Analysis of microbial communities associated with hydrogen production obtained from wastewater

Chacón-Carrera, R.A.[1], Zavala-Díaz de la Serna, F.J.[1], Ramos-Sánchez, V.H.[1], Camacho-Dávila, A.[1], Robles-Venzor, J.C.[1].

(1)Universidad Autónoma de Chihuahua, Facultad de Ciencias Químicas, Nuevo Campus Universitario s/n, Chihuahua, Chih. C.P.31125,
México. Tel: 6142366000 Ext.4298 email: vramos@uach.mx

ABSTRACT

Microbial Fuel Cells (MFCs) are devices capable to supply energy from organic substrates. Although MFCs offer low current densities, they have proven to be a feasible option for wastewater treatment. When a suitable overpotential is applied to MFCs, hydrogen is produced, these devices are known as Microbial Electrolysis Cells (MECs). México has a huge potential for solar energy, particularly Chihuahua exhibits a solar irradiance of 18kJ/m2. Bearing this in mind, this project aims to feed the overpotential for a MEC through a solar cell, in order to assess the potential to produce hydrogen through wastewater effluents in public universities. The microorganisms responsible of hydrogen production in these devices have not been fully characterized, as a matter of fact; the occurrence of microbial consortia able to optimize the operation of MECs is still under study. Therefore, at this stage of research, a wastewater sample was growth in differential cultures; and communities were compared by molecular techniques using a Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis (PCR-DGGE) in order to compare their electrochemical performance and hydrogen yield within a MEC.

Keywords: wastewater, MEC, PCR-DGGE

Sociedad Mexicana del Hidrógeno A. C.
Mexican Hydrogen Society
CONACYT
ITC

1. Introduction

The use of fossil fuels in recent years has accelerated the depletion of non-renewable resources. Furthermore, the unprecedented increase in greenhouse gas emissions due to combustion of fossil fuels is greatly contributing to the climate change.[1] 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. Although hydrogen is produced from natural gas, oil or coal, these methods are not sustainable due to CO2-emissions and exploitation of fossil fuels.[2] Among the technologies currently available for hydrogen production, biological methods are generally preferred over chemical and thermal methods, since organic wastes can be used as substrates. As a result, potential pollutants such as wastewater (WW) are now being regarded as potential commodities for bioenergy and biochemical production rather than useless residues.[3] A new alternative process for biohydrogen production is electrohydrogenesis, which uses exoelectrogenic bacteria in a microbial electrolysis cell (MEC).[4] MECs have attracted considerable attention over the last years as a promising technology for the production of biohydrogen on account of its great hydrogen yield compared to conventional hydrogen producing-dark fermentation.[5] In a MEC, microorganisms oxidize organic compounds at the anode to CO2, protons and electrons. At the cathode, protons conducted by an ion selective membrane, and electrons derived from the anode, together with those externally supplied, are combined to produce H2.[6] Within this cell, electrochemically active bacteria transfer electrons to the anode and protons are released to the aqueous media. In order to produce hydrogen at the cathode, a potential of at least -0.414 V is required under standard biological conditions.[7]

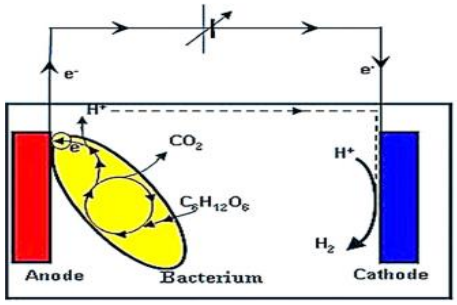


Figure 1. Schematic of typical single chamber MEC construction and operation [7]

