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Dark fermentation effluents exploited as inoculum and substrate for electricity production in microbial fuel cells

Efluentes de fermentación obscura aprovechados como inóculo y substrato para producción de electricidad en celdas de combustible microbianas

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Technological innovation: Utilization of organic matter effluents with bio-electrochemical systems technology.

Industrial application area: Treatment plants for industrial effluents from bioprocesses such as the brewing industry, dairy processing industry.

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Resumen

La vinculación directa de la fermentación obscura con las celdas de combustible microbianas (MFC) podría verse limitada por la variabilidad en la composición de los efluentes de fermentación oscura (DFE) y por el uso de un inóculo externo. En esta investigación se probaron dos tipos de DFE reales en tres series para determinar la viabilidad de usar directamente los efluentes como inóculo y sustrato en una MFC. En cada prueba se investigó la composición del DFE real, la relación inóculo-sustrato (ISR), la comunidad microbiana y la resistencia a la transferencia de carga en circuito abierto y cerrado. La mayor densidad de potencia (0.4 a 0.5 mW m^{-2}) se atribuyó al contenido de sólidos volátiles y a un DFE menos complejo con una ISR de 0.35 y 0.52. El porcentaje de abundancia relativa de microorganismos electroactivos fue solo del 30%, lo que indica la necesidad de un procedimiento de enriquecimiento de este tipo de microorganismos. Las mediciones de resistencia a la transferencia de carga fueron más precisas cuando las MFC estaban

en circuito abierto, y la distribución de resistencias reveló que el cátodo era el electrodo limitante. La vinculación directa de la fermentación oscura con la MFC fue factible; sin embargo, aún son necesarias mejoras que conduzcan al enriquecimiento de la comunidad electroactiva y a la disminución de la resistencia en el cátodo. Los resultados de esta investigación contribuyen al aprovechamiento de las aguas residuales procedentes de bioprocesos para producción de electricidad.

Palabras clave: Efluentes de fermentación obscura, Celdas de combustible microbianas, Comunidad microbiana, relación inoculo-substrato, Resistencia a la transferencia de carga.

Abstract

Direct linkage of dark fermentation with microbial fuel cells (MFCs) might be limited by the variability in the composition of actual dark fermentation effluents (DFE) and the use of an external inoculum. In this research two types of actual DFE were tested in three runs to determine the viability of the direct use of effluents as both inoculum and substrate in an MFC. The composition of the actual DFE, the inoculum-to-substrate ratio (ISR), microbial community, and charge transfer resistance in open and closed circuits were investigated in each test. The highest power density (0.4 to 0.5 mW m^{-2}) was attributed to the content of volatile solids and a less complex DFE with an ISR of 0.35 and 0.52. The percentage of relative abundance of electroactive microorganisms was only 30%, indicating a need for an enrichment procedure for this type of microorganism. Measurements of charge transfer resistance were more accurate when the MFCs were in open circuit, and the distribution of resistances revealed that the cathode was the limiting electrode. Direct linkage of dark fermentation with MFC was feasible; nevertheless, improvements leading to enrichment of the electroactive community and decrease of the resistance at the cathode are still needed. The results of this investigation contribute to the use of wastewater from bioprocesses for electricity production.

Keywords: Charge transfer resistance, Dark fermentation effluents, Inoculum-to-substrate ratio, Microbial community, Microbial fuel cell.

1. Introduction

Dark fermentation exploits a wide variety of biomass sources that microorganisms utilize to produce hydrogen. However, in dark fermentation, a maximum of one-third of the biomass is converted into hydrogen, and the remaining organic matter is converted to fermentation products such as volatile fatty acids and solvents [1]. In recent years, dark fermentation has been coupled to electricity production in microbial fuel cells (MFCs) to increase the energy yield from a biomass feedstock [2]. In addition to organic matter, dark fermentation effluents (DFE) offer a quantity of microbial biomass that can be exploited as an inoculum in other bioprocesses such as MFCs. MFCs are devices that convert the chemical energy present in organic compounds to electrical energy via the catalytic activity of microorganisms. MFCs usually require an external inoculum source that may or may not receive a pretreatment; dilution, heat shock, acetate enrichment, and re-culturing are some

of the reported pretreatments [3]. Nevertheless, the use of an external inoculum and its pretreatment does not facilitate the direct linkage of dark fermentation to MFCs since the inoculum is often obtained from other facilities (wastewater treatment plants). Therefore, an adaptation period of the microorganisms to the new environment is necessary before reaching the full operation of the MFC. Because the composition of DFE varies widely as a function of the bioreactor operation, the power output of DFE FUELLED MFCs also varies from a few milliwatts to hundreds of milliwatts [4]. Our research group demonstrated in a previous work that the cell voltage decreased as function of the synthetic mixture composition fed to MFCs. The MFCs were fed acetic acid, followed by acetic-butyric acid mixture and finally an acetic-butyric-propionic acid mixture. The acetic-butyric acid mixture significantly reduced the MFC voltage to 2 mV compared to acetic acid alone (60 mV), but the acetic-butyric-propionic mixture increased the potential up to 30 mV [5]. Although the synthetic mixtures enabled the most favorable feeding strategy to be elucidated, the agreement of these results with actual DFE remained unanswered.

As in other bioprocesses, the inoculum-to-substrate ratio (ISR) likely influences the performance of an MFC; unfortunately, this ratio is not often reported for bio-electrochemical systems, including DFE fed MFCs. Because DFE contains microorganisms in addition to organic compounds, it can be hypothesized that this type of effluent is exploitable both as inoculum and as a substrate in the production of electricity in MFCs.

Although different studies have shown the benefits of using DFE, most of those studies utilized an external inoculum (Table 1).

Table 1. Overview of MFC-fed actual fermentation effluents.

