Hydrogen evolution in microbial electrolysis cells treating landfill leachate: dynamics of anodic biofilm

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Abstract

Two microbial electrolysis cells (MEC-1 and 2) under 30 °C and 17±3 °C temperatures, respectively were operated using simulated landfill leachate as substrate. The system achieved a projected current density of 1000–1200 mA m^{-2} (MEC-1) and 530–755 mA m^{-2} (MEC-2) coupled with low cost hydrogen production rate of 0.148 L La^{-1} d^{-1} (MEC-1) and 0.04 L La^{-1} d^{-1} (MEC-2) at an applied voltage of 1.0 V. Current generation led to a maximum COD oxidation of 73±8% (MEC-1) and 65±7% (MEC-2) with ≥100% energy recovery. The system also exhibited a high hydrogen recovery (66–95%), pure hydrogen yield (98%) and tremendous working stability during two months of operation. Electroactive microbes such as *Pseudomonadaceae*, *Geobacteraceae* and *Comamonadaceae* were found in anodophilic biofim, along with *Rhodospirillaceae* and *Rhodocyclaceae*, which could be involved in hydrogen production. These results demonstrated an energy-efficient approach for hydrogen production coupled with pollutants removal.

Keywords: Microbial electrolysis cells (MECs), energy efficiency, landfill leachate, Pyrosequencing, Hydrogen

Nomenclature:

BES	bioelectrochemical system
MEC	microbial electrolysis cell
DNA	deoxyribo nucleic acid
rRNA	ribosomal ribonucleic acid
PBS	phosphate buffered solution
PCR	polymerase chain reaction
r_{cat}	cathodic hydrogen recovery
OTUs	operational taxonomic units
Anammox	anaerobic ammonium oxidation
CEM	cationic exchange membrane
HRT	hydraulic retention time

Glossary:

- CE (Coulombic efficiency): the ratio of total coulombs actually transferred to the anode from the substrate to the maximum possible coulombs if all substrate removal produces electricity.
- E_{sp} (Specific energy consumption): the electrical energy consumed per kg of COD removed.
- η_E (Energy recovery efficiency): ratio of electrical energy output in terms of H_2 and CH_4 gases to electrical energy input.
- MEC (Microbial electrolysis cell): bioelectrochemical system, which is used to produce hydrogen (H_2) and other value - added chemicals.
- QH_2 (Volumetric hydrogen evolution): the total amount of hydrogen produced per day and per liter of anodic chamber.

Highlights

- Simulated landfill leachate was employed as substrate for H_2 production in MEC.
- The system showed a high H_2 recovery (66-95%) and pure H_2 yield (98%).
- Applied potential, temperature and organic load imparted clear affect on system efficiency.
- Low cost and pure H_2 yield was attained having less energy consumption.
- Anodophlic communities shared 33.7% of the total OTUs showing quite stable over time

1. Introduction

Environmental pollution and energy shortages are two major global puzzles of the time. The present energy mainly comes from burning fossil fuels; however, depletion of fossil reserves with burgeoning global energy demand and increasingly environmental pollution is a matter of concern. Hence, the demand of renewable energy resources to replace fossil fuels has attracted worldwide attention. Hydrogen gas (H_2) is a preferred alternate energy source since it is clean and renewable energy carrier. Nowadays, H_2 gas is mostly produced from certain processes such as gasification, pyrolysis, thermochemical water splitting, steam reformation, electrolysis, fermentation and photo-fermentation [1]. One very promising technology to generate bio hydrogen gas is the use of microbial electrolysis cell (MEC), a viable alternative for wastewater treatment as well [2–4]. The H_2 evolution rates are significantly higher in MECs (80 – 100%) as compared to the fermentation process and water electrolysis [5]. In an MEC, a group of electroactive bacteria utilize the potential energy stored in the organic compounds to metabolize and grow, donating electrons to the anode which then transport to the cathode with anaerobic environment, through electrolyte in a circuit and generate H_2 gas electrochemically (i.e. electrohydrogenesis) by certain endergonic reactions [3,6], as described in Eq. (1).

$$2H^+ + 2e^- \longrightarrow H_2 \qquad E^0 = -0.414\,V \qquad (1)$$

The voltage produced by the exoelectrogenic bacteria is not sufficient for H_2 gas evolution, therefore in most cases, MECs a certain external voltage is added to the circuit to drive the redox reactions because no oxygen (or oxidative agent) is available in the cathode chamber electrochemical H_2 production [7]. Theoretically, a very low potential input (-0.414 V) is required to drive the process (Eq. 1), but in practice it is substantially

increased (i.e. 0.5 – 1.0 V) due to over potentials of the system. The H_2 production increases with the amplitude of applied potential (*E*ap, 0.7 – 1.0 V), which still is quite lesser than required for water electrolysis (1.8 – 2.0 V) [4,6,8].

The electrohydrogenesis proficiencies differ significantly with the nature of substrates employed. The MEC system with easily biodegradable organic compounds would have high hydrogen yield and energy output. Nevertheless, upon employing wastewater as substrate, the performance might be worse due to the high vulnerability of electrochemically active microbiota [9]. As shown in Table 1, beside simple substrates, a broad range of complex substrates have been tested so far in MECs for electrohydrohenesis such as human urine, glycerol, starch, winery, food processing, domestic and industrial wastewaters. However, less attention was given to use landfill leachate as anodic substrate in MECs. Landfill leachate is a high strength wastewater with excessive COD, NH_4-N and volatile fatty acid contents (VFAs). Organic content present in landfill leachate and its composition vary depending on the type of landfill waste materials and age of the leachate. High COD (>5 g L^{-1}) and NH_4-N (> 0.4 g L^{-1}) content, and low BOD_5/COD ratio (< 0.1) makes biological treatment of landfill leachate very difficult [10,11].

<Table 1>

Employing landfill leachate as raw material for production of energy and chemicals is a novel approach. It is likely that landfill leachates with unique physiochemical characteristics and more complex components have higher difficulties in hydrogen production. Whether or not MEC system can catalyze landfill leachate to produce H_2 gas is still dubious owing to least attention. Anode respiring bacteria (ARB), the key microbes that colonize the anode of BES, can oxidize only a few simple compounds as electron donors. In this regard, Mahmoud et al. [12] employed pre-

fermentation of mature landfill leachate (BOD_5/COD ratio of 0.32) for enhanced current density, CE and organics removal in MEC. The fermentation reactions produce the mixture of simpler compounds that ARB can oxidize. During fermentation, the complex organic compounds in the leachate were converted to simple VFAs, mainly succinate and acetate in batch tests, but mostly acetate in semi-continuous fermentation. The degree of conversion to VFAs improved by 4-fold, which led to a 68% increase in CE and a maximum current density of 23 A m^{-3} (or 1.7 mA m^{-2}). In another study [13], Fenton-based pre-treatment was employed to improve the biodegradability of landfill leachate that is subsequently fed to an MEC. It led to higher MEC performance: 52 ± 10% BOD_5 removal, 29 ± 3% CE, and 1.42 ± 0.27 A m^{-2} current density as compared to 3 ± 0.3% BOD_5 removal, 1.8 ± 0.5% CE, and 0.11 ± 0.06 A m^{-2} current density for the raw leachate.

Kargi et al. [14] employed landfill leachate for H_2 production applying DC voltage in the range of 0.5 – 5.0 V (i.e. electrohydrolysis) using aluminum electrodes. The highest cumulative H_2 evolution (5 L), H_2 yield (2.4 LH_2 g^{-1} COD), daily H_2 gas production (1.27 Ld^{-1}), and percent H_2 (99%) in the gas phase coupled with 77% COD removal were attained applying 4 V DC voltage. However, only 22 mL H_2 gas was generated within 96 h applying 0.5 V DC voltage. It indicated that the H_2 production was driven by electrohydrolysis other than bacterial decomposition of the leachate.

Since no MEC study has been employed for hydrogen production using landfill leachate as substrate. Herein, we investigate the potential opportunities of efficient clean hydrogen production treating simulated landfill leachate without any pre-treatment approach either biologically, physico-chemically or electro-chemically. To the best of our knowledge, this is the first study for H_2 evolution using landfill leachate as substrate. Series of batch scale experiments were conducted under different operational and

electrochemical conditions to see the overall system performance including organic and nitrogen content removal, energy consumption and yield. At the end, microbial community was studied for a better understanding of the H_2 generation mechanism.

