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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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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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