Substrate	Inoculum	MFC type	Maximum power density (mW m^{-2})	Coulombic efficiency (%)	Reference
Dark fermentation effluents	Farm manure	Two-chamber	165	48	[3]
Leachate from pressed municipal solid waste	Mesophilic anaerobic sludge	Two-chamber	Nr	4.2	[6]
Waste fermentation effluents (food, distillery, lignocellulose)	Fly ash leachate	One chamber	6[a]	9.3 – 11.2	[7]
Diluted dark fermentation	Anolyte from MFC	One chamber, air cathode	439	24	[8]
Acetate rich wastewater Butyrate rich wastewater	Photosynthetic bacteria	One chamber, air cathode	112.2 10.5	Nr	[9]
	Farm manure	MFC stack,	19.7	9.8	[10]

Dark fermentation effluents		dual gas diffusion cathode			
Filtered dark fermentation effluents	Biofilm from previous electrode formed with fly ash leachate	Two-chamber	85	13	[11]
Diluted fermented primary sludge	MFC effluent	One chamber, air cathode	870	60	[12]
Fermented distillery wastewater	Sediment	Tank	80	Nr	[13]
Effluents from a hydrogen producing bioreactor	Effluent from a hydrogen producing reactor	One chamber, air cathode	1.4-3.3[a]	Nr	[14]

Nr: not reported.
a. Units $W\ m^{-3}$

To our knowledge, only one study has reported the use of DFE as both inoculum and substrate in an MFC. Li et al. [14] placed a MFC between two hydrogen-producing bioreactors to buffer the pH of the DFE leaving the first reactor and entering the second reactor, but no comprehensive characterization of the input and output liquid currents was provided.

To achieve effective exploitation of DFE in MFCs, it is necessary to first determine the chemical, microbiological and electrochemical characteristics of the DFE-MFC system and then to test optimization strategies. It can be assumed that any DFE-MFC system will be distinctive due to the variability in the composition of the DFE and the diversity of MFC designs.

The success of the integration of MFCs in the chain of processes for the use of wastewater depends on demonstrating the feasibility of direct linkage of bioprocesses.

Based on these considerations and on the lack of information on the direct use of actual DFE as both inoculum and substrate in MECs, the aim of this work was to evaluate various actual hydrogen-production reactors effluents as both inoculum and substrate sources in a two-chamber MFC and to identify the relevant parameters and operation ranges as a means of determining the viability of direct linkage dark fermentation with MFCs.

2. Experimental methods

2.1 MFC design and operation

Cylindrical two-chamber MFCs (40 mL each chamber) were constructed utilizing PVC connectors. The PVC pipe fittings, one male and one female, were placed horizontally and sealed with acrylic on the open side to construct the anodic and cathodic compartments (Ø = 5.5 cm). The distance between the electrodes was 4.5 cm, and the total length of the MFC was 6.5 cm. Two ports were placed in each chamber for sampling and external connections. The chambers were separated by a cation-exchange membrane (Ø = 5 cm, CMI-7000 Ultrex, Membranes Int., USA). Carbon felt (2 cm x 2 cm x 0.5 cm, Grupo Rooe, Mexico) was used as both the anode and the cathode electrodes (Figure 1).

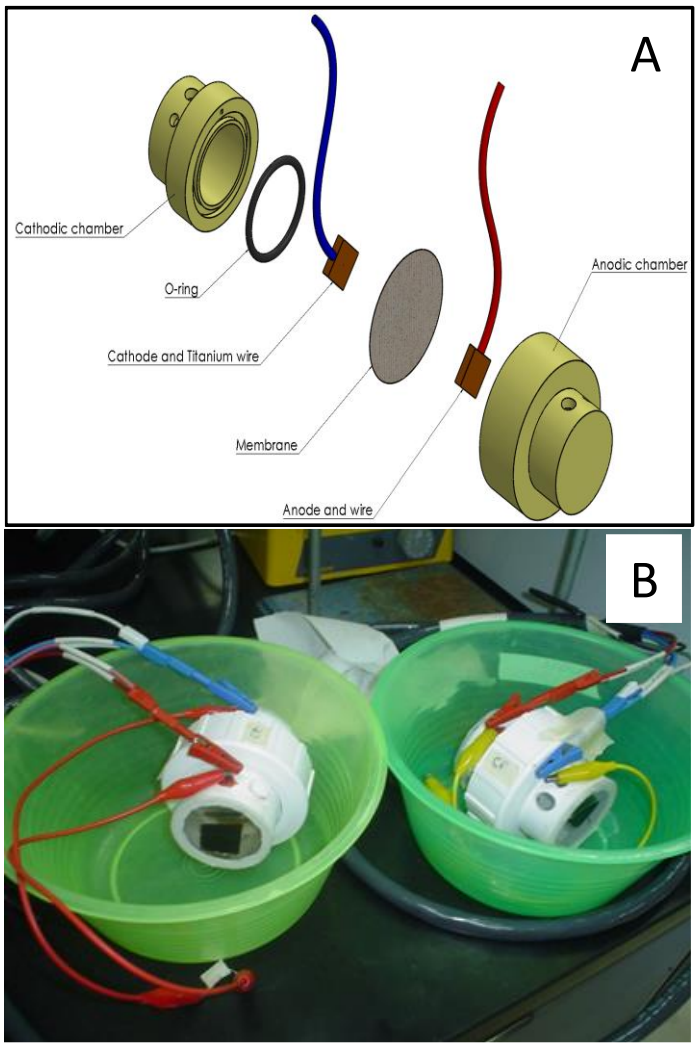

Figure 1. Design of the MFC. A) Schematic representation of the components. B) Examples of experimental units.