2. Materials and Methods

2.1 MEC set up

A set of dual chambered rectangular reactors (MEC-1 and MEC-2) having 1.0 L and 0.5 L of total working volume, respectively as explained in previous work [15] was employed in this study. The schematics of experimental set-up is illustrated in Fig. 1. For hydrogen collection and measurement, the gas port at the top of cathode chamber was connected to an inverted measuring cylinder in a water tub. The anode of MEC-1 and MEC-2 consisted of 1.0 cm thick carbon felt (Sigratherm soft felt GFD 2, SGL Carbon Group, Wiesbaden, Germany), and the cathode was a perforated stainless steel plate with staggered-hole pattern, both with dimensions of 34.5 × 14.5 cm and 24.5 × 9.5 cm, respectively. The electrodes were separated by cation exchange membrane (CEM, CMI-7000, Membranes Int., USA). A titanium wire as current collector was connected with electrodes (two electrode system). The anolyte and catholyte in both systems was mixed with an external recirculation loop using a peristaltic pump (Dosiper C1R; Leon, Spain) at a recirculation rate of 8 L h^{-1}, during batch and continuous mode of operation. Helium was purged into the anode and cathode stock solution bottles (5 L working volume) to ensure anaerobic conditions. A100 W heating pad (20×150 mm) (RoHS, UK) was fixed on the outer wall of anode chamber of MEC-1 to control the temperature at 30 °C.

<Fig. 1>

2.2 MEC Operation

The experiment was extended to a total duration of 62 days (MEC-1) and 58 days (MEC-2), operating under batch and continuous modes. Table 2 shows the various

conditions applied in MEC-1 and MEC-2. During the batch mode, the system was operated at a controlled temperature (30 °C, MEC-1) and room temperature (17±3 °C, MEC-2).

<Table 2>

During the inoculation period, the system was operated for 15 days (data not shown). Acetate (9.4 mM), propionate (1.8 mM) and glucose (1.5 mM) were used as carbon source with 1.0 mL^{-1} of a trace metal solution, prepared according to Moreno et al. [16]. Nutrient buffer solution was (in g L^{-1}) KH_2PO_4: 0.68, K_2HPO_4: 0.87, KCl: 0.74, NaCl: 0.58, $MgSO_4.7H_2O$: 0.1 and NH_4Cl: 0.18, whereas 100 mM phosphate buffered solution (pH 7) was employed as catholyte. A 50 mL anaerobic digester sludge collected from municipal wastewater treatment plant of Leon (Spain) was used as inoculum in each reactor per liter of anode medium. A mean 75% of the media was replaced at the end of each batch cycle until a steady response of current production was observed.

After inoculation period, the simulated landfill leachate was employed as substrate prepared according to Rowe et al. [17] with minor modifications. The chemical characteristics of the simulated landfill leachate are presented in Table 3. In batch mode, 100 mM PBS at pH 7 was used as catholyte and in continuous mode carbonate buffer (0.25 M $NaHCO_3$ and Na_2CO_3, pH 10) was applied.

<Table 3>

2.3. Measurements and analytical determinations

The MEC electrical outputs of each module were monitored separately. An adjustable DC power supply was used to maintain the voltage at a predetermined set point. The power supply was computer controlled using an analog output board (PCI-6713; National Instruments, Austin, TX). Data were recorded at 10 min intervals. The

produced gas in the cathode chambers was collected in 0.2 L gasbags and gas production rate was measured by inverted gas jar method.

Gas composition was analyzed by gas chromatography as described by Martinez et al. [18]. Ammonium nitrogen (NH_4-N) was determined using an ion-selective electrode (781 pH/Ion Meter, Metrohm). The pH and conductivity were measured using pH meter and conductivity meter, respectively. Total organic carbon (TOC), total carbon (TC) and total nitrogen (TN) content were determined using a TOC analyzer (multi N/C 3100, AnalytikJena).

2.4. MEC performance parameters

The MEC performance was evaluated in terms of (i) volumetric hydrogen yield (QH_2) per liter of the reactor volume (La^{-1} d^{-1}), (ii) coulombic efficiency (CE, %), the ratio between the total coulombs actually transferred to the anode from the substrate, and the theoretical maximum, (iii) cathodic H_2 recovery (r_{cat}, %), calculated as the ratio of the electrons recovered as hydrogen gas to the total number of electrons that reach the cathode and (iv) specific energy consumption (E_{sp}) expressed as the electrical energy consumed per kg of COD removed (kW h kg-COD^{-1}), (v) net energy consumption (kW h kg-COD^{-1}) and (vi) energy recovery efficiency (η_E, %). The computation methods for the aforementioned performance parameters have been explained in supplementary materials and derived from Ref. [4,19,20].

2.5. Microbial community analyses

In order to study the microbial community attached to the electrodes at three different periods (PI, PII and PIII), three different spots of the anode carbon felt were sampled corresponding to 30, 42 and 62 days of operation. Samples PI and PII were taken during the continuous mode operation whereas, PIII corresponds to batch mode during the five months of total operation period. Further operating conditions are given in

Table S1 (supplementary material). Total genomic DNA was extracted with a PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The quantity and quality of the extracted DNA was checked measuring them in a NanoDrop 1000 (Thermo Scientific).

The DNA extracted was used for 454-pyrosequencing purposes. *16S rRNA* genes were amplified from each sample. The primer set used was 27Fmod (5-AGRGTTTGATCMTGGCTCAG-3`)/519R modBio (5-GTNTTACNGCGGCKGCTG-3`) for the eubacterial population. For amplification, 2µl of each DNA was used and a reaction was carried out in 50 µl containing 0.4 mM of fusion primers, 0.1 mM of dNTPs, 2.5 U of Taq ADN polymerase (Qiagen), and 5 µl of the reaction buffer (Qiagen). The PCR amplification was carried out in a thermocycler GeneAmp_PCR system 9700 (Applied Biosystems) and operated with the following protocol: 30 s at 95 °C, followed by 30 cycles at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 10 min. The obtained DNA reads were compiled in FASTq files for further bioinformatic processing. These pyrosequencing data were also taxonomically classified using the Ribosomal Database Project Group (RDP), in order to compare the results obtained in both databases. The Venn diagram was performed using VENNY software, and XLSTAT software package was used for performing a Correspondence Analysis (CA). The data from pyrosequencing datasets was submitted to the Sequence Read Archive of the National Centre for Biotechnology Information (NCBI) for eubacterial populations.

2.6 Statistical analysis

Analysis of variance (ANOVA) with a significance level of 5% ($p < 0.05$) was performed using Excel for Mac 2011 to determine statistical differences in the results obtained from different conditions.

3 Results

3.1. Current generation

Current response under each condition was monitored over time to evaluate the overall performance of the MEC configuration. Fig. 2A, B illustrates the variations of current intensity at various cycles in MEC-1 and MEC-2. Applied voltage (E_{ap}) statistically influenced the current density in both systems ($p < 0.05$). As can be seen, the current density in MEC-1 rose to the highest value of 1000 – 1200 mA m^{-2} in cycle 1 and 2 (day 1–7), followed by 800 mA m^{-2} in cycle 3 (day 7–16). Upon reducing the E_{ap} to 0.8 V (cycle 4, day 17–22), a maximum current density of only 250 mA m^{-2} was noticed followed by a two-fold surge in cycle 5. When the system was again run at E_{ap} of 1.0 V (cycle 6 and 7, day 32 – 47), the current density exhibited to a highest value of 800 mA m^{-2}.

Similar trend was observed in MEC-2: the maximum current density attained was 530 and 755 mA m^{-2} (cycle 1–2, day 1–12) at 1.0 V E_{ap}, while only 485 – 510 mA m^{-2} was registered at 0.8 V (cycle 3–4, day 13–24). The current intensity again increased to 600 – 650 mA m^{-2} at 1 V (cycle 5–6, day 25–40).

<Fig. 2>

3.2. Organic and nitrogenous content removal

Fig. 3A/B explains the COD removal rate at various operating cycles. The COD removal in MEC-1 (cycle 1–3) was in the range of 42 - 50% (1.12 – 1.17 g COD L^{-1} d^{-1}) (Fig. 3A). At 0.8 V, the COD removal efficacy substantially decreased to 15.1 – 32% (cycle 4–5) from an initial concentration of 6.74 – 11.11 g L^{-1} d^{-1}. The COD elimination further increased to 47±11 – 73±8% equating to 2.68±1.26 g COD L^{-1} d^{-1} (cycle 6–7) at 1.0 V, describing that E_{ap} has statistical effect on COD removal ($p < 0.01$). MEC-2 demonstrated the same outcome ($p < 0.01$), where 28.5 – 53.3% (cycle 1–2) was abated

from the similar initial concentrations as that of MEC-1 (Fig. 3B). In cycle 3 and 4 (0.8 V *E*ap) only a mean 13–15% COD removal was noticed which increased to 62±6% and 65±7% COD abatement at a degradation rate of 1.53 g L^{-1} d^{-1} at *E*ap of 1.0 V.

