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\textit{anode}) 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.

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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\textsubscript{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\textsubscript{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 phylogenotypes 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...
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 phytophagous 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/cm², 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 (GWNstek, model GPS–4303). The experiments were carried out on 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 (QIME) 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, 1948), 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
hydrogenotrophic methanogens orders *Firmicutes* and *Firmicutes* (MBT) and *Methanomicrobiales* (MMB), two aceticlastic methanogen families *Methanosarcinaeaceae* (MSC) and *Methanosaetaceae* (MST) within the order *Methanosarcinales*. These three orders have been found to cover the majority of methanogens in bioelectrochemical systems (Chae et al., 2010; Lee et al., 2009; Parameswaran et al., 2010). Homo-acetogenic bacteria were quantified by targeting the gene encoding for the formyltetrahydrofolate synthetase (FTHS), a conserved gene involved in their CO₂ fixation pathway. All the tests were performed with Eppendorf Realplex 4S real-time thermal-cycler using a 20 μL total volume reaction. For SYBR green, each reaction contained: 8.6 μL of PCR grade water, 10 μL of SYBR green mix (TakaRa Bio Inc., Japan), 0.2 μL of forward and reverse primers and 1 μL of DNA template, whereas for TaqMan assays, each reaction was performed using: 6.94 μL of PCR grade water, 10 μL of TAQ PCR supermix, 0.06 μL TAQMAN probe, 1 μL of forward and reverse primers and 1 μL of DNA template. Assay details, primers and probes used are summarized in Table S2. All tests were performed as described by Parameswaran et al. (2010).

### 3. Results

#### 3.1. MEC performance

During all the experiments hydrogen content in the gas was comprised between 80% and 98% and no methane was detected. Two different blocks of experiments were performed at different periods using the same source of inoculum, but collected from the wastewater treatment plant at a different time in the year (Table S3). The best H₂ production rates were obtained in the first block of experiments. The highest H₂ production value was 265 mL/d/Lreactor. This was achieved by the MEC (A1500/P250C1) fed with 1500 mg/L acetic acid and 250 mg/L propionic acid, followed by MEC A1843/P325 with a H₂ production value of 256 mL/d/Lreactor. In the second block of experiments, the highest H₂ production was also achieved with a mixture of acetic acid and propionic acid of 1500 mg/L and 250 mg/L (A1500/P250C6), respectively, but this time the maximum H₂ production was only 187 mL/d/Lreactor. The highest H₂ production rates were reported in MECs A2000/P250 and A950/P250. The H₂ production rates obtained in our study are higher than those obtained using a similar system configuration, but with acetic (2.2 mL/d/Lreactor) and propionic (11.9 mL/d/Lreactor) acids provided as single substrates (Sun et al., 2008, 2010), suggesting a synergistic effect provided by the mixtures of acetic and propionic acids used in our study, a better inoculum than the one used by Sun et al. (2008, 2010), or a combination of both. Blank tests demonstrated that no hydrogen production was observed when the MEC were run only with mineral media. However, when VFA were added to the MEC, hydrogen production was observed. CD values varied from 0.29 to 2.91 A/m², with a maximum value of 2.35 A/m², when 1500 mg/L acetic acid and 250 mg/L propionic acid were the substrates provided (Table S3). Those results are in accordance with the H₂ produced in each MEC and the acids uptake by microorganisms. Hydrogen recovery, Rₜₜ, evaluated as the percentage of substrate used in producing hydrogen on the cathode, was calculated as proposed by Ditziga et al. (2007). The Rₜₜ varied from 9% to 40%, and the highest values were obtained at the maximum hydrogen production rates for each inoculum used.

#### 3.2. Microbial ecology

We obtained after rarefaction to 2500 sequences per sample a maximum number of operational taxonomic units (OTUs) of 533 for A1500/P250 (C1) and a minimum of 82 OTUs for A1500/P250 (C5) (Table S3). The total number of OTUs estimated by Chao1 estimator for samples with the best H₂ production were 469.16 ± 42.13 (A1843/P325), 968.37 ± 50.71 (A1500/P250C4), 558.59 ± 64.86 (A1500/P250C5) and 632.92 ± 33.44 (A1500/P250C6), while for samples with the lowest H₂ production were 551.38 ± 64.26 (A2000/P250) and 374.76 ± 58.05 (A950/P250). Furthermore, we estimated the Shannon diversity index of each sample and we found that A1500/P250C1 had the highest diversity (Shannon = 7.43 ± 0.02 and Simpson = 0.98) and also the best H₂ production rate. Sample A950/P250 presented the lowest H₂ production rate and had the 2nd lowest diversity (Shannon = 2.10 ± 0.07 and Simpson = 0.39). This indicates that for our system H₂ production rate was somehow positively correlated with microbial diversity.