The external connections were made with titanium wire, and the electrodes were connected via an external 1000 Ω resistor to close the circuit. The MFCs were operated in batch mode. The cathodic compartment was filled with a phosphate buffer solution (50 mM, pH 7) supplemented with KCl (38 mM). This compartment was flushed with air for 1 min prior to starting the operation. Two different types of fermentation effluents were fed separately into the anode chamber of two different MFCs. This procedure was repeated in three runs of 9, 10 and 13 days. The voltage drop in the first run determined the duration of the following two runs. The anode chamber was inoculated and fed two different actual dark fermentation effluents; the effluents were tested in three independent experimental runs. The actual DFE were obtained from two hydrogen-producing bioreactors whose operation is detailed in a previous report [15]. Briefly, two anaerobic fluidized bed reactors were fed with a powered cheese whey solution (20 g L^{-1}) for 112 days. The reactors were started-up with different strategies; reactor 1 (R1) was operated with a hydrogen-producing inoculum enriched by retention time washout, and reactor 2 (R2) was operated with a thermal pretreated inoculum.

The anode compartments were purged with nitrogen gas for 2 min prior to starting the operation. The characterization of the actual DFE is shown in the Section 3.1.

2.2 Chemical, electrochemical and microbial analysis

The inoculum biomass was determined using the volatile solids standard method, and the substrate concentration was quantified as the total and soluble chemical oxygen demand (COD) using the closed reflux method [16]. The inoculum-to-substrate ratio (ISR) was calculated as the volatile solids/total COD ratio. The concentrations of acetic, propionic, and butyric acids in the media were determined via capillary electrophoresis (Agilent Technologies G1602A, U.S.A.) by the method described previously [17].

The MFC potential was measured using a high impedance multimeter. The current density, power density, coulombic efficiency percentage, and polarization curves were determined as described by Rosales-Sierra et al. [5] Electrochemical impedance spectroscopy was performed to measure internal resistance and the charge transfer resistance at the electrodes in the MFCs. The distribution of resistances was compared at open and closed-circuit operation. The bioanode was connected as the working electrode and the cathode as the reference and counter-electrodes. The analysis was performed over the frequency range from 10 mHz to 100 kHz with an amplitude of 10 mV in a potentiostat-galvanostat (Bio-Logic SAS, EC-Lab ver. 10.23). Model circuits were used to model and estimate the ohmic resistance (R0) and the charge transfer resistance at the anode (Ra) and cathode (Rc).

The microbial communities in the DFE samples were analyzed as previously reported [18]. The characterization was performed through denaturing gradient gel electrophoresis of 16S rRNA gene fingerprints. The predominant bands were excised, and the DNA in the bands was extracted and amplified by the polymerase chain reaction (PCR). Bacterial specific primers were used with a nested PCR technique using a Taq DNA polymerase (Invitrogen, U.S.A.); bacterial denaturing gradient gel electrophoresis was performed and stained as reported elsewhere [18,19]. Relative genera abundances were estimated after denaturing gradient gel electrophoresis of the DNA and determination of band intensities using Quantity One analysis software (Bio-Rad, U.S.A.), and data were grouped at the genus level. Shannon-Wiener diversity indexes (H index) were calculated based on the intensities of the bands.

3. Results and discussion

3.1 Effect of composition of actual dark fermentation effluents on MFC start-up

Three experimental runs were performed with two different actual DFE using an external of 1000 Ω resistor. Run 1 had a duration of 9 d; in this run, the potential of the effluents fed by the R1 reactor (MFC-R1) reached 3 mV in the first 3 days, whereas the MFC-fed effluents from reactor R2 (MFC-R2) reached a 4.5 mV peak in the same period. After peaking, the cell potential of both MFCs decreased to 2 mV. Run 2 persisted for 10 d; the potential in MFC-R1 decreased continuously, and peaks in potential were not observed in this period. The potential in MFC-R2 decreased from 2.5 mV to approximately zero and then increased to 2 mV on the 10th day. Run 3 had a duration of 13 d; throughout this run, the MFCs generated a comparatively stable cell potential. The dissimilar MFC performances of the three runs and the fact that Run 3 gave the highest performance were explained by the volatile solid content in the DFE. The volatile solid content of the R1 and R2 effluents that were used in Run 3 was the highest (Table 2); this observation agreed with previous results in our laboratory showing that the concentration of volatile solids correlates with the current density produced by bioanodes [20].

This behavior has been confirmed by other authors. Liu & Li, (2007) studied the effect of different inoculum concentrations, 5%, 10% and 15% in an MFC and found that the higher the inoculum concentration, the higher the growth rate. Moreover, the output power increased from 78 µW to 80 µW and to 84 µW with the increase of the inoculum concentration [21].

Table 2. Characterization of actual dark fermentation effluents from reactors R1 and R2 utilized in Runs 1-3.

Parameter (mg L^{-1})	**Run 1**		**Run 2**		**Run 3**	
	R1	**R2**	**R1**	**R2**	**R1**	**R2**
pH	6.0	5.8	6.0	5.6	5.3	5.7
Volatile solids	88	37	222	180	564	336
Total solids	367	149	426	590	841	563
COD_T[a]	769	441	948	150	1083	953
COD_S[a]	576	391	872	130	870	805
Volatile solids/COD_T[b]	0.11	0.08	0.23	1.2	0.52	0.35
Formic acid	701	1090	427	1111	0	0
Acetic acid	1329	1208	577	1507	1874	3110
Propionic acid	2091	3176	0	432	4289	10494
Butyric acid	757	328	2037	1685	0	0
H index	2.688	2.545	2.466	2.56	2.055	2.688

a. Units in g L^{-1}.
b. Ratio that corresponds to the inoculum to substrate parameter (ISR).

The inoculum to substrate ratio (ISR) is commonly utilized to describe bioprocesses. A high ISR value increases productivity, whereas a low ISR value leads to accumulation of substrates, which in turn can cause inhibition of the microbial activity [22]. It is known that anaerobic processes operate with ISR ranging from 0.2 to 2.5 [23]; therefore, the ISR values ranging from 0.08 to 1.2 in the present work for the two DFE and the three runs could be classified in the low-medium range of ISR. The test that resulted in comparatively stable performance (Run 3) presented ISRs of 0.52 and 0.35, values that are in the medium range. Because the ISR can vary from one DFE to another, it should be determined regularly to characterize the operation of specific DFE-MEC systems.

