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ANALYSIS OF MICROBIAL DIVERSITY OF INOCULA USED IN A MICROBIAL FUEL CELL

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Abstract

A microbial fuel cell (MFC) is a device that converts chemical energy into electrical energy with the aid of biocatalytic reactions of electrochemically active bacteria (EAB) (Kim et al., 2003). A MFC can be used to enrich a microbial consortium using wastewater as the electron donor. Molecular techniques are now widely applied to assess the diversity of microbial communities by analyzing the 16S rDNA sequence (Phung et al, 2004). Since it has been suggested that different microorganisms are enriched in MFCs fed with different inocula, this research seeks to experimentally confirm this hypothesis by characterizing the bacterial communities in MFCs fed with sulphate-reducing inoculum and enriched inoculum.

The sulphate inoculum was cultured in a complete mix sulfate-reducing bioreactor fed a mixture of sucrose, acetic acid, and sodium sulfate. The enriched inoculum was obtained with serial transfers of a sediment sample cultivated in a medium containing ferric citrate as electrons acceptor and sodium acetate as electron donor. The biofilm formed on the carbon cloth electrodes from the anodes was used for DNA extraction according to specific literature. Total genomic DNA was used as template for PCR amplification of approximately 1500 bp of 16S rDNA with a forward and reverse primers. The PCR products were purified, cloned, transformed into competent cells of *E. coli* XL1-Blue as described by in literature. Tansformants were transferred to plates containing LB broth spiked with antibiotics and the plasmids were isolated and digested for the presence of inserts.

The bacterial population obtained from biofilm of the MFC loaded with sulphate-reducing inoculum and enriched inoculum was analyzed in terms of identity, abundance, phylum (class). *Clostridiales bacterium* was present in both inocula.

There was a significant difference in community composition between both inocula. *Clostridia* predominated in the community of the biofilm of the MFC fed with sulphate reducing inoculum, whereas in the the biofilm of the cell loaded with enriched inocula the predominant microbes belonged to *Deferribacteres* class.

Our results were similar to those observed in literature, with enriched electrochemically active bacteria in a MFC using glucose and glutamate (copiotrophic conditions); the enriched population consisted of *γ-Proteobacteria* (36.5%), followed by *Firmicutes* (27%) and *δ- Proteobacteria* (15%). Other researchers observed that the bacterial communities that develop in MFC show great diversity, ranging from primarily *δ-Proteobacteria*, that predominate in sediments MFCs to communities composed of α- , β-, *γ-* or *δ-Proteobacteria*, Firmicutes and uncharacterized clones in other types of MFCs. On the other hand *Geovibrio ferrireducens, Geovibrio thiophilus* and *Denitrovibrio acetiphilus* are known to contain c-type cytochromes. Current evidence suggests that a series of c-type cytochromes associated with the inner membrane, the periplasm, and the outer membrane might interact to transfer electrons to the outer membrane surface.

The bacterial population in the anodic biofilms of our cells was not as rich as found from other types of inocula. For instance, diversity given by Shanon-Weaver index was 1.27 and 1.38 and the species evenness given by Pielou´s evenness index was 0.66 and 0.71, for the sulphate reducing and enriched inoculum, respectively. These values mean that diversity of inocula was low and the evenness was low-to-moderate, respectively.