



Methodology

Simple and inexpensive DNA extraction protocol for studying the bacterial composition of sludges used in microbial fuel cells

B. Canto-Canché¹, M. Tzec-Simá¹, J.I. Vázquez-Loría¹,
H. Espadas-Álvarez¹, B.H. Chí-Manzanero¹, R. Rojas-Herrera²,
R. Valdez-Ojeda³ and L. Alzate-Gaviria³

¹Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, Mérida, Yucatán, México

²Universidad Autónoma de Yucatán, Facultad de Ingeniería Química, Mérida, Yucatán, México

³Unidad de Energía Renovable, Centro de Investigación Científica de Yucatán, Mérida, Yucatán, México

Corresponding author: B. Canto-Canché

E-mail: cantocanche@cicy.mx

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ABSTRACT. Bacteria oxidize organic matter and nutrients to produce electric energy in microbial fuel cells (MFC) - a technology of increasing importance because of its sustainability. To improve the performance of MFCs, it is necessary not only to gain a better understanding of MFC engineering designs, but also to improve the understanding of the composition of the microbial communities in MFCs. Fast and efficient DNA extraction protocols that are suitable for extracting diverse bacterial genomes are necessary to identify the bacterial diversity present in MFCs and to further monitor the dynamic changes of microbial communities. This study focused on testing different direct cell lysis protocols to extract DNA from a microbial sludge harvested

from an MFC. The protocol that achieved the best results was based on a previous study, but was modified by eliminating a chaotropic salt and the special columns used for nucleic acid purification. The efficiency of this less expensive and more straightforward protocol was confirmed by PCR amplification of the 16S rRNA gene and denaturing gradient gel electrophoresis analysis, which confirmed the extraction of multiple genomes. The sequences of 10 clones revealed the presence of phyla, Proteobacteria, Firmicutes and Actinobacteria, comprising both Gram-negative and Gram-positive bacteria. Some of these bacteria were identified at the genus level, e.g., *Clostridium*, *Pseudoxanthomonas*, *Tistrella*, and *Enterobacter*; these genera have been described in active sludges from wastewater treatment, supporting the congruency of our results. Therefore, this protocol is a useful tool for analysis of the bacteria responsible for energy production in MFCs.

Key words: DNA extraction; Bacterial consortium; 16S rRNA; Microbial fuel cell; Inexpensive DNA protocol; Electrical energy