As seen in Fig. 1, at the phyla level, the first set of experiments (with the exception of A1843/P325) had generally even distribution of three major phyla, *Proteobacteria, Firmicutes* and *Bacteroidetes*. *Synergistetes* and *Tenericutes* were less abundant than the other three phyla. For the second set of reactors, the majority of the population belonged to *Proteobacteria*, followed by *Firmicutes* and *Bacteroidetes*. *Synergistetes, Tenericutes*, and *Spirochaetes* were also found in a lesser percentage. *Synergistetes* were more abundant in the first set of reactors than in the second set. As seen in Fig. 2A from the *Proteobacteria* phylum, *Rhodocyclales* and *Campylobacteriales* dominated the second set of reactors and *Campylobacteriales* only dominated A1843/P325 in the first set. During the two experimental stages the inoculum source was the same (wastewater feed to a treatment plant), however, because the two experimental stages were run in different months (September and December), because...
the water treated in the plant that provided the inoculum treats industrial-type waste, some conditions in the wastewater such as temperature and water composition vary from month to month, resulting in the enrichment of these two specific orders from Proteobacteria. Proteobacteria species are metabolically diverse and can dominate wastewater treatment plants (Yang et al., 2011), therefore it is very possible that the inoculum used for the sets of experiments, had Proteobacteria in different abundance and compositions. Rhodocyclales and Campylobacterales have been detected before in wastewater and MFCs studies. In particular, Rhodocyclales isolated from activated sludge showed metabolic versatility by oxidizing many fatty acids such as propionate and butyrate as well as aromatic hydrocarbons (Hesselsou et al., 2009), can reduce many electron acceptors including nitrate (Ontiveros-Valencia et al., 2013), and have been shown to dominate anode chambers of MFCs fed with acetate (Borole et al., 2011). Members of Campylobacterales have been also shown to thrive as a response to acetate amendments (Handley et al., 2012), and we have detected them previously in reactors with multiple acceptors, under sulfur cycling conditions (Zhao et al., 2013). Clostridiales and Bacteroidales were the main orders from Firmicutes and Bacteroidetes, respectively, in all reactors (Fig. 2B).

The abundance of orders in the Proteobacteria differed immensely among the first and second set of reactors. Besides A1843/P325, the first set of reactors was dominated by 3 orders of Deltaproteobacteria (Desulfobacterales, Desulfovibionales and Desulfomonadales) (Fig. 2A). The most abundant genera (>1%) of first set of reactors and second set of reactors are illustrated in Fig. 3A and B, respectively. Arcobacter and Azovibrio dominated the second set of reactors while Arcobacter was solely the dominant genera of A1843/P325 (from the first set). Aciinetobacter was more abundant in A1106/P195 from the first set and in A1500/P360 and A2000/P250 from the second set in comparison to other reactors. Desulfovibrio, Desulfoabibus, and Desulfomicrobium were more abundant in the first set of reactors in comparison to second set; however, they were not completely diminished in the second set of reactors. Geobacter was not one of the most abundant genera among all in all reactors (0.01–2.00%); nevertheless, it was present in all reactors. Genera from Firmicutes, Bacteroidetes and Synergistetes were mainly unclassified (Fig. 3B) besides Parabacteroides and vadin CA02. Parabacteroides was one of the most abundant phylotypes at the genera level in the first set of reactors.

Finally, to assess the B diversity (diversity compared between samples), we performed unifrac analysis. It was found that samples naturally are separated in two groups, each containing samples from a different inoculation date (Fig. 4). Here, we can appreciate how the samples can be cluster together depending of the date of inoculation, the upper data represent the samples from the first set of experiments (experiments performed in September) and the bottom data correspond to the samples from the second set of experiments (experiments performed in December). Strengthening what was observed also in the reactors performance. To identify possible clusters between samples, weighted and un-weighted principal coordinates analysis (PCoA) were performed from jack-knifed OTU tables. A PCoA generated with weighted unifrac analysis is presented in Fig. S1. This PCoA also shows that the inoculum source was a more critical variable to determine the community structure than the substrate concentration or type.