The volatile fatty acid composition of the DFE also contributed to the performance of Run 3. The volatile fatty acids mixture used in Run 3 was deficient in formic acid and butyric acid; this favored the cell potential even though the chemical oxygen demand was the highest (Table 2). Moreover, the superior propionic acid content in R2 than R1 likely caused the decrease in performance since a negative effect of propionic acid has been previously observed for MFCs in our research group [5]. These simultaneous conditions, that is, a high concentration of a less complex substrate, resulted in reasonably increasing MFC performance (Figure 2A).

The composition of actual DFE cannot be controlled unless the operation conditions of the fermenter are changed; nevertheless, the viability of exploiting these actual DFE to feed MFCs can be anticipated by considering the chemical composition and the ISR.

3.2 MFC potential and power density

The two MFCs produced a comparatively stable and high cell potential in run 3, so this run was analyzed in depth via polarization

curves. The MFC potential showed a lag time followed by a rapid increase to a peak within 5 days; thereafter, the potential declined, only to increase again to a plateau of 6 mV for MFC-R1 and 5 mV for MFC-R2 (Figure 2A). The lag time was related to biofilm development on the electrodes (day 1). This extremely short start-up time may represent an advantage compared to the extended period that is regularly required to operate MFCs. Multiple feeding cycles with a duration of several days each constitute a start-up procedure that is frequently utilized for MFCs [3]. The shorter start-up time in the present study was attributed to the unchanged environment in which the microbial community developed. The direct feeding of DFE to the MEC clearly obviates the need for the extended acclimation period that is required when an external inoculum is utilized.

The initial potential peak (days 2-5) occurred due to bacterial consumption of available and easily biodegradable substrates such as short-chain organic acids in the actual DFE (Table 2). It is worth noting that one of the advantages of using actual DFE as the substrate is the uninterrupted supply of short-chain carbonaceous compounds that originate from the degradation of more complex molecules present in the effluent. The stable cell potential observed from day 8 and day 10 for MFC-R1 and MFC-R2, respectively, reflected the presence of a well-established biofilm.

The maximum power density was 117 $\mu W\ m^{-2}$ for MFC-R1 and 78 $\mu W\ m^{-2}$ for MFC-R2 when the MFCs were operated with a fixed external resistor of 1000 Ω. The power output increased to 422 $\mu W\ m^{-2}$ and 500 $\mu W\ m^{-2}$ when the performance was determined by polarization curves for MFC-R1 and MFC-R2, respectively (Figure 2B). These results suggest that more power might be extracted from the MFCs by tracking the maximum power point with electronic circuits [24]. Diverse electronic circuits are being developed to harness and boost the energy from MFCs to overcome apparent limitation on power output. For instance, 0.05 V from a sediment-based MFC has been boosted to 3.3 V [24] and the voltage output from a compost-based MFC stack was increased from 0.8 V to voltage peaks of 3.3 V in our laboratory [25].

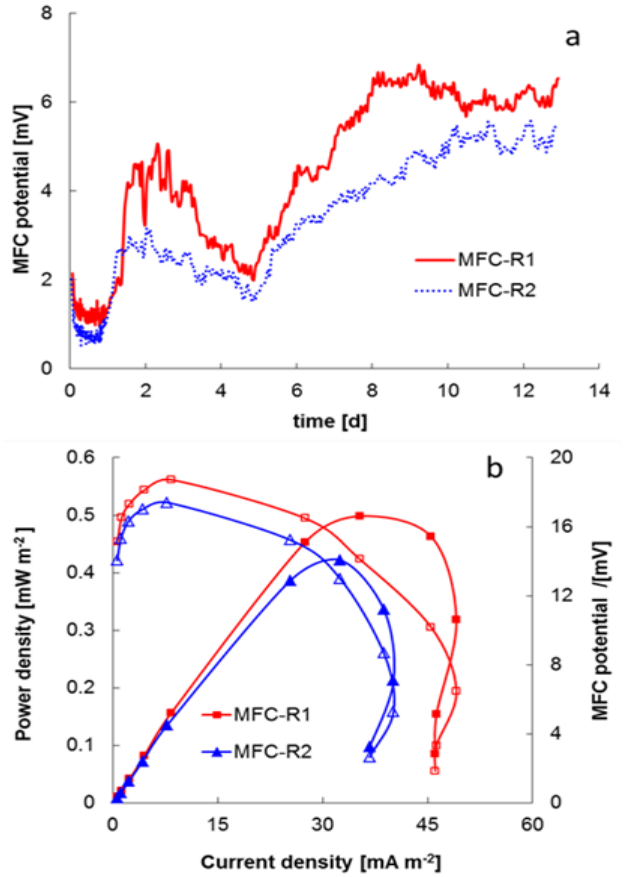

Figure 2. Performance of MFC inoculated and fed with two actual fermentation effluents (R1, R2) during Run 3. A) MFC potential with an external resistor of 1000 Ω. B) Power density measurements based on polarization curves at 13 days of operation.

One of the aims of the present research was to determine the viability of direct linkage between dark fermentation and MFCs. The results presented here demonstrate that the two-stage process is feasible. Several strategies have been reported in the literature to increase the power density in MFC from fermentation effluents, but most of these strategies do not take advantage of the direct coupling between two bioprocesses. For example, an MEC installed with a preformed bioanode achieved 85 mW m^{-2} [11]; the use of farm manure as an external inoculum allowed the production of 165 mW m^{-2} [3]; and dilution of fermentation effluent by 50% increased the power density up to 439 mW m^{-2} [8].