It has been demonstrated that cathodic biofilms inoculated with microorganisms originated from wastewater treatment plants exhibit good electron transfer. Indeed the ability of some bacteria to transfer electrons as part of their bacterial respiration process has been exploited; such bacteria are known as exoelectrogens. To date, reported exoelectrogens include members from diverse bacteria genre, such as *Geobacter*, *Shewanella*, *Pseudomonas*, *Clostridium*, *Desulforomonas*, *Escherichia* and *Klebsiella*.[9] With the fast progress of molecular biological technologies in the last decade, some powerful tools, such as denaturing gradient gel electrophoresis of polymerase chain reaction (PCR-DGGE) and fluorescent *in situ* hybridization (FISH) etc., have been developed, and now it is possible to look into bacterial community structures without relying on time-consuming bias-bearing cultivation methods. To date, most studies of MECs and MFCs have investigated the microbial composition, using methods such as 16S rDNA sequencing and denaturing gradient gel electrophoresis [10].

México has a high level of solar radiation. It receives, for example, twice as much solar radiation as Germany. Across México, daily radiation varies between 4.4 kWh/m2 and 6.3 kWh/m2 of solar energy. Mexico's six northern Border States cover nearly half of the country's total surface. These states have a predominantly arid desert climate with high level of solar irradiation. Chihuahua is recognized as having one of the highest solar irradiation levels (18kJ/m2), which exhibits the potential to supply the abovementioned voltage required for operation of a MEC using photovoltaic panels.[8] Based on this geographical context, in the first stage of this research, wastewater was taken from a public university and then enriched culture media were prepared with salts in order to promote the growth of exoelectrogens microorganisms and to compare the communities using PCR-DGGE. In a second and final stage, not yet reported, the performance of a MEC inoculated with the culture media richest in exoelectrogens will be assessed to prove the feasibility to promote this sustainable wastewater treatment in Chihuahua's public sector.

2. Experimental

2.1 Sampling

Samples were collected from a septic tank in the Universidad Autonoma de Chihuahua. The samples contained water and also soil, pH (Table 1) was recorded to observe initial conditions.

Table 1.pH of wastewater samples

	Sample 1	*Sample 2*	*Sample 3*	*Sample 4*	*Sample 5*	*Sample 6*
pH	6.388	6.287	6.601	6.912	5.862	5.767

2.2 Preparation of enriched culture media.

To make enrichment culture, samples were cultured in a Winogradsky's column, which is a plastic container with one-third of the solid sample (sediment) and two-thirds of liquid sample (water). Two cultures were prepared with 3 g of $CuSO_4$ and the two remaining with 3 g of Na_2SO_4. The columns were kept under normal conditions of temperature and pressure during two months for further observation (Figure 2).

Figure 2: Enriched culture media.

2.3 DNA Extraction and PCR

Wastewater samples were filtered in order to collect as many organisms as possible. The filter was placed in a phosphate buffer solution pH 8. The DNA extraction was developed by the DNAzol protocol with previous modifications, while 16S rDNA was amplified using PCR technique with the 3R and 3F oligonucleotides. The mix used for PCR is described in Table 2.

The conditions in the thermocycler were a) denaturalization 5 min 94° C; b) amplification: 35 cycles, each one with a denaturalization step 94° C for 1 min, alignment step 55° C for 1 min and synthesis step 72° C for 1 min; d) the final extension step 72° C for 5 min.

Table 2. PCR Components for 18 samples with no dilution.

Component	Final Conditions
Reaction Buffer 10X	1X
$MgCl_2$ 50 mM	2 mM
Dntps 10 mM	0.4 mM
Primer F 10X	0.8 mM
Primer R 10X	0.8 mM
Taq pol	1.5 µL
DNA	5 µL
Water	12 µL

2.4 Denaturing gradient gel electrophoresis

In this case two solutions were prepared, one of a gradient of 100 % and other with a 0 % gradient. In Table 3, the components of each solution are shown.