3.3. Presence of homo-acetogens, Geobacteraceae and Archaea population in biofilm samples

We confirmed the presence of homo-acetogens, Geobacteraceae and Archaea (Fig. 5), three important groups of microorganisms previously detected in MECs (Parameswaran et al., 2010) and involved in important syntrophic relationships (Parameswaran et al., 2011, 2012). Homo-acetogens were detected and quantified with qPCR targeting the FTHFS gene. The highest number of FTHFS copies was recorded for A1500/P250C2 with a value of 3.84 × 10^4 gene copies/cm^2, while the lowest value was only 7.8 × 10^2 gene copies/cm^2.
copies/cm² in sample A1843/P195. Sample A1500/P250C5 presented the highest number of Geobacteraceae sp. 16S rRNA gene copies/cm² with a value of $3.65 \times 10^7$ while the lowest value was obtained in sample A1843/P325 with $5.69 \times 10^4$ gene copies/cm². **Fig. 5** shows that Geobacteraceae sp. gene copies are at least 2 orders of magnitude lower than general bacteria. Although these numbers are not absolute, this in general terms would indicate that Geobacteraceae sp. are in the order of 1% of the overall community, hence, all else equal, Geobacteraceae sp. would not show up as one of the abundant members of the community during pyrosequencing.

Finally, the Archaea population present in biofilm samples was quantified, as these can consume the acetate added like substrate or the H₂ produced in the anode chamber. Although anodes were exposed to air during 20 min at the end of each cycle of operation, hydrogenotrophic and acetoclastic methanogens were detected in all the samples. The number of copies of the Archaea 16S rRNA gene ranged from $6.88 \times 10^4$ gene copies/cm² (A1843/P195) to $1.96 \times 10^6$ gene copies/cm² (A1500/P250C1). *Methanosacetaceae, an acetoclastic methanogen* dominated the Archaea population (**Fig. S2**). Other hydrogenotrophic and acetoclastic methanogens such as *methylotroadiales*, *methanomicrobiales* and *methanosarcinaceae* were also present but less abundant in biofilm samples. Furthermore, *Methanomicrobiales* species were not detected in samples A1500/P250C4, A1500/P250C5 and A950/P250. The low hydrogenotrophic quantities found in the samples suggest that H₂ losses due to methanogens were controlled effectively; however,

**Fig. 3.** Distribution of the 10 most abundant genera of the anodic biofilm samples from the (A) first and (B) second set of experiments. *Azovibrio* or *Arcobacter* were found to be mostly abundant in the second set of experiments.
the presence of *Methanosacetaceae* indicates that methanogens were competing with ARB for acetate and decreasing the efficiency of these systems because some of the electrons in acetate were directed to methane formation. These results confirm that an effective method to control methanogenesis is necessary to further improve the system’s performance.

4. Discussion

This study demonstrated that the inoculum plays a major role in the development of MEC microbial consortia and that different microbial consortia develop depending on acetic acid and propionic acid concentrations fed in microbial electrolysis cells. Un-weighted unifrac analysis allowed us to clearly visualize that the inoculum source was a more critical factor determining community structure than the substrate concentration or type (Fig. 4). Our MECs performance results corroborated that the composition and diversity of the microbial consortia in anodic biofilms can significantly influence hydrogen production rates of MECs. H₂ production rates were higher during the first block of experiments, this generally correlated to more diverse ARB biofilms in all samples, excluding A1500/P250C5 which belonged to the second block of experiments, demonstrating the significance of functional redundancy and development of important syntrophic interactions among bacteria.

