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The source of inoculum plays a defining role in the development of MEC microbial consortia fed with acetic and propionic acid mixtures

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ABSTRACT

Microbial electrolysis cells (MECs) can be used as a downstream process to dark fermentation to further capture electron in volatile fatty acids that remain after fermentation, improving this way the viability of the overall process. Acetic and propionic acid are common products of dark fermentation. The main objective of this work was to investigate the effect of different initial concentrations of a mixture of acetic and propionic acids on MECs microbial ecology and hydrogen production performance. To link microbial structure and function, we characterized the anode respiring biofilm communities using pyrosequencing and quantitative-PCR. The best hydrogen production rates (265 mL/d/$L_{reactor}$) were obtained in the first block of experiments by MEC fed with 1500 mg/L acetic acid and 250 mg/L propionic acid. This reactor presents in the anode biofilm an even distribution of *Proteobacteria*, *Firmicutes* and *Bacteroidetes* and *Arcobacter* was the dominant genera. The above fact also correlated to the highest electron load among all the reactors. It was evidenced that although defined acetic and propionic acid concentrations fed affected the structure of the microbial consortia that developed at the anode, the initial inoculum played a major role in the development of MEC microbial consortia.

1. Introduction

Hydrogen is recognized as an impermanent renewable energy carrier of the future with many advantages such as the high-energy yield of 122 kJ/g (Kapdan and Kargi, 2006). Although hydrogen is produced from natural gas, oil or coal, the applied techniques are not sustainable because they cause CO_2-emission and are based on non-renewable energy sources. Biological production of hydrogen via dark fermentation has been widely studied (Cheng et al., 2001; Fang et al., 2001; Hallenbeck and Ghosh, 2009). Volatile fatty acids (VFAs) are common products of dark fermentation (Kapdan and Kargi, 2006). Microbial electrolysis cells (MECs) can be used as a follow-up process to dark fermentation to transform the VFAs in further hydrogen, improving by this way the viability of the overall process. In both microbial fuel cell (MFC) and MEC the organic matter is decomposed, by the microorganisms present at the anode, into CO_2, electrons, and protons. In a MFC the electrons pass through a circuit to the cathode, and oxygen is combined with the protons to form water generating energy. In MECs, the application of a low potential (>0.2 V), leads to water/protons being reduced to hydrogen at the cathode. In both cases, microorganisms transfer the electrons directly to the electrode instead of a terminal electron acceptor.

The inoculum and operational conditions immensely affect the community composition in MECs. For example, using anaerobic sludge as the inoculum (Jung and Regan, 2007; Torres et al., 2009), showed that the anode biofilm community of MEC was dominated by phylotypes similar to *Geobacter sulfurreducens*, whereas a more diverse community was present when digester sludge was used as inoculum, with relatively fewer numbers of *Geobacteraceae* (Chae et al., 2009). Miceli et al. (2012) showed that *G. sulfurreducens* were not the only efficient anode respiring bacteria (ARB) in inocula from diverse locations, and that biofilm diversity was not directly correlated to high current densities. Some other studies documented the presence of *Pseudomonas* and *Rhodopseudomonas* at a higher abundance than the *Geobacter* species (Xing et al., 2009). It was also observed that when the anodes, fed with acetate as the electron

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donor, were transitioned from MFCs to MECs, the MEC communities changed, resulting in less microbial diversity due to a loss of facultative microorganisms and an increase in phylotypes with high similarity to *G. sulfurreducens* (Chae et al., 2008; Kiely et al., 2010). This is understandable, as the anaerobic *Geobacter* populations have a selective advantage in MECs compared to MFCs due to the lack of oxygen intrusion into the system. Community profiling using clone libraries targeting the 16S rRNA gene revealed that large populations of fermenting microorganisms (*Firmicutes*) and smaller populations of *Geobacteraceae* were present in anode respiring biofilms of MFCs when supplied with propionate (Chae et al., 2009; Jang et al., 2010).

Since anode respiring reactions in MECs can be completed through direct oxidation by ARB or syntrophic interactions among microorganisms in the biofilm consortia, examining the microbial community structures of anode respiring biofilms is necessary to understand the complex interactions occurring at the anode (Torres et al., 2010). This understanding of the microbial community structure specially when linked to function is critical to improve the design and operation of scale-up systems. The feasibility of MECs as a coupled system with dark fermentation will depend on the presence and abundance of microorganisms that can metabolize VFAs to either produce hydrogen and acetate (fermenters) or by transferring electrons to the anode (ARB).

Until now, most MEC studies have focused on microbial community characterization when a variety of single substrates, such as formate, ethanol, acetate, propionate, butyrate, succinate, lactate, glucose, or cellulose are used (Chae et al., 2009; Cheng et al., 2001; Jung and Regan, 2007; Kiely et al., 2011; Parameswaran et al., 2009, 2010; Xing et al., 2009; Liu et al., 2012). Nevertheless, hydrogen production rates and microbial community structure of MECs have not been extensively studied in the presence of defined volatile fatty acid mixtures such as acetate and propionate in specific proportions. The objective of this study was to characterize the performance and structure of anode respiring biofilm communities in MECs, operated at different initial concentrations of a mixture of acetic and propionic acids.