It is found that the consumption order of complex ingredients in simulated leachate was acetic acid > butyric acid > propionic acid (data not shown), which is in accordance with Escapa et al. [15], where acetic and butyric acids were easily consumed and propionic acid exhibited a refractory behavior.

As expected, nitrogen revealed quite similar abatement trend as that of COD removal. At 1.0 V applied voltage, MEC-1 decreased the NH_4^+-N concentration in the anolyte from 788±163 to 232±120 mg L^{-1}, representing a removal rate of 196±27 mg L^{-1} d^{-1} (72±5% efficiency) coupled with 36±11% NO_3^--N reduction (12±2 mgL^{-1} d^{-1}) corresponding to a TN removal scale of 265±98 mg L^{-1} d^{-1} (Fig. 3C). The nitrogen at 0.8 V exhibited slightly lower removal trend in both systems with a TN removal rate of 62±12% and 48.5±0.8% in MEC-1 and 2, respectively. On the other hand, MEC-2 presented 31 – 76% NH_4^+-N removal (106±33 mgL^{-1} d^{-1}) followed by 29.3±18.3% NO_3^--N reduction (9.5±5.3 mgL^{-1} d^{-1}) constituting a TN degradation of 56.5±5.3% at a rate of 146±83 mg L^{-1} d^{-1} (Fig. 3D).

<Fig 3>

3.3. Hydrogen production

In both MECs, hydrogen production in the cathode chamber followed the same trend as organic compounds removal regarding applied external voltages ($p < 0.05$). As shown in Table 4, higher hydrogen recoveries (r_{cat}) in the range of 66 – 95% occurred with H_2 evolution of 0.08 – 0.148 L^{-1} La^{-1} d^{-1} (at 1.0 V, MEC-1). However, at 0.8 V, r_{cat} reduced to only 44 – 51% producing a mean Q_{H_2} of 0.024 - 0.043 L La^{-1} d^{-1}. MEC-2 exhibited low r_{cat} of only 21 – 25% (cycle 1 and 2), followed by a surge to 120 and

114%, respectively with high rate of H_2 production in the last two subsequent cycles. When gas evolution started to enhance, pH and electrical conductivity (EC) in both systems rose up to 8.8 and 28 mS cm^{-1} (MEC-1) and 10.19 and 26 mS cm^{-1} (MEC-2) (results not shown).

<Table 4>

3.4. Energy balance and coulombic efficiency

Applied voltage exhibited a clear influence over energy balance as shown in Fig. 4 At 1.0 V ≥ 100% energy recovery was achieved based on energy demand of 8.1×10^{-4} to 0.698 kWh-kg COD^{-1} (MEC-1); whereas >100% energy efficiency was observed at 0.8 V having energy demand of 0.323 and 0.374 kWh-kg COD^{-1} (MEC-1). The highest energy demand at cycle 3 might be due to very long cycle time of 230 h i.e. high-accumulated charge. In MEC-2, < 100% energy efficiency was observed at 1.0 and 0.8 V except cycle 5 and 6, where highest efficiency of 139% was noticed with least energy consumption. The overall energy demand in MEC-1 was observed higher than MEC-2 having a similar trend in hydrogen production rate (Fig. 4).

<Fig 4>

Despite higher current densities, MEC-1 experienced low CEs (calculated with ΔCOD) in the range of 12 – 14.5% except cycle 3 (41%) at 1.0 V, and 18 – 20% at 0.8 V. On the other hand, MEC-2 exhibited 31% CE at the initial two cycles and increased to 32.7 – 35.6% at *E*ap of 0.8V, but declined later (11 – 12.5%) at the terminal cycles (*E*ap = 1.0V) (Table 4).

3.5. Dynamics of anodic biofilm

Three anode samples were taken from the MEC-1 reactor at three different periods (PI, PII and PIII) along with inoculum sample in order to study the microbial

community composition attached onto the electrodes, and the similarities and differences between the eubacterial populations.

Firstly, to have an overall overview of the dynamics of the inoculum and the anode samples of MEC-1, correspondence analyses (CA) based on pyrosequencing results were carried out (Fig. 5A). The CA based on the relative abundances of the all genus presented in all samples. The sum of components (F1 and F2), explains 95% of the total variation among the samples. Over 100 genera were identified in all samples and used to do the matrix for the analysis, but just the main genera were represented in Fig. 5A. Three clusters were obtained during these analyses. The inoculum cluster clearly differentiated from the other two, and the cluster composed for the MEC-1_PI and MEC-1_PII samples, which evolved towards a third cluster formed by MEC-1_PIII sample.

To gain insights about the community structure of all samples that makes the differences between them, the phylogenetic composition at family level was analyzed and represented in Fig. 5B. The native microbiota of the initial inoculum from anaerobic digester showed differences respect to the anodophilic communities (MEC-1_PI, MEC-1_PII and MEC-1_PIII). However, the three anodophilic samples taken showed quite similarities. Moreover, the family diversity is quite high such in the inoculum (represented by 17 families), as in the anode samples (18-21 different families) (Fig. 5B).

In the initial inoculum, 57% of the total community was accounted for four families i.e. *Eubacteriaceae* (22.2%), *Clostridiaceae* (14.5%), *Rhodocyclaceae* (11.0%) and *Comamonadaceae* (9.0%). In terms of composition, the three anodiphilic communities were similar between them, although the relative abundance of these families changed depending on the operational conditions. The dominant phyla identified in the anodic biofilm was *Proteobacteria*, represented by 11 families, some of them previously described in BES, such as *Pseudomonadaceae*, *Geobacteraceae*,

Comamonadaceae, *Rhodospirillaceae* and *Rhodocyclaceae* among others. *Bacteroidetes* and *Clostridia* were the other two dominant phyla identified.

<Fig 5>

In order to find out which OTUs showed a greater trend to establish themselves in anode biofilms, the presence of the common OTUs in the three different samples were compared among them (Fig. S1, supplementary material). The total amount of the observed OTUs in the three anodophilic biofilms were 1,572. The most part of these OTUs (604) were shared between MEC-1_PI and MEC-1_PII, representing to 38.4% of the total OTUs. Besides, a total of 529 OTUs, equivalent to 33.7% of the total community were shared between the three samples (MEC-1_PI, MEC-1-PII and MEC-1_PIII). These OTUs, which prevailed attached onto the anode electrodes despite the different operational conditions, could be considered the core microbiome.

4. Discussions

As it is clear from Fig. 2–4 and Table 4, the applied external voltage, temperature and influent COD content exhibited a clear impact on the overall system performance i.e. current density, substrate removal, energy consumption and hydrogen production. MEC current was generated by microbial oxidation of organic matter at the anode, and the presence of some microorganisms previously identified in BES as *Pseudomonadaceae, Comamonadaceae and Geobacteraceae*. The COD and nitrogen removal in the anode chamber increased with increasing current density [21], but prolong cycle durations as in cycle 3 (MEC-1) not only slow down the substrate removal rate but also the hydrogen production [22]. The system eliminated a maximum 77.5 and 65.7% COD, equating to treatment rate of 2.68 g COD L^{-1} d^{-1} and 1.53 g COD L^{-1} d^{-1} in MEC-1 and 2, respectively at *E*ap of 1.0 V. As can be seen in Figure 3A/B, influent organic load has influenced the COD removal efficiency, suggesting that it may reach the saturation

conditions. Gil-Carrera et al. [23] found similar result in a semi-pilot scale MEC applying various organic loading rates.

On the basis of existing literature and operating conditions it is hypothesized that certain pathways might be involved in nitrogen removal in this study. As can been seen in Fig. 3C/D, the anolyte nitrogen removal was mainly attributed by NH_4^+-N in both systems. Ammonium transport to the cathode rises with increasing current density and it also depends on concentration gradient between anolyte and catholyte [21,24]. Although cathodic nitrogen content was not monitored in this study, however, the conductivity profiles in the anode and cathode chambers support this hypothesis. It was found that the conductivity of anolyte decreased from 14.6±2.3 to 10.3±1.2 mS cm^{-1}, whereas catholyte conductivity increased from 21.3±1.1 to 28.6±7.0 mS cm^{-1}. Nevertheless, this phenomenon was not merely attributed by NH_4^+-N migration but also ensured by other cations such as Na^+, K^+ and protons [25]. A little fraction of partial nitrification cannot be ruled out in the system as the reactor was exposed to oxygen prior to replenishment of the electrolyte in each batch cycle. In MECs, limited ammonium removal was observed with micro-aerobic conditions increasing the nitrification rate [26,27]. NH_4^+-N removal can also be accomplished through bioelectrochemical denitrification and anammox process, since the anoxic/anaerobic anode chamber is best suited for such processes [28]. In an ammonium-fed MEC system, Zhan et al. [27] also observed ammonium oxidation in the presence of nitrite or nitrate under an applied voltage. Nitrate removal might be due to denitrification in the anode chamber as the presence of pseudomonads corroborates the process. The possible pathways of nitrogen removal from anodic chamber in MFC has been explained in our previous work [24], where young and old landfill leachate was employed as substrate.