Pyrosequencing analysis revealed that reactors from the first set of experiments had an even distribution of *Proteobacteria*, *Firmicutes* and *Bacteroidetes* while the second set of experiments had *Proteobacteria* overwhelmingly dominant in each reactor. Overall, the first set of reactors had the highest hydrogen production rate, in particular reactors A1500/P250C1 and A1843/P325. These results suggest that even though microbial diversity is important for high hydrogen production rate, the presence and abundance of certain species also has an effect on hydrogen production rate. *Proteobacteria* and *Firmicutes* are well-known fermenters and syntrophic bacterial partners that can ferment volatile fatty acids, such as acetate, propionate and butyrate (Freguia et al., 2010; Kiely et al., 2011). *Actinobacteria* and *Bacteroidetes* have been associated with biofilm anodes, but with low fractions of total Bacteria which is in accordance with this study (Torres et al., 2009; Xing et al., 2009). *Synergistetes* was slightly more abundant in the first set of reactors than the second set of reactors; however, A1500/P250C4 and A1500/P250C6 had comparable levels to the first set of reactors. At the genera level, *Arcobacter* was the dominant genera in A1843/P325 and A2000/P250. These two reactors had the highest electron load among all the reactors (Table S3), *Arcobacter* are known to be fermentative and acidogenic bacteria which can be involved in the degradation of organic and amino acids to produce suitable substrates that can be metabolized by ARB for electricity generation or hydrogen production (Holt et al., 1994; Matthies et al., 2000; Tully et al., 1994).

The second set of experiments was overwhelmingly enriched with *Azovibrio* independent from hydrogen production rate. *Azovibrio* sp. has been identified as possible electro-chemically active Bacteria in previous studies with MFC and MECs (Freguia et al., 2010; Li et al., 2011; Mehanna et al., 2010); however, in this study it was the most abundant genera in the samples presenting both the highest and lowest hydrogen production rates (A1500/P250C4, A1500/P250C5 and A950/P250). *Desulfobulbus*, *Desulfovibrio* and *Desulfovibrio* were present in all reactors but were more abundant in the first set of experiments. *Desulfobulbus* sp. can oxidize propionate to acetate as the end product (Holt et al., 1994) hence, here they probably contributed to generate acetate which can be used by ARB. On the other hand, *Desulfovibrio* has not been

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**Fig. 5.** Distribution of *Geobacteraceae* sp., FTHFS and general bacteria in anode biofilms (gene copies reported are calculated based on the total area of electrodes (50 cm²)).
shown to grow using acetate or propionate (Thevenieau et al., 2007).
Even when the same acetic and propionic acid concentrations were used in some of the MECs that were inoculated with the same inoculum (for example A1500/P250C4, A1500/P250CS and A1500/P250CB), a different microbial consortia developed (Fig. S1). These differences could be due to a number of factors, such as the source of collection of the inoculum (even when the source of the inoculum was the same, the season was different generating some possible changes in the microbiota in the wastewater), instead of the volatile fatty acids concentrations. Previous studies have demonstrated the predominance of Geobacter species in biofilm anodes (Call et al. 2009; Jung and Regan, 2007; Parameswaran et al., 2009; Torres et al., 2009); however, in this study we found that some other bacteria like Arcobacter, Desulfiomicrobiurn, Desulfobulbus, Pseudomonas, Macromonas and Azovibrio were the most representatives and could be associated with hydrogen production in MECs.

Using qPCR analysis we observed that samples with the highest number of FTHS gene copies/cm² (targeting acetogens) were also the ones that had the highest number of Geobacteraceae gene copies/cm² suggesting a previously proposed syntrophic interaction between homoacetogens and ARB such as Geobacteraceae. However, we were not able to find a direct relationship between volatile fatty acid concentrations and the gene copies/cm² of FTHS and Geobacter sp. In addition, qPCR analysis indicated that methanogens were at low abundances in all biofilm anodes. Methanosarcinaaceae sp. dominated the Archaea population, representing a possible loss of acetic acid to produce trace amounts of methane (below detection limit). Although methanogens were detected, the amount of methane was not significant and can be explained by the operation of the cells. At the end of each cycle operation, the anodes were exposed to air when new substrate was added, causing inhibition to the majority of methanogens, limiting its proliferation and, in consequence, only low amounts of methane were produced.

The above findings indicate that in order to enhance hydrogen production rates, control strategies to select for robust microbial communities containing efficient anode respiring bacteria with appropriate microbial partners are required during inoculation and start-up of the cells.

Acknowledgements
This research was supported by the International Found of the Instituto de Ingeniería – UNAM, the CONACYT – Mexico (project 100298) and by NSF career award #1053939. The authors gratefully acknowledge the Swette Center for Environmental Biotechnologies at ASU by the support in the accomplishment of the microbiological analyses. We also wish to thank Dr. Pratap Parameswaran for his technical support and advice to enrich this work.

Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbiotec.2014.04.016.


