Assessment of biotic and abiotic graphite cathodes for hydrogen production in microbial electrolysis cells

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Abstract
Hydrogen represents a promising clean fuel for future applications. The biocathode of a two-chambered microbial electrolysis cell (biotic MEC) was studied and compared with an abiotic cathode (abiotic MEC) in order to assess the influence of naturally selected microorganisms for hydrogen production in a wide range of cathode potentials (from −400 to −1800 mV vs SHE). Hydrogen production in both MECs increased when cathode potential was decreased. Microorganisms present in the biotic MEC were identified as Hoeflea sp. and Aquiflexum sp. Supplied energy was utilized more efficiently in the biotic MEC than in the abiotic, obtaining higher hydrogen production respect to energy consumption. At −1000 mV biotic MEC produced 0.89 ± 0.10 m³ H₂ d⁻¹ m⁻³ NCC (Net Cathodic Compartment) at a minimum operational cost of 3.2 USD kg⁻¹ H₂. This cost is lower than the estimated market value for hydrogen (6 USD kg⁻¹ H₂).

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1. Introduction

Hydrogen is a sustainable energy carrier, which releases water as the only product when it is burnt and it can be produced from a variety of sources. Nowadays, most of produced hydrogen comes from large-scale processes such as gasification, pyrolysis, thermochemical water splitting, steam reforming and electrolysis [1]. These processes use fossil fuels, consume large amounts of energy or both. For this reason they contribute significantly to global warming, mainly due to carbon dioxide emissions and large electric consumption, especially when it comes from non-renewable sources. Even though these methods are energy intensive, at present they are the only way of supplying large amounts of H₂ for industrial application.

One of the most promising technologies for a future sustainable production of hydrogen is the use of microbial electrolysis cells (MEC). Theoretically, a relatively low amount of voltage (<414 mV), which could be produced from renewable sources, is required to drive the process. But in practice this voltage is substantially increased due to overpotentials of the system [2].

In the conventional MEC configuration, microorganisms are used in the anode chamber to recover energy contained in organic matter. This oxidation generates protons and...
electrons, which are transferred to an electrochemical cathode containing a metal catalyst (i.e. platinum, nickel or stainless steel) enhancing hydrogen production [3–8]. However, the use of metal catalysts requires high capital and operational costs and they have to be constantly replaced, mainly due to corrosion or deactivation problems. An alternative approach is the use of microorganisms as a biological catalyst in the cathode chamber. Electrotrophic microorganisms are able to accept electrons directly or indirectly and may use them to reduce protons to hydrogen, as described in Equation (1).

\[2H^+ + 2e^- \rightarrow H_2. \quad E^0 = -414 \text{ mV}\]  

(1)

Some recent studies have been focused on hydrogen production and its coproduction using microorganisms in the biocathode of a MEC [9–14]. Table 1 summarizes some literature studies using MECs. Generally, higher volumetric hydrogen productions were achieved using a metal catalyst in an abiotic cathode. Cheng and Logan [3], who obtained 17.80 m³ H₂ m⁻³ MEC d⁻¹, demonstrated that Platinum (Pt) showed a good performance. But the highest production rate was achieved by Jeremiass et al. [8] using Nickel (Ni) foam as cathode catalyst. They produced hydrogen at a maximum rate of 50.00 m³ H₂ m⁻³ MEC d⁻¹, which decreased during operation due to anode and cathode overpotentials.

Hydrogen production rates by microorganisms in a biocathode are usually one order of magnitude lower than those obtained with metal catalysts [9–11,13,14]. The highest volumetric hydrogen production rate obtained by Jeremiass et al. [12] with a biocathode was 2.20 m³ H₂ m⁻³ Net Cathode Compartment (NCC) d⁻¹, being the only example in which a biocathode yielded hydrogen production rates in the range of those obtained by metal catalysts.

On the other hand, in terms of cathodic hydrogen recovery (i.e. electrons from the anode recovered in the form of hydrogen at the cathode), up to date biocathodes are found to reach lower values than metal catalyzed cathodes. Using a biocathode, Rozendal et al. [9] reached a maximum cathodic hydrogen recovery of 57%, which was much higher than the control electrode used in the same study (25%). Meanwhile values up to 93% were obtained using nickel [8], 84% using stainless steel [7] and 96% using platinum as catalyst [4]. However, other authors noted [15] that the use of biocathodes could reduce costs of construction and operation of the system. Moreover, it could overcome most of the problems related to the use of metal catalysts such as corrosion or deactivation.

There are still some gaps in the knowledge about how biocathodes work and which microorganisms are involved in hydrogen production. A better understanding of microorganisms and its metabolic pathways could improve hydrogen production rate and energy recovery in the near future. Thus it could make MEC biocathodes a promising cost-effective production platform for hydrogen gas [10].

Although optimistic results have been obtained in recent studies, further efforts are needed to improve MECs for hydrogen production and make this an economically feasible process [8,12]. In this study, a biotic and an abiotic (pure electrochemical reactions) MEC for hydrogen production were thoroughly studied. Measurements of hydrogen production were conducted to compare both systems in a wide range of cathode potentials. Although Rozendal et al. [10] already compared a naturally selected culture (biocathode) with a control (abiotic), they did it at an unique cathode potential of –700 mV vs SHE. As far as we know, this is the first time that a naturally selected culture in a biocathode and an abiotic cathode are compared in a wide range of cathode potentials.