Although MEC-2 consumed lower energy than MEC-1 but based on other efficiencies, like COD removal and hydrogen production, the later has been taken as a reference system during comparative evaluation with other systems/technologies. The reason why MEC-1 performed better than MEC-2 is because the former was controlled at 30 °C whereas, the later at a wide range of room temperature (17±3 °C). Literature suggest that in BES, electrochemically active microbes rely upon an operational temperature of 30 °C [29,30]. At mesophilic conditions a clear specialization in anodophilic microorganisms were observed from cluster formed for inoculum sample to cluster formed for MEC-1_PI and PII and the third group formed for MEC-1_PIII (Fig. 5A). Although the microbial diversity results similar both in the inoculum and in the biofilms samples, a clear enrichment in *Proteobacteria* phylum was observed. Some of well-known electroactive bacteria belong to this group as *Pseudomonadaceae*, *Comamonadacea* and *Geobacteraceae* (Fig. 5B). The reason for such substantial decline in performance of MEC-2 could be the slower growth of electroactive bacteria. At cycle 5 and 6 of MEC-2 there was an increase of the ambient temperature that can explain the improvement in the overall system efficiency. In this way not only applied voltage but also temperature exercised a clear impact on the system performance.

Coulombic efficiency (CE, %) is a parameter for the assessment of efficiently converting chemical energy stored in the substrate/wastewater into electrical energy, or in other words how much of the removed COD was converted to electrons. However in MECs, COD abatement is the main goal of wastewater treatment coupled with minimizing the overall energy demand for the process [22,31]. Low electrical energy input is more beneficial than high consumption of chemical energy (i.e. high CE) [30]. The reason of low CEs at *E*ap of 1.0 V despite higher current densities may reveal that a fraction of COD removed was due to certain other non-electrogenic activity. Another

possible reason might be the aerobic degradation of substrate sustained by oxygen intrusion, as the system was exposed to oxygen for 15 – 20 min prior to substrate replenishment in order to suppress methanogens [32]. In general, the long cycle time resulted in higher CEs, for example 41% in cycle 3 with total duration of 230 h. This phenomenon was in accordance with Wagner et al. [33] where highest CE was achieved in the long batch test of 184 h, having low recovery of hydrogen at the cathode (29±2%).

The high hydrogen recoveries in the MEC system was not only from high current densities but also owing to high conversion rate of electrons to hydrogen (i.e. cathodic hydrogen recoveries, r_{cat}), meaning that 66 – 95% of the electrons captured from the substrate were transferred into current at *E*ap of 1.0 V (Table 4). The gas production rate in both systems was highly variable based on applied voltage and cycle duration, for example at cycle 3 of MEC-1, long cycle time (230 h) resulted in a comparatively low recovery of hydrogen (76%), similar trend as observed by Wagner et al. [33]. However, the gas composition was consistent and highly enriched in H_2 (97 – 99%) along with 0.5–1.5% CH_4 and 0.5 – 1.0% CO_2, revealing that the MEC system produced pure hydrogen gas. The intermittent oxygen exposure between batches may suppress methanogenic growth [19,34], hence very negligible portion of CH_4 appeared in the cathode. Moreover, operating MEC at a relative higher *E*ap (> 0.6 V) is able to reduce methane production and improve the H_2 recovery [35], but higher than 1.0 V *E*ap exhibit decrease in H_2 and increase in methane production [36]. The higher r_{cat} might also be linked to the phenomenon that no hydrogen recycling effect occurred between anode and cathode, as it is exhibited by lower CEs (< 100%).

4.3. Comparative evaluation with other studies

Table 5 shows the comparative evaluation of this study with other existing hydrogen producing technologies. Our results exhibited least energy requirements of 2.7

– 10.1 kWh-kg H_2 and higher energy recovery (MEC-1, 1.0 V *E*ap) than other technologies i.e. partial oxidation of heavy oils, coal gasification, steam methane reforming and grid water electrolysis. Moreover, the specific energy was below the net energy consumption threshold traditionally associated with aerobic treatment of domestic wastewater (0.7 - 2.0 kWh kg^{-1} COD) [37]. The data of energy recovery suggested that MEC is an energy-producing device at 1.0 V *E*ap.

The estimated cost of hydrogen production via electrohydrogenesis due to the electrical energy input was lower than reported in literature. For example, using B-glycerol substrate, the energy cost was 0.945 € kg^{-1} H_2 at applied voltage of 0.5 V and € 1.11 kg^{-1} H_2 at 0.9 V *E*ap, having energy demands of 2.32 kWh/m^3 H_2 (0.5 V *E*ap) and 2.60 kWh/m^3 H_2 (0.9 V *E*ap), respectively [38]. These calculations were based on current wholesale electricity prices of € 38.65 per MWh, tax not included. Similarly, Batlle-Vilanova et al. [39] estimated hydrogen production cost of 3.02 € kg^{-1} H_2 at poised cathode potential of –1.0 V vs Ag/AgCl, and Cusick et al. [40] obtained comparatively low cost of 2.84 € kg^{-1} H_2 for a MEC decontaminating domestic wastewater using a Pt catalyst. In this study, the energy cost was estimated to be 0.67±0.24 € kg^{-1} H_2 (electricity prices of 150 € per MWh, tax included; www.omie.es) at energy demand of 0.376±0.179 kWh per m^3 H_2, which was even lower than H_2 production via steam methane reforming (0.71€ kg^{-1} H_2). In this way our system is accredited to produce low cost and pure hydrogen (≈99%) with low energy consumption.

Although MEC proved to be energy efficienct technology but certain other parameters also need to be taken into consideration for practical applications of MECs like operating and capital costs, sludge production, environmental factors, HRTs and organics strength etc. Further research is required treating real wastewaters under realistic conditions in order to make this ttechnique more viable. Moreover, correlating this

technology with certain other hydrogen producing technologies may give an understanding towards economical balanced hydrogen.

4.4. Challenges and future perspectives

MEC technology is still in its infancy due to several inherent factors. The gap between rhetoric and reality in MEC research is substantial. This technology is mostly limited to the bench-scale studies ranging from a few milliliters to several liters at most. Although few researchers applied this technology in pilot-scale dealing with real wastewaters but were unable to attain a rational throughput. One more challenge which MECs confront is the start-up time ranging from weeks to months, which limits its practical application. Electrode materials and cathode catalysts are another concern in scaling-up this technology. Certain noble elements like platinum etc. have proved to be effective, however their high cost and short life time make them unreasonable for industrial-scale application. So far carbon-based materials are the most frequently employed electrodes due to their low cost and good biocompatibility, however high overpotentials and large ohmic/voltage loss make them less viable.

In this way, certain parameters need to be taken into consideration for practical applications of MECs like operating and capital costs, sludge production, environmental factors, HRTs and organics strength etc. Moreover, it needs further research treating real wastes under realistic conditions using plausible materials in order to make this technology reliable. As for as energy output in terms of hydrogen generation is concerned, correlating this technology with other existing techniques may give an understanding towards economical balance of the hydrogen.

Additive manufacturing or three-dimensional (3D) technology can be applied for electrode configuration, ion exchange membrane manufacturing or reactor designing. In MFCs, flat 2D porous anodes have small pore sizes, and bacteria can only clog on the surface and are out-of-the-way to the interior of the anode. This subsequently limits the anode efficacy. To overcome this glitch, 3D structured anodes have been devised, which provide larger bio-accessible area which promotes formation of active biofilm and effective substrate transfer simultaneously [41]. Moreover, Modeling and simulations may help design and refine such studies.