3.3 Microbial community structure

The conversion of energy was achieved by autochthonous microorganisms; therefore, the distribution of electroactive microorganisms in the DFE samples is of paramount importance, so it was investigated.

The microbial community structure varied as a function of the reactor (R1, R2) and the run (1 to 3). *Clostridium*, *Lactobacillus*, and representatives of Enterobacteriaceae were dominant in the DFE samples, as shown in Figure 3.

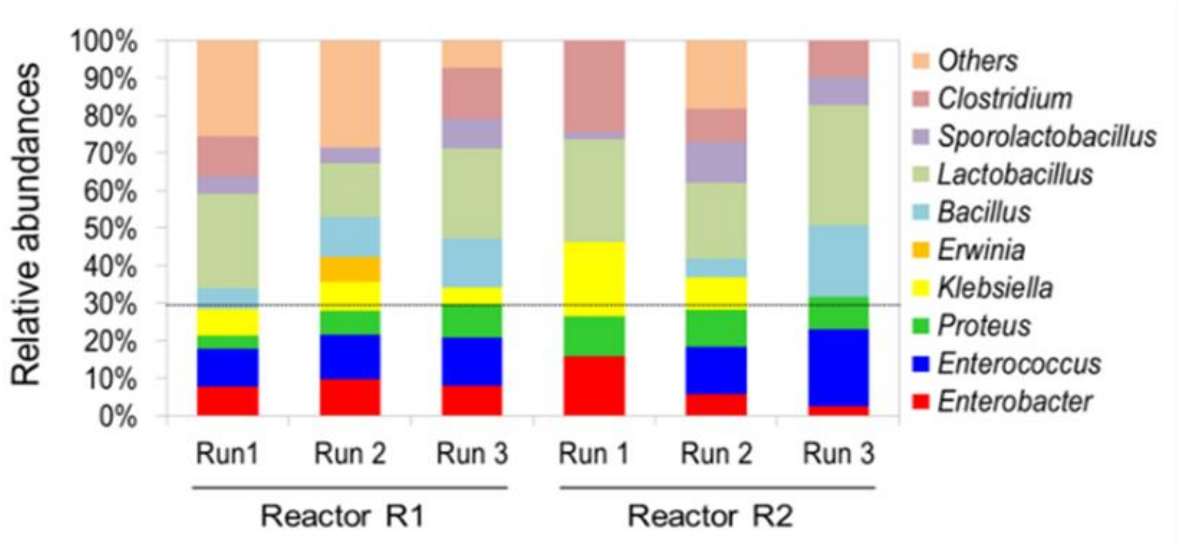

Figure 3. Relative abundance of microorganisms grouped at the genus level in the actual dark fermentation effluents samples from reactors R1 and R2 for Runs 1 to 3.

Genera similar to *Klebsiella*, *Proteus*, *Enterococcus*, and *Enterobacter* were found in the DFE samples and are likely to be related to the MFC performance. These genera belong to the phylum *Proteobacteria*, which has been identified repeatedly in electroactive communities [26]. The accumulative abundance of the electroactive genera in the microbial community was approximately 30%. This result indicated that a strategy for enrichment of genera having electroactive capabilities might be necessary. Diverse enrichment processes such as inoculation with effluent obtained from a previously operated MFC, scratching the biofilm from previously formed bioanodes, and performing multiple feeding cycles have been reported [27]. Nevertheless, in order to retain the advantages of a direct linkage between dark fermentation and MFCs, installation of packed bed bioreactors [28] is another strategy for enrichment of the amount of bioactive material.

The H index indicates the microbial diversity of DFE samples. This index was similar for the MFC that showed stable, high performance (MFC-R2 in Run 3) and for an MFC with unstable, low performance (MFC-R1 in Run 1) as shown in Table 2.

However, the relative abundance of the identified genera with electroactive properties differed between the two cells. The better performing MFC showed a higher abundance of *Enterococcus* and *Proteus*, which were found to produce 144 mW m^{-2} and 22-50 mW m^{-2} respectively [29, 30]. *Enterobacter* has produced from 16 to 1936 mW m^{-2} [31].

In contrast, *Klesbsiella*, which was also found in the low performing MFC, has produced only 30-67 mW m^{-2} [30]. Considering that there are differences in energy production between species, and especially between MEC operating conditions, the high performance in the MFC-R2 in Run 3 could be due to the higher relative abundance of *Enterococcus*.

In addition, previous studies shown that feeding a MFC with diluted fermentation effluent allowed the proliferation of *Geobacter* and *Pseudomonas*, in contrast to feeding unfermented whey, in which case *Clostridium* and *Lactobacillus* predominated

[8]. This result is in agreement with those obtained in the present work, since *Clostridium* and *Lactobacillus* were found in high abundance in most of the runs (Figure 3). Therefore, it can be assumed that the dilution of the fermentation effluents prior to cell feeding could favor the presence of other electroactive species.

Overall, the two different DFE showed variations in their chemical and microbial composition during the three runs. The DFE that contained COD concentrations in the low range (150 mg L^{-1} to 948 mg L^{-1}) limited the power density; in addition, the presence of formic, acetic, propionic, and butyric acids instead of only acetic and propionic acids also seemed to contribute to low MFC performance. Hence, high COD concentration (953 mg L^{-1} and 1083 mg L^{-1}) of a less complex DFE (acetic and propionic acids) facilitated favorable operation of the MFC.

Volatile solids content in the range 88 mg L^{-1} to 222 mg L^{-1} was not satisfactory for power production; in contrast, the test operated with 336 mg L^{-1} to 5645 mg L^{-1} volatile solids showed high and sustained power density production. Not only did the amount of microbial biomass affect the MFC performance but also the relative abundance of electroactive species should be surveilled as an optimization factor.