### Table 1 – Results obtained with different cathode catalysts from previous studies.

<table>
<thead>
<tr>
<th>Operational mode</th>
<th>Cathode material</th>
<th>Cathode potential (mV vs SHE)</th>
<th>Applied potential (mV)</th>
<th>Qₜₜ (m³ H₂ m⁻³ cathode liquid volume day⁻¹)</th>
<th>Coulombic efficiency, CE (%)</th>
<th>Cathodic hydrogen recovery rₕₕ (％)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>n/a</td>
<td>500</td>
<td>0.24⁹</td>
<td>n/a</td>
<td>21</td>
<td>[11]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>–700</td>
<td>n/a</td>
<td>2.20⁸</td>
<td>n/a</td>
<td>50 ± 2.3</td>
<td>[12]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>–590</td>
<td>n/a</td>
<td>0.29⁹</td>
<td>54</td>
<td>n/a</td>
<td>[13]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>–710</td>
<td>500</td>
<td>0.04</td>
<td>92 ± 6.3</td>
<td>57 ± 0.1</td>
<td>[2]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>–700</td>
<td>n/a</td>
<td>0.63</td>
<td>n/a</td>
<td>49</td>
<td>[3]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>–750</td>
<td>n/a</td>
<td>0.01⁸</td>
<td>~80</td>
<td>n/a</td>
<td>[14]</td>
</tr>
<tr>
<td>Single-chamber MEC</td>
<td>Carbon cloth with Pt (0.5 mg cm⁻²)</td>
<td>n/a</td>
<td>800</td>
<td>3.12 ± 0.02¹</td>
<td>96.8 ± 1.4</td>
<td>96 ± 1.1</td>
<td>[6]</td>
</tr>
<tr>
<td>Single-chamber MEC</td>
<td>Carbon cloth with Pt (0.5 mg cm⁻²)</td>
<td>n/a</td>
<td>1000</td>
<td>17.80</td>
<td>n/a</td>
<td>93</td>
<td>[5]</td>
</tr>
<tr>
<td>Single-chamber MEC</td>
<td>Stainless steel type 304#60 mesh</td>
<td>n/a</td>
<td>900</td>
<td>1.40 ± 0.13³</td>
<td>87 ± 5</td>
<td>n/a</td>
<td>[8]</td>
</tr>
<tr>
<td>Single-chamber MEC</td>
<td>Stainless steel brush cathodes type 304</td>
<td>n/a</td>
<td>600</td>
<td>1.70 ± 0.1¹</td>
<td>n/a</td>
<td>84</td>
<td>[9]</td>
</tr>
<tr>
<td>Single-chamber MEC</td>
<td>60 mg Ni in 267 μL Nafion on carbon cloth</td>
<td>n/a</td>
<td>600</td>
<td>1.30 ± 0.3³</td>
<td>92.7 ± 15.8</td>
<td>79 ± 10</td>
<td>[7]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Ni foam</td>
<td>n/a</td>
<td>1000</td>
<td>50.00</td>
<td>n/a</td>
<td>90</td>
<td>[10]</td>
</tr>
</tbody>
</table>

⁹ Referred to MEC total liquid volume.

¹ Calculated from given data; n/a: non-available.
(from -400 to -1800 mV vs SHE) for hydrogen production. Due to relative lack of information about which microorganisms are involved in hydrogen production, we identified dominant members of the naturally selected communities in the biocathode using high-throughput molecular methods.

2. Materials and methods

2.1. Experimental setup

A two-chambered MEC was constructed using a previously described design [16]. The MEC consisted of an anode and a cathode placed on opposite sides of a single methacrylate rectangular chamber. The anode and cathode chambers were filled with granular graphite (model 00514, diameter 1.5–5 mm, EnViRo-cell, Germany), which decreased the volumes to 400 mL net anodic compartment (NAC) and 390 mL NCC, respectively. The electrodes were previously washed in 1 M HCl and 1 M NaOH to remove possible metal and organic contamination. Two thinner graphite electrodes (130 × 4 mm [anode] and 130 × 4 mm [cathode], Sofacel, Spain) were introduced on each chamber. A cation exchange membrane (CMI-7000, Membranes International Inc., USA) was placed between the anode and cathode frames. Synthetic water was continuously fed at 1.75 and 1.51 L d⁻¹ between the anode and cathode frames. Synthetic water was constructed, resulting in an NCC of 400 mL, and operated as a control.

The cathode potential was monitored with an Ag/AgCl reference electrode (+197 mV vs Standard Hydrogen Electrode, model RE-5B, BASI, United Kingdom). All voltages are expressed to ±10 mV. Working Electrode (WE) was the anode electrode. Cathode potential was poised, and current demand was monitored with a potentiostat (BioLogic, France). All the experiments were duplicated.

2.2. Influent characteristics

Bicarbonate was used as a carbon source in the cathode to promote the growth of autotrophic microorganisms. In the anode, bicarbonate improves