5. Conclusion

The system eliminated a maximum 73±8 % COD, equating to treatment rate of 2.68 g COD L^{-1} d^{-1} (MEC-1) and 65±9% (MEC-2) with least energy consumption. The overall energy demand in MEC-1 was observed higher than MEC-2. Applied voltage, temperature and HRT imparted a clear impact on the overall system efficiency. At 1.0 V *E*ap, a maximum 0.148 L H_2 La^{-1} d^{-1} (MEC-1) and 0.04 L H_2 La^{-1} d^{-1} (MEC-2) was produced in batch mode. Nitrogen removal (mostly NH_4^+-N) was occurred in the system obeying different removal pathways. The system also exhibited a high hydrogen recovery (66 – 95%), pure hydrogen yield (98%) and tremendous working stability during two months of operation. Regarding the eubacterial community, the native microbiota of the initial inoculum showed differences respect to the anodophilic communities which remain quite stable along different operational conditions, sharing 33.7% of the total OTUs. Besides, both electroactive and hydrogen producing bacteria were enriched at the anodic biofilm. The results of this study specified that MEC is an energy-efficient technique to deal with certain waste streams; however further research is needed treating real leachate under realistic conditions to make this technology reliable.

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FIGURE CAPTIONS

Fig 1: Schematics of the experimental set-up.

Fig 2: Current density profiles of MEC-1 (A) and MEC-2 (B) operating at various applied potentials (batch mode). MEC-1 was operated at controlled

Fig. 3: COD removal of MEC-1 (A) and MEC-2 (B) and Nitrogen removal efficiency of MEC-1 (C) and MEC-2 (D) along various cycles in batch mode operation.

Fig 4: Energy balance and efficiency of MEC-1 (A) and MEC-2 (B).

Fig. 5: Correspondence analysis (CA) of eubacterial communities from Inoculum, MEC-1_PI, MEC-1_PII and MEC-1_PIII samples, based on pyrosequencing of eubacteria *16S rRNA gene* with genus level taxonomy matrix (B): Taxonomic classification of pyrosequencing of *16S rRNA gene* from eubacterial communities at family level

TABLE CAPTIONS

Table 1: Employing complex substrates in MEC.

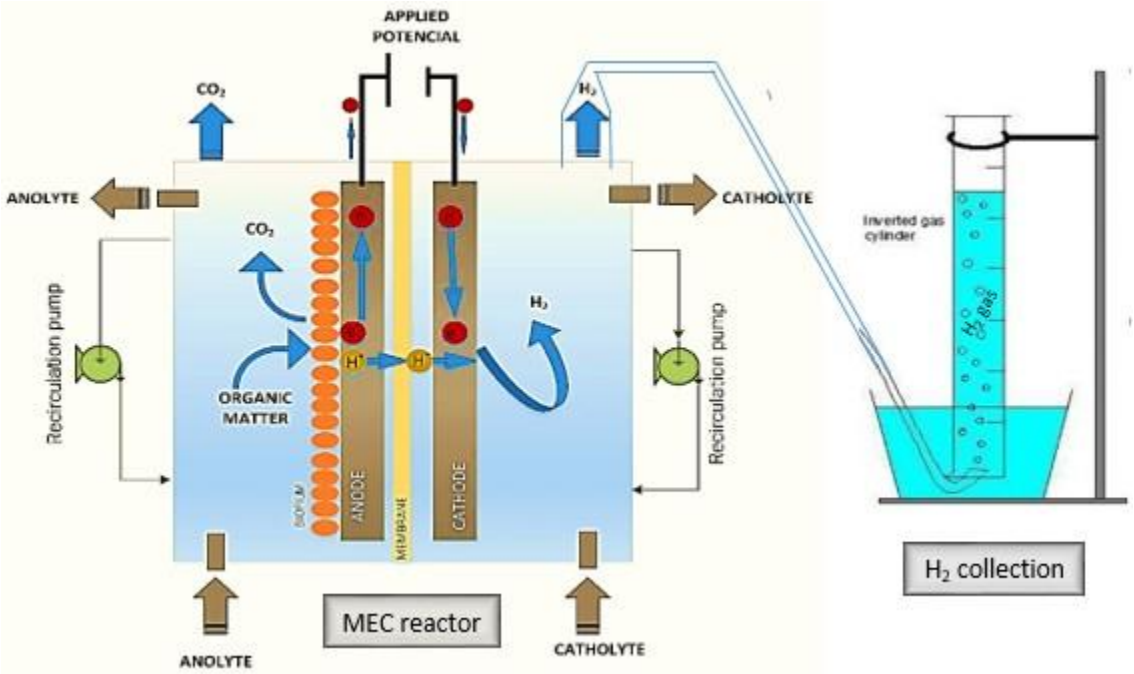
Table 2: Applied conditions for MEC-1 and MEC-2

Table 3: Chemical characteristics of simulated leachate employed in this study (n = 10)

Table 4: MEC system performance along different cycles and applied voltages (*Eap*) at batch mode operation

Table 5: Comparative evaluation of the results obtained in this study with other existing hydrogen producing technologies.

Figures



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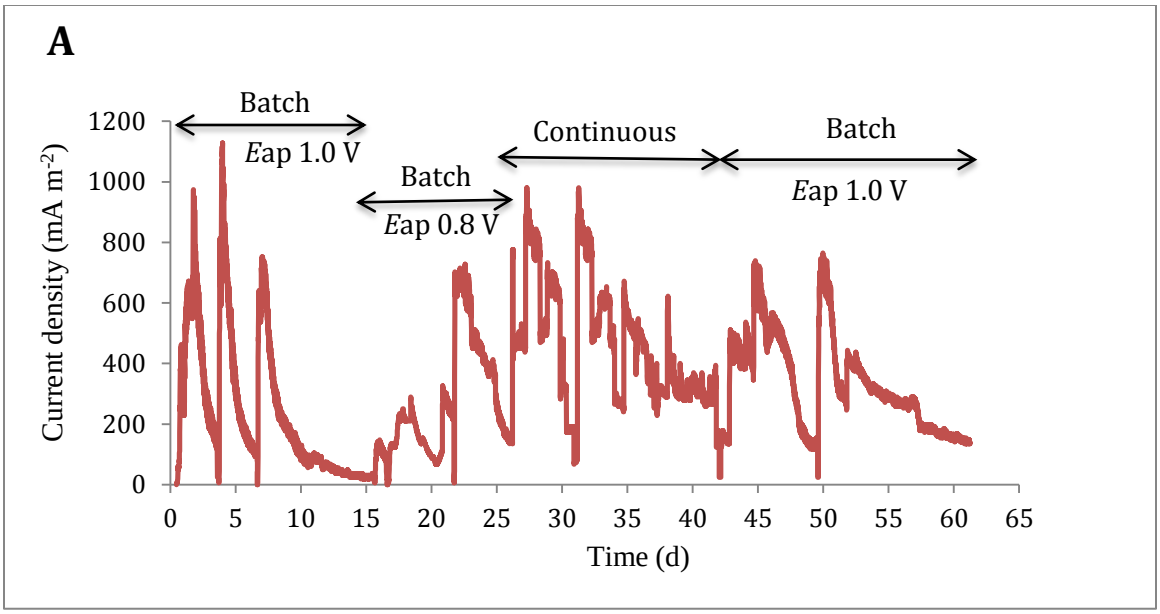