2. Materials and methods

2.1. MECs configuration and operation

Seven MECs were constructed using a two-chamber (H type) configuration. The anode and cathode chambers had each 450 mL. The chambers were separated by an anionic exchange membrane (AEM) (AMI 7001, Membranes International, Glen Rock, NJ). The cathode electrode was made of carbon paper with Pt (5 cm × 5 cm, 0.5 mg Pt/cm^2, ElectroChem, Inc., Woburn, MA). Carbon paper without wet proof (5 cm × 5 cm, TorayTM, ElectroChem, Inc., Woburn, MA) was used as anode. The anode of each reactor was inoculated using a (75:25) mixture of municipal wastewater from the wastewater treatment plant of Campus Juriquilla, UNAM and sodium acetate (20 mM) in a 50 mM phosphate buffer solution and vitamins and minerals (Lovley and Phillips, 1988). The catholyte was a 50 mM phosphate buffer solution. MECs were operated in fed-batch mode (cycles of 24 h) at room temperature (18–22 °C). Once inoculated the MECs, several mixtures of acetic acid and propionic acid at different concentrations were used to evaluate the effect of both acids in the hydrogen production in the cathode and the anode microbial community. A voltage of 0.6 V was applied using a power source (GWInstek, model GPS-4303). The experiments were carried out in two separate blocks performed at different periods of time using the same source of inoculum, but collected from the plant at a different time in the year (the first block was performed on September and the second one on December). Each block was composed of seven experiments, giving a total of 14 experiments arranged in a Central Composite Design (CCD). At the end of each operational cycle, the volume of gas produced at the cathode of the MECs was measured in an inverted cylinder filled with water. The gas composition (hydrogen, methane and carbon dioxide) was analyzed using a gas chromatograph (model SRI 8619C) as described by Hernández-Mendoza and Buitrón (2014). The average hydrogen production rate obtained during seven cycles (48 h) of operation of the MECs. The oxidation–reduction potential (Endress + Hauser, Orbipac CPF82, Ag/AgCl reference system) measured in the anodic chambers was between −193 and −200 mV. Table S1 shows the different concentrations supplied to the anode chamber of the MECs according to the CCD.

2.2. DNA extraction

At the end of each block of experiments, the entire biofilm formed on the surface of the anode was scraped using a sterile pipette tip. DNA was extracted using an Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories, Inc.) according to the manufacturer's instructions. The quality and quantity of the extracted DNA were verified by measuring absorbance at 260 and 280 nm using a Nanodrop spectrophotometer. Dilutions of the extracted DNA were prepared in order to compare the different tests made using quantitative real-time PCR (the dilutions were prepared in order to have 5 ng/μL of DNA in each sample).

2.3. Pyrosequencing and sequence analysis of bacterial DNA from anode biofilms

Extracted anodic biofilm DNA samples were sent to the Research and Testing Laboratories (TX, USA) for 454 Titanium sequencing (Sun et al., 2011). V2–V3 regions of 16S rDNA were amplified using 104 F and 530 R primers. A total of 129,182 sequences were generated for 14 samples. Sequence analysis was performed using Quantitative Insights into Microbial Ecology (QIIME) suite 1.6 (Caporaso et al., 2010). Sequences that were shorter than 250 bps or had primer mismatches, average quality score of 25, and homopolymers of 6 bps were excluded. Operational taxonomic units (OTUs) were selected at 97% sequence similarity using Uclust (Edgar, 2010) and the most abundant sequence of each cluster was chosen as the representative sequence. The representative sequences were aligned using PyNAST alignment tool to Greengenes database (Caporaso et al., 2010; DeSantis et al., 2006). Chimeric sequences were identified and removed using Chimera Slayer (Haas et al., 2010) then taxonomy was assigned to the sequences using RDP aligner at 80% confidence level (Wang et al., 2007). A biom-formatted OTU table was built for downstream analysis (McDonald et al., 2012). OTUs with less than 2 were removed prior to downstream analysis. All samples were rarefied to 2500 sequences in order to reduce heterogeneity in the comparison. A Newick-formatted phylogenetic tree was built using FastTree (Price et al., 2010). For alpha diversity, the richness of each sample was estimated using the Chao1 index (Chao, 1987), the diversity using the Shannon index (Shannon, 1984), the Phylogenetic Distance Whole Tree (Faith, 1992) metrics, and the evenness with the equitability metrics. To assess how the diversity compares between samples (beta diversity), weighted and un-weighted unifrac metric (Lozupone and Knight, 2006) were used. The sequence data were deposited into the NCBI Sequence Read Archive under the accession numbers SAMN02389562 to SAMN02389575 (Table S1).

2.4. Quantitative real-time PCR analysis

Quantitative real-time PCR (qPCR) was used to quantify 16S rDNA copy number of Bacteria, Archaea, *Geobacteraceae*, two