to the MEC circuit (see please Appendix). During