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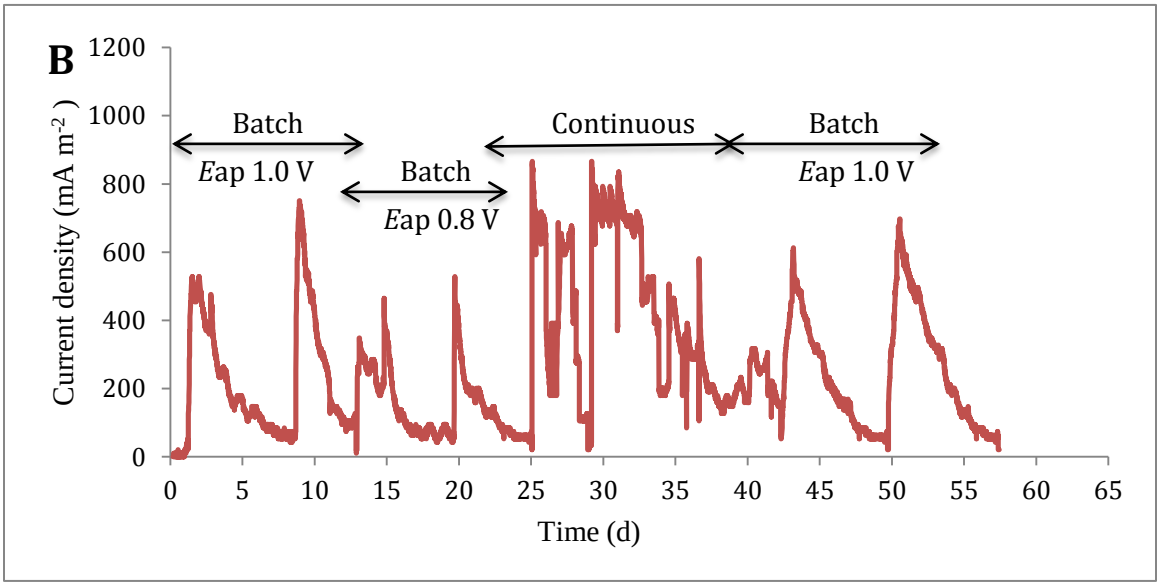
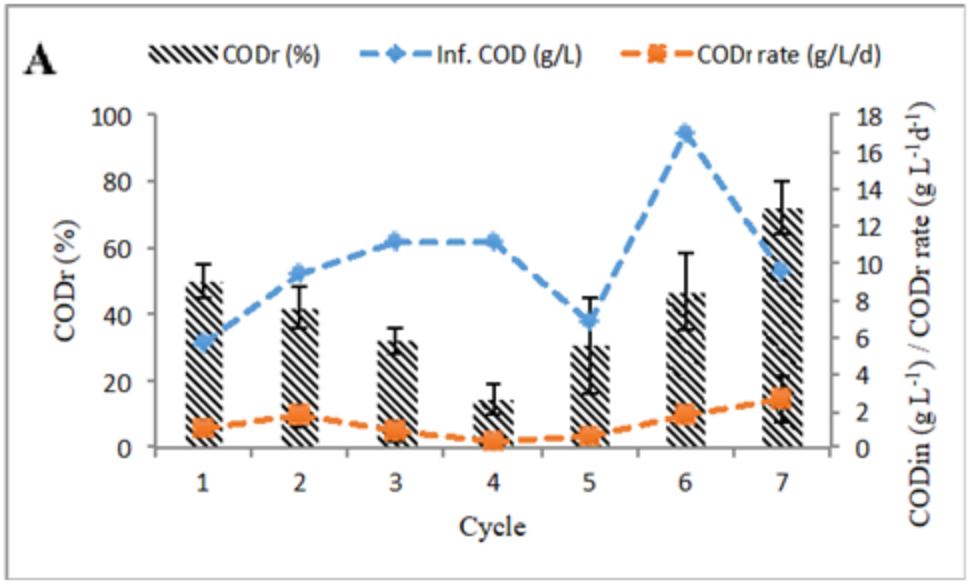
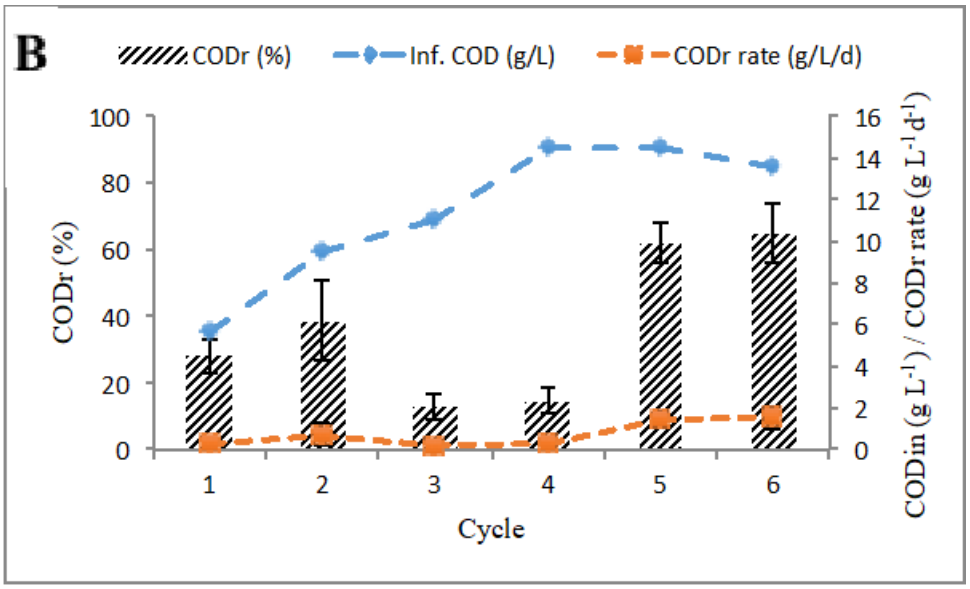


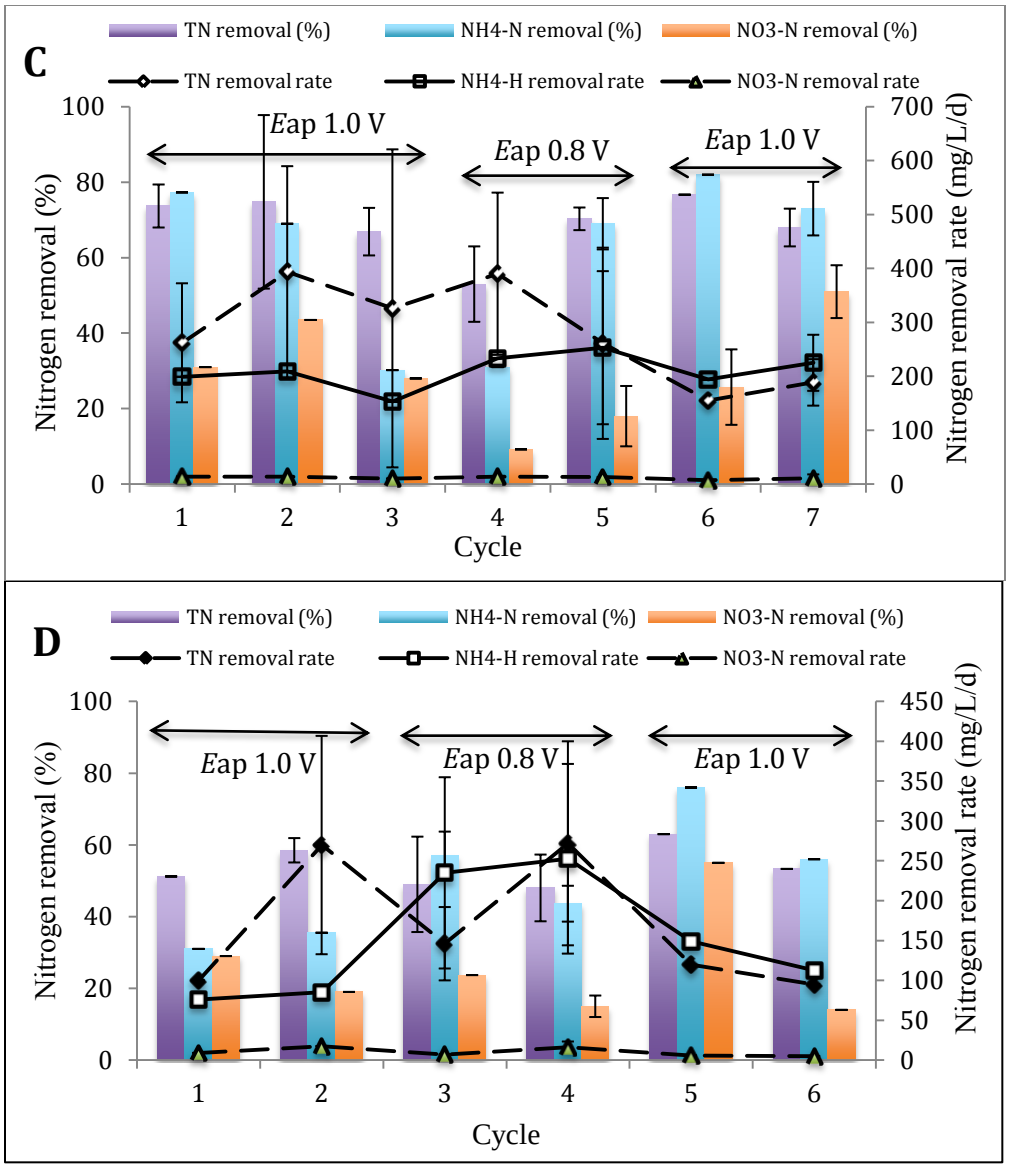
686 **Fig 2:** Current density profiles of MEC-1 (A) and MEC-2 (B) operating at various
687 applied potentials (batch mode). MEC-1 was operated at controlled temperature (30 ℃)
688 and MEC-2 at room temperature (17±3 ℃).