Table 3. Components of the solutions for DGGE for 50 mL

	Solution A (100 %)	*Solution B (0 %)*
Urea	22.16 g	-
Formamide	20.00 mL	-
Polyacrilamide	26.65 mL	26.65 mL
TAE 50X	1.00 mL	1.00 mL

3. Results and discussion

3.1 Media Analysis

Table 4 shows the pH data of the enriched media culture. Data suggest that there is a significant difference between wastewater and wastewater in the culture enriched media. pH measurements show that the media rich in sodium are the most suitable for a MEC, according to Ya-Peng *et al* [11] that found an optimal pH of 9 for hydrogen production in MEC, which suggests that exoelectrogenic activity is favored at this pH.

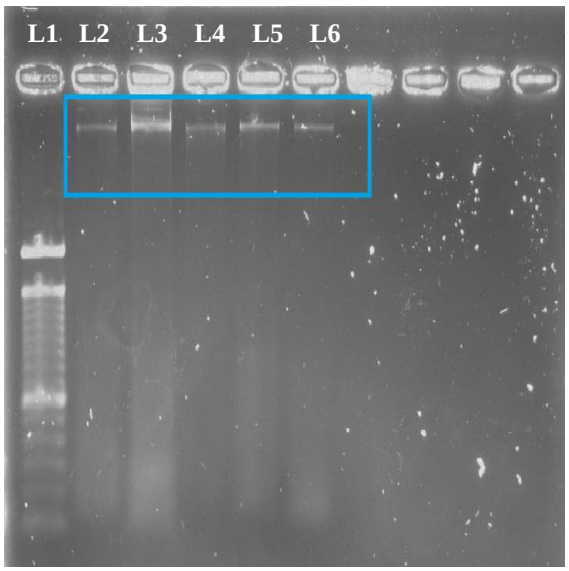
Table 4. pH of enriched media culture

	Media Na_2SO_4 *(1)*	*Media* $NasSO_4$ *(Bis)*	*Media* $CuSO_4$ *(1)*	*Media* $CuSO_4$ *(Bis)*
pH	8.831	8.861	7.356	7.683

3.2 DNA Extraction

Extraction was successfully accomplished in an agarose gel 1%. DNA was obtained of good size and quality, as shown in Figure 3.

Figure 3. DNA Extraction: In lane 1(L1) the molecular weight marker was placed; whereas samples were set in lanes 2 to 6 (L2-L6).



3.3 Polymerase Chain Reaction.

The amplification by PCR showed fragments of an approximate size of 300 pbs, which correspond to expected sizes. Figure 4 shows samples amplified before enriched media culture and those obtained from the enriched culture

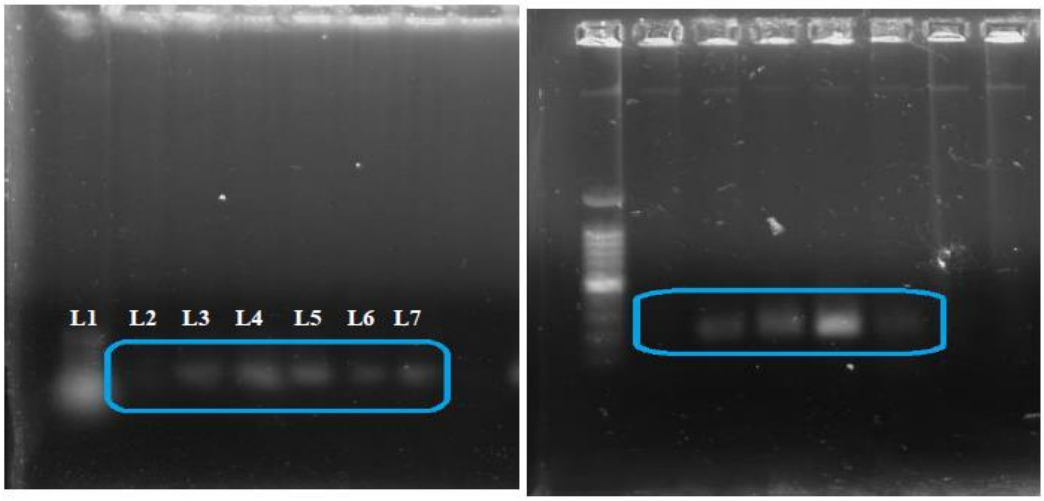


Figure 4. Amplification with sample distribution as in Figure 3, except lane 2 (L2) contains a negative control: *Left*: samples before enrichment; *Right*: samples after enrichment.