3.4 Electrochemical characterization of the MFC

Although DFE are exploitable both as inoculum and as substrate in MFCs, improvements in the process could be oriented to an optimized MFC design. The electrochemical cell design used in this study was closer to a parallel plate reactor; at first glance, it is necessary to increase the volume of the anode chamber to contain a volumetric electrode and support a large amount of biofilm. Nevertheless, to improve the MFC design, it is first necessary to determine the components that result in low efficiency such as the internal resistance of the MFC and the charge transfer resistance at the electrodes. To investigate these factors, electrochemical impedance spectroscopy was conducted in an open and in a closed-circuit mode in the whole MFC.

The distribution of resistances in MFC-R1 and MFC-R2 was investigated in closed and open circuits at the end of Run 3. Impedance measurements were made in a two-electrode configuration, i.e., the response included the contribution of the anode, the cathode, the dissolutions, and the membrane. Two models were proposed for both open and closed operation. The model circuit for closed operation included an external resistance coupled in parallel to the circuit elements. The Nyquist plots obtained from electrochemical impedance spectroscopy and the simulation obtained with the model circuits are shown in Figure 4.

The small semicircle or loop had a deformed side in the high-frequency region (R(Z) near zero) that approached a linear tendency to 45°. This is typical behavior of porous materials [32] and would be expected for an electrode covered by a biofilm. In addition, cathodic reactions have been reported to limit MFC performance [33]; therefore, the loops in the low- and high-frequency ranges were attributed to the anode and cathode contributions, respectively.

When the electrode processes had been assigned to the observed loops, the models of the equivalent circuits were fitted to the experimental data for the open and closed-circuit installations. A very good fit was obtained in every case, as shown by the continuous lines in the Nyquist representation (Figure 4) and by the $\chi 2$ parameter, which ranged from 0.002 to 0.000095. This

goodness of fit supported the correctness of the proposed equivalent circuits.

There were no significant differences in the values of equivalent circuit parameters estimated in the open and closed circuits with the exception of the Rc values in the open circuit, which were 4.58 x 10^3 Ω for MFC-R1 and 4.38 x 10^{16} Ω for MFC-R2. These differences were attributed to the inaccuracy of the fitting used to estimate this parameter because the corresponding semi-loop was incomplete and not well defined; nevertheless, this problem did not occur in the case of the other parameters.

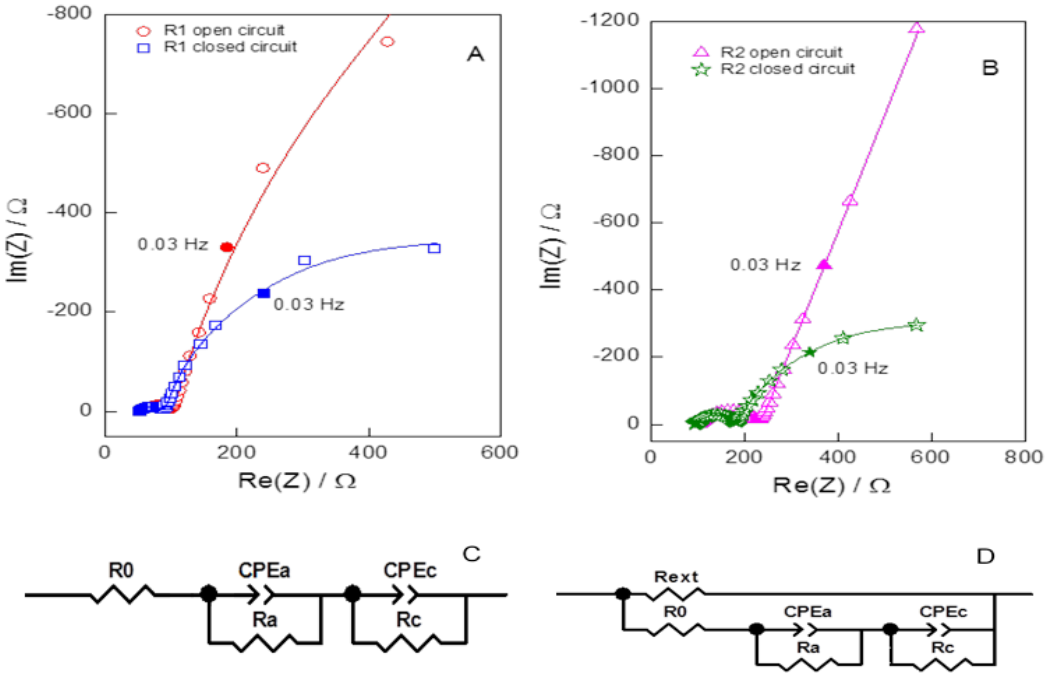

Figure 4. Nyquist plots for microbial fuel cells (MFCs) at the end of Run 3. MFCs were inoculated and fed with actual dark fermentation effluents from reactors (R1, R2). A) MFC-R1 at open and closed circuits. B) MFC-R2 at open and closed circuits. C) Model circuit for MFC installed in open circuit. D) Model circuit for MFC installed in closed circuit.

The ohmic resistance (R0) in MFC-R1 (53 Ω) was approximately half of that observed in MFC-R2 (102 Ω), indicating that the solution in MFC-R1 was more conductive. The charge transfer resistance at the anode (Ra) was also lower for MFC-R1 than for MFC-R2 (48 ± 1.9 Ω vs. 128 ± 6 Ω). Both low resistance values (ohmic and charge transfer) contributed to the better performance of MFC-R1 (Figure 2).

Interestingly, the Ra values were slightly lower in the closed circuit than in the open circuit. This was attributed to the external resistance, which provoked shrinking of the loops. This effect was checked by simulation, and it was found that smaller external resistance values resulted in smaller loops.