A
CODr (%)
Inf. COD (g/L)
CODr rate (g/L/d)
CODr (%)
CODin (g L-1) / CODr rate (g L-1 d-1)
Cycle

689
690


B
CODr (%)
Inf. COD (g/L)
CODr rate (g/L/d)
CODr (%)
CODin (g L-1) / CODr rate (g L-1 d-1)
Cycle

691



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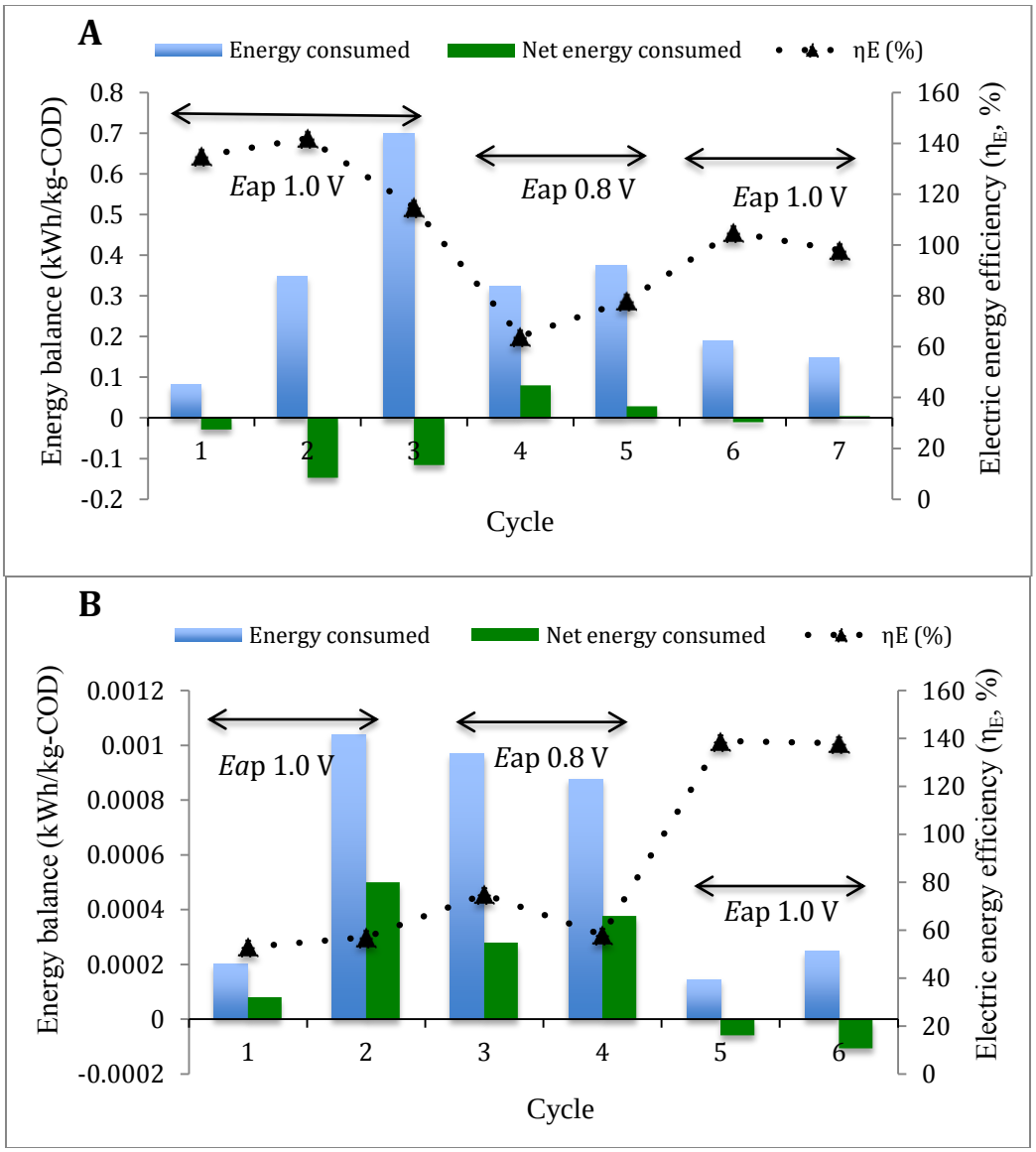
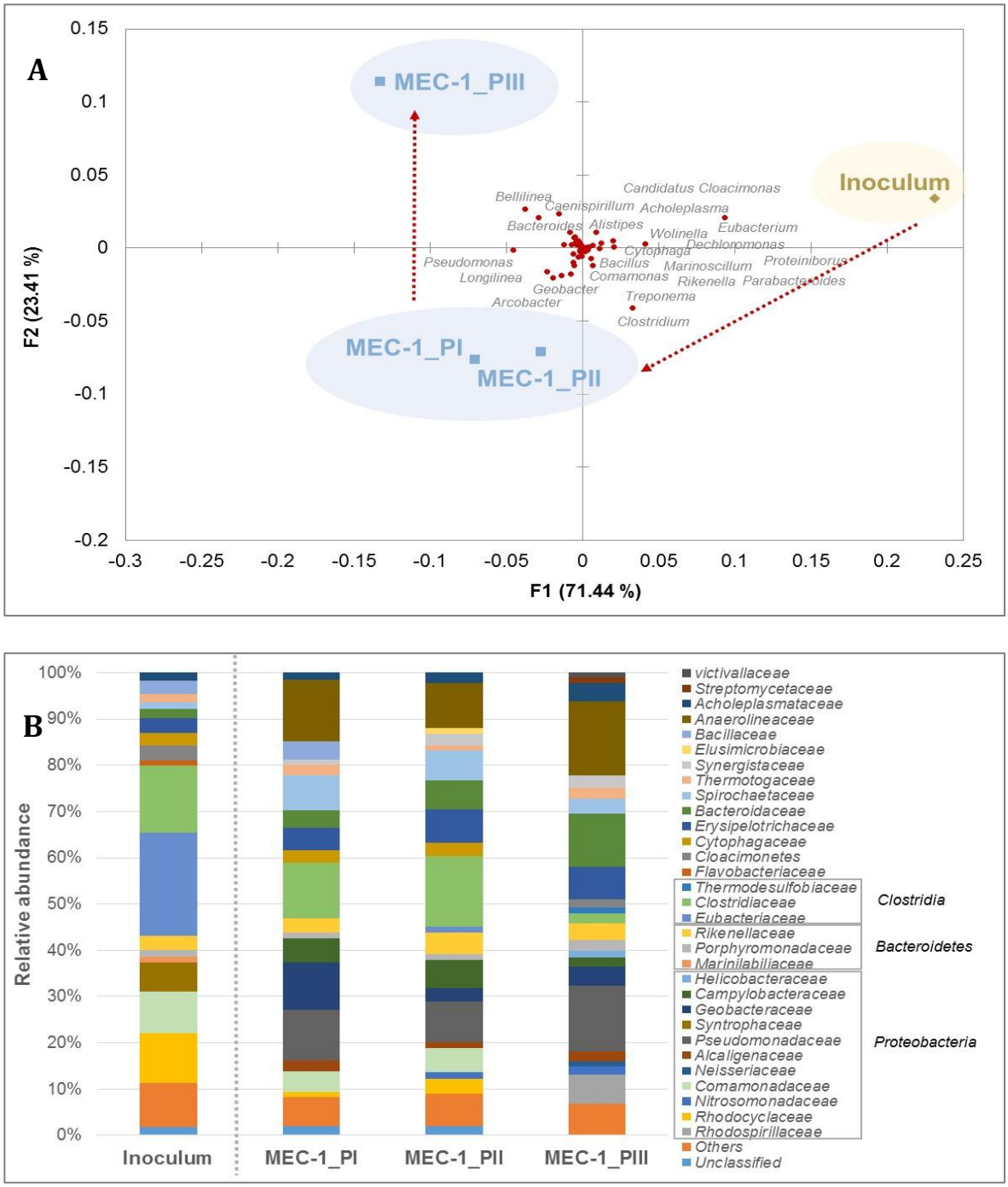


699

700
701
702 **Fig. 4:** Energy balance and efficiency of MEC-1 (A) and MEC-2 (B). E_{ap} of all cycles
703 are 1 V, except 0.8 V in cycle 3 and 4 (MEC-1) and cycle 4 and 5 (MEC-2).



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Tables

Table 1: Employing complex substrates in MEC.