Summary and perspectives

The enrichment culture media showed that the most suitable media to use in a MEC is the one with sodium, also the conditions provided for the media are those reported as optimal for hydrogen production, which is an advantage for the following stage of this research.

The DNA extraction and amplifying by PCR is the first phase to make a comparison between microbial communities in wastewater and those communities favored in the enrichment culture media. This comparison is in process and will be developed by DGGE. Such analysis might indicate whether a variation among individuals is occurring or if there is a diverse population. The latter would favor isolation to achieve a suitable microbial consortium; whereas the former condition would require maintaining a suitable culture media to promote growth of exoelectrogenic species.

References

[1]Zhang, Y., Angelidaki, I. Microbial electrolysis cells turning to be versatile technology: recent advances and future challenges. J. Water Research 2014; 56: 11-25

[2]Ruiz, V., Esra, Z., Kang, D.,Krajmalnik, R., Buitrón,G.

[3]Escapa, A., Gil-Carrera, L., García, V., Morán, A. Performance of continuous flow microbial electrolysis cell (MEC) fed with domestic wastewater. Biosource technology 2012; 117: 55-62

[4]Chae, K., Choi, M., Kim, K., Ajayi, F. Chag, I., Kim, I. Selective inhibition of methanogens for the improvement of biohydrogen production in microbial electrolysis cell. International Journal of Hydrogen Energy 2010; 35: 13379-13386

[5]Croese, E., Jeremiasse, A., Marshall, Ian., Spormann, A., Euverink, G., Geelhoed, J., Stams, A., Plugge, C. Influence of setup and carbon source on the bacterial community of biocathodes in microbial electrolysis cells. Enzyme and Microbial techonology 2014; 61-62: 67-75

[6]Varroné, C., Van, J., Liu, W., Zhou, B., Wang, Z., Liu, W., He, Z., Wu, L., Zhou, J., Wang, A. Metagenomic-based analysis of biofilm communities for electrohydrogensis: from wastewater to hydrogen. International Journal of Hydrogen Energy 2014: 39; 4222-4233.

[7]Kadier, A., Simayi, Y., Sahaid, M., Abdeshanian, P., Abdul, A. A review of the substrates used in microbial electrolysis cells (MECs) for producing sustainable and clean hydrogen gas. Renewable Energy 2014: 71; 466-472.

[8]Romero-Hernández, S., Rodriguez-Granada, B., Romero-Hernández, O., Wood, D. Re-energizing the border: renewable energy, green Jobs and border infrastructure Project; solar energy potential in Mexico's northern border states. 2012. Mexico Institute. 1-2 pp.

[9]Croese, E., Jeremiasse, A., Marshall, I., Spormann, A., Euverink, G., Geelhoed, J., Stams, A., Plugge, C. Influence of setup and carbon source on the bacterial community of biocathodes in microbial electrolysis cell. Enzyme and Microbial Techonology

[10]Liu, X., Zhang, Y., Yang, M, Wang, Z., Wen, L. Analysis of bacterial community structures in two sewage treatment plants with different sludge properties and treatment performance by nested PCR-DGGE method. Journal of Environmental Sciences 2007: 19; 60-66

[11]Ya-Peng, L., Yung-Hai, W., Bai-Shi, W., Qing-Yung, Chen. Effect of anolyte pH and cathode Pt loading on electricity and hydrogen co- production performance of the bio-electrochemical system. International Journal of Hydrogen Energy 2014; 1-5