Nyquist spectra composed of two loops were previously discussed by Martin et al. [34]. The authors explained the two loops as the result of a possible secondary redox reaction

in the biofilm. The results obtained through the simulation performed in the present work demonstrated that two loops can be associated with the anodic and the cathodic processes when the impedance analysis is performed in a two-electrode configuration for the whole MFC. Because the Nyquist spectrum is affected by the external resistor value, electrochemical impedance analysis in an open circuit will be more accurate and could facilitate the comparison of data obtained in systems that are operated with diverse external resistors. These findings certainly contribute to the understanding and interpretation of electrochemical impedance spectroscopy spectra for two-chamber MFCs.

In the present work, it was demonstrated that the simplest effluents composition results in the highest power density production; actual DFE that lacked butyric acid yielded the highest MFC performance. The ISR is a parameter that is often used to compare startup and operation between bioreactors, here the highest and most stable performance in MFCs was observed with ISRs of 0.35 and 0.52; these values are in the medium-low range reported for anaerobic reactors.

The most promising result obtained in this study was that the autochthonous microbial community in the DFE allowed the production of energy, thus reducing the start time and excluding the use of external inoculum.

4. Conclusion

The direct linkage of dark fermentation and MFCs for electricity production was investigated. The MFC performance was superior with a less complex mixture of volatile fatty acids (acetic and propionic acids), a high content of volatile solids (953 mg L^{-1} to 1083 mg L^{-1}) and a high chemical oxygen demand (336 mg L^{-1} to 564 mg L^{-1}) in the DFE. The inoculum to substrate ratio was proposed as a parameter for comparison; values of 0.52 and 0.35 enabled the power density to reach 0.5 mW m^{-2} and 0.4 mW m^{-2}, respectively. Microbial community composition was a factor that partially explained the MECs performance; because representatives of *Proteobacteria*, were present at only 30% relative abundance in the DFE samples. The analysis of electrochemical impedance spectroscopy revealed that this analysis should be performed under open circuit conditions to obtain accurate data and to facilitate comparison among research studies. The feasibility of generating electrical energy from DFE with no external inoculum was demonstrated and the results provide a foundation for others DFE to be evaluated.

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References

[1] Sinha, P., Pandey, A. “An evaluative report and challenges for fermentative biohydrogen production”. *International Journal of Hydrogen Energy*. 36.13 (2011):7460-78.

[2] Mohanakrishna, G., S. V. Mohan, and P. N. Sarma. "Utilizing Acid-Rich Effluents of Fermentative Hydrogen Production Process as Substrate for Harnessing Bioelectricity: An Integrative Approach". *International Journal of Hydrogen Energy* 35.8 (2010): 3440-49.

[3] ElMekawy, A., Srikanth, S., Vanbroekhoven, K., De Wever, H., Pant, D. "Bioelectro-Catalytic Valorization of Dark Fermentation Effluents by Acetate Oxidizing Bacteria in Bioelectrochemical System (BES)". *Journal of Power Sources* 262 (2014): 183-91.

[4] Choi, Jin-dal-rae, Ho Nam Chang, and Jong-In Han. "Performance of Microbial Fuel Cell with Volatile Fatty Acids from Food Wastes". *Biotechnology Letters* 33.4 (2011): 705-14.

[5] Rosales-Sierra, A., Rosales-Mendoza, S., Monreal-Escalante, E., Celis, L. B., et al. "Acclimation Strategy Using Complex Volatile Fatty Acid Mixtures Increases the Microbial Fuel Cell (Mfc) Potential". *Chemistryselect* 2.22 (2017): 6277-85.

[6] Rozsenberszki, T., Kook, L., Bakonyi, P., Nemestothy, N., et al. "Municipal Waste Liquor Treatment Via Bioelectrochemical and Fermentation (H-2 + Ch4) Processes: Assessment of Various Technological Sequences". *Chemosphere* 171 (2017): 692-701.

[7] Varanasi, J. L., P. Sinha, and D. Das. "Maximizing Power Generation from Dark Fermentation Effluents in Microbial Fuel Cell by Selective Enrichment of Exoelectrogens and Optimization of Anodic Operational Parameters". *Biotechnology Letters* 39.5 (2017): 721-30.

[8] Wenzel, J., Fuentes, L., Cabezas, A., Etchebehere, C. "Microbial Fuel Cell Coupled to Biohydrogen Reactor: A Feasible Technology to Increase Energy Yield from Cheese Whey". *Bioprocess and Biosystems Engineering* 40.6 (2017): 807-19.

[9] Chandra, Rashmi, J. Annie Modestra, and S. Venkata Mohan. "Biophotovoltaic Cell to Harness Bioelectricity from Acidogenic Wastewater Associated with Microbial Community Profiling". *Fuel* 160 (2015): 502-12.

[10] Pasupuleti, S. B., Srikanth, S., Mohan, S. V., Pant, D. "Continuous Mode Operation of Microbial Fuel Cell (Mfc) Stack with Dual Gas Diffusion Cathode Design for the Treatment of Dark Fermentation Effluent". *International Journal of Hydrogen Energy* 40.36 (2015): 12424-35.

[11] Varanasi, J. L., Roy, S., Pandit, S., Das, D. "Improvement of Energy Recovery from Cellobiose by Thermophillic Dark Fermentative Hydrogen Production Followed by Microbial Fuel Cell". *International Journal of Hydrogen Energy* 40.26 (2015): 8311-21.

[12] Yang, F., Ren, L., Pu, Y., Logan, B. E. "Electricity Generation from Fermented Primary Sludge Using Single-Chamber Air-Cathode Microbial Fuel Cells". *Bioresource Technology* 128 (2013): 784-87.

[13] Mohan, S. Venkata, G. Mohanakrishna, and P. Chiranjeevi. "Sustainable Power Generation from Floating Macrophytes Based Ecological Microenvironment through Embedded Fuel Cells Along with Simultaneous Wastewater Treatment". *Bioresource Technology* 102.14 (2011): 7036-42.