Substrate	Reactor type	Volume (mL)	E_{ap} (V)	j (A/m^3)	CE (%)	$\mathbf{r}_{cat}$ (%)	Biogas composition (% H_2)	Q_{H_2} (m^3 m^{-3}.d)	Reference
Glycerol	SC	28	0.8	100	35->100	4	5	0.021	[42]
Starch	SC	28	0.8	25	35->100	0	0	0	[42]
Milk	SC	28	0.8	75	30->100	13	76	0.086	[42]
Diluted LPW	SC	100	3.0	115.9±7.2*	118-152	5 - 21	16-50	0.12 - 0.38	[43]
Food processing	SC	280	0.7	1.0-2.4[b]	29±2		32±4	0.12±0.02	[44]
Food processing	SC	280	0.7	1.2-2.1[b]	12±2		86	0.8-1.8	[44]
Raw activated sludge	DC	250	-	8	28±10	69±9	n.a.	0.056±0.008	[45]
Swine WW	DC	28	0.5	106-112	29-70	29-61	58-64	0.9-1.0	[33]
Domestic WW	SC	120000	1.1	0.135*		60	100±6.4	0.015	[20]
Landfill leachate	SC	320	-3.0[a]	0.11±0.06 [b]	1.8±0.5	n.a.	n.d.	n.d.	[13]
Fenton-treated leachate	SC	320	-3.0[a]	1.42±0.27 [b]	29±0.5	n.a.	n.d.	n.d.	[13]
Landfill leachate	DC	320	0.3[a]	2.5	56	n.a.	n.d.	n.d.	[46]
Pre-fermented leachate	DC	320	0.3[a]	23	68	n.a.	n.d.	n.d.	[46]
Domestic WW	SC	2000	1.0	0.22 [b]	28-30	18.6	100	0.05	[47]
Simulated landfill leachate	DC	1000	1.0	1.0-1.2 [b]	12-41	66-95	98	0.04±0.06	This study

Index * implies that the parameter has been computed from the actual existing data in respective publication. *LPW*: Liquid faction of pressed municipal solid waste; *SC:* Single-chambered; *DC*: Dual-chambered

[a] Anodic poised potential (V) vs Ag/AgCl

[b] j (A/m^2)

720 **Table 2**: Applied conditions for MEC-1 and MEC-2

Reactor	Cycle	Operation mode	Operation time (d)	*Eap* (V)	Temperature (°C)
MEC-1	1	Batch	1-4	1.0	30
	2	Batch	5-8	1.0	30
	3	Batch	9-15	1.0	30
	4	Batch	16-20	0.8	30
	5	Batch	21-26	0.8	30
		Continuous	26-42	**	30
	6	Batch	42-50	1.0	30
	7	Batch	50-62	1.0	30
MEC-2	1	Batch	1-8	1.0	17±3
	2	Batch	9-13	1.0	17±3
	3	Batch	14-19	0.8	17±3
	4	Batch	20-25	0.8	17±3
		Continuous	26-42	**	30
	5	Batch	42-50	1.0	17±3
	6	Batch	50-68	1.0	17±3

721 ** *Eap* ranging from 0.5-1.0 V
722

723 **Table 3:** Chemical characteristics of simulated landfill leachate employed in this study (*n*
724 = 10)
725

Parameter	Unit	Value (± Standard deviation)
pH		7.86 ± 0.63
Electrical conductivity (EC)	mS cm^{-1}	16.11 ± 3.01
Oxidation-Reduction Potential (ORP)	mV	200.63 ± 31.72
Chemical Oxygen Demand (COD)	g L^{-1}	14.33 ± 4.42
Total Organic Carbon (TOC)	g L^{-1}	4.81 ± 1.31
Inorganic Carbon (IC)	g L^{-1}	0.44 ± 0.17
Total Carbon (TC)	g L^{-1}	5.59 ± 1.61
Total Nitrogen (TN)	g L^{-1}	0.80 ± 0.13
Ammonium Nitrogen (NH_4 - N)	g L^{-1}	0.72 ± 0.10
Nitrate Nitrogen (NO_3 - N)	g L^{-1}	0.04 ± 0.01
Phosphate (PO_4–P)	g L^{-1}	0.012±0.008
Volatile Fatty Acids (VFAs):		
– Acetic acid	g L^{-1}	4.86 ± 1.39
– Propionic acid	g L^{-1}	3.64 ± 0.76
– Butyric acid	g L^{-1}	0.98 ± 0.06

726

727 **Table 4:** MEC system performance along different cycles and applied voltages (*Eap*) at
728 batch mode operation
729

Reactor	Cycle #	1	2	3	4	5	6	7
MEC-1	*Eap* (V)	1.0	1.0	1.0	0.8	0.8	1.0	1.0
	Cycle time (h)	85	75	230	72	189	153	115
	CE (%)	14.5	13.4	41	20	18	12	14
	r_{cat} (%)	91	96	76	44	51	89	66
	Q_{H_2} (L $La^{-1}d^{-1}$)	0.148±0.116	0.132±0.103	0.109±0.102	0.024±0.01	0.043±0.01	0.08±0.04	0.10±0.05
MEC-2	*Eap* (V)	1.0	1.0	0.8	0.8	1.0	1.0	
	Cycle time (h)	192	124	163	110	156	128	
	CE (%)	31	31	35.6	32.7	11	12.5	
	r_{cat} (%)	21	25	39	23	120	114	
	Q_{H_2} (L $La^{-1}d^{-1}$)	0.004±0.002	0.012	0.01±0.01	0.01±0.01	0.03±0.03	0.04±0.06	

730

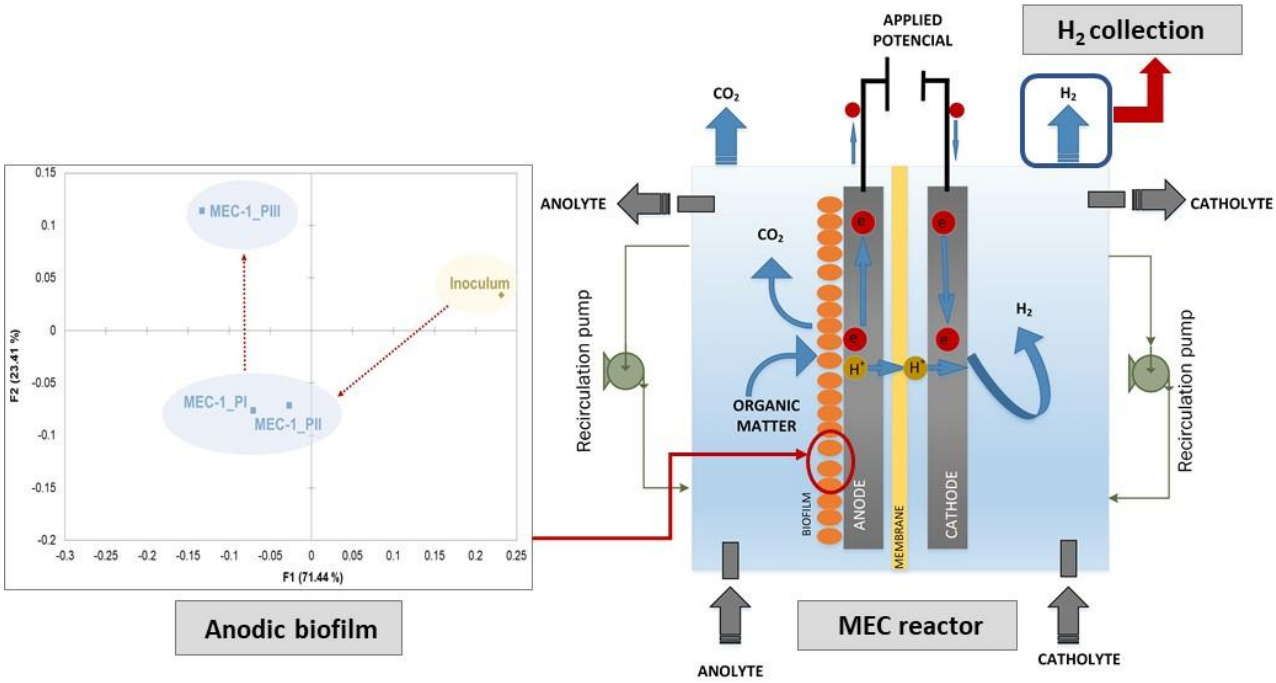
731 **Table 5:** Comparative evaluation of the results obtained in this study with other existing
732 hydrogen producing technologies.

Specifics	This study	Biotic MEC	Steam methane reforming	Partial oxidation of heavy oil	Coal gasification	Grid electrolysis of water
Energy efficiency (η_E, %)	46 − 142	53 – 175	70 – 80	70 – 74	60	27
Energy consumption (kWh kg^{-1} H_2)	2.7 – 10.1	33.2 – 117	22.4	54.9	96.3	54.9
H_2 production cost (€ kg^{-1} H_2)	0.67 ± 0.24	3.04	0.71	1.32	1.15 – 2.30	2.43 -2.82

733 Data obtained from Ref. [1,39,48]
734

735 **Graphical Abstract**

736



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Supplementary Material

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