[14] Li, J., Zou, W., Xu, Z., Ye, D., et al. "Improved Hydrogen Production of the Downstream Bioreactor by Coupling Single Chamber Microbial Fuel Cells between Series-Connected Photosynthetic Biohydrogen Reactors". *International Journal of Hydrogen Energy* 38.35 (2013): 15613-19.

[15] Cisneros-Perez, C., Etchebehere, C., Celis, L.B., Carrillo-Reyes, J., Alatriste-Mondragon, F., Razo-Flores, E. “Effect of inoculum pretreatment on the microbial community structure and its performance during dark fermentation using anaerobic fluidized-bed reactors”. *International Journal of Hydrogen Energy*. 42.15 (2017):9589-99.

[16] Eaton, A. D., Clesceeri, L. S., Rice, E. W., & Greenberg, A. E. (2005). Standard methods for the examination of water and wastewater. 22nd ed., 5220-5310. American Public Health Association/American Water Works Association/Water Environment Federation. Washington DC, USA.

[17] Davila-Vazquez, G., Alatriste-Mondragon, F., de Leon-Rodriguez, A., Razo-Flores, E. "Fermentative Hydrogen Production in Batch Experiments Using Lactose, Cheese Whey and Glucose: Influence of Initial Substrate Concentration and Ph". *International Journal of Hydrogen Energy* 33.19 (2008): 4989-97.

[18] Cisneros-Perez, C., Carrillo-Reyes, J., Cells, L. B., Alatriste-Mondragon, F., et al. "Inoculum Pretreatment Promotes Differences in Hydrogen Production Performance in Egsb Reactors". *International Journal of Hydrogen Energy* 40.19 (2015): 6329-39.

[19] Carrillo-Reyes, J., Celis, L. B., Alatriste-Mondragon, F., Razo-Flores, E."Different Start-up Strategies to Enhance Biohydrogen Production from Cheese Whey in Uasb Reactors". *International Journal of Hydrogen Energy* 37.7 (2012): 5591-601.

[20] Cercado, B., Felipe Chazaro-Ruiz, L., Ruiz, V., de Jesus Lopez-Prieto, I., et al. "Biotic and Abiotic Characterization of Bioanodes Formed on Oxidized Carbon Electrodes as a Basis to Predict Their Performance". *Biosensors & Bioelectronics* 50 (2013): 373-81.

[21] Liu, Z., Li, H. “Effects of bio- and abio-factors on electricity production in a mediatorless microbial fuel cell”. *Biochemical Engineering Journal* 36 (2007): 209-214.

[22] Shah, F. A., Mahmood, Q., Shah, M. M., Pervez, A., Asad, S. A. "Microbial Ecology of Anaerobic Digesters: The Key Players of Anaerobiosis". *Scientific World Journal* (2014).

[23] Moset, Veronica, Nawras Al-zohairi, and Henrik B. Moller. "The Impact of Inoculum Source, Inoculum to Substrate Ratio and Sample Preservation on Methane Potential from Different Substrates". *Biomass & Bioenergy* 83 (2015): 474-82.

[24] Wang, H. M., J. D. Park, and Z. J. Ren. "Practical Energy Harvesting for Microbial Fuel Cells: A Review". *Environmental Science & Technology* 49.6 (2015): 3267-77.

[25] Garita-Meza, M. A., Ramirez-Balderas, L. A., Contreras-Bustos, R., Chavez-Ramirez, A. U., Cercado, B. "Blocking Oscillator-Based Electronic Circuit to Harvest and Boost the Voltage Produced by a Compost-Based Microbial Fuel Cell Stack". *Sustainable Energy Technologies and Assessments* 29 (2018): 164-70.

[26] Miceli, J. F., Parameswaran, P., Kang, D. W., Krajmalnik-Brown, R., Torres, C. I. "Enrichment and Analysis of Anode-Respiring Bacteria from Diverse Anaerobic Inocula". *Environmental Science & Technology* 46.18 (2012): 10349-55.

[27] Hasany, M., M. M. Mardanpour, and S. Yaghmaei. "Biocatalysts in Microbial Electrolysis Cells: A Review". *International Journal of Hydrogen Energy* 41.3 (2016): 1477-93.

[28] Tchobanouglous, G., Burton, F. L., (1991). Wastewater engineering. Treatment, disposal, and reuse. McGraw-Hill. Singapore.

[29] Parihar, P., Keshavkant, S., Jadhav, S. “Electrogenic potential of *Enterococcus*

faecalis DWW1 isolated from the anodic biofilm of a dairy wastewater fed dual chambered microbial fuel cell". *Journal of Water Process Engineering* 45 (2022):102503.

[30] Ng, I. S., Hsueh, C. C., Chen, B. Y. "Electron transport phenomena of electroactive bacteria in microbial fuel cells: a review of *Proteus hauseri*". *Bioresources and Bioprocesses*. 4.53 (2017).

[31] Feng, C., Li, J., Qin, D., Chen, L., et al. "Characterization of exoelectrogenic bacteria *Enterobacter* strains isolated from a microbial fuel cell exposed to copper shock load". *Plos One* 9.11 (2014): e113379.

[32] Bisquert, J., Garcia-Belmonte, G., Fabregat-Santiago, F., Ferriols, N. S., et al. "Doubling Exponent Models for the Analysis of Porous Film Electrodes by Impedance. Relaxation of TiO_2 Nanoporous in Aqueous Solution". *Journal of Physical Chemistry B* 104.10 (2000): 2287-98.

[33] Borole, A. P., Aaron, D., Hamilton, C. Y., Tsouris, C. "Understanding Long-Term Changes in Microbial Fuel Cell Performance Using Electrochemical Impedance Spectroscopy". *Environmental Science & Technology* 44.7 (2010): 2740-44.

[34] Martin, E., Savadogo, O., Guiot, S. R., Tartakovsky, B. "Electrochemical Characterization of Anodic Biofilm Development in a Microbial Fuel Cell". *Journal of Applied Electrochemistry* 43.5 (2013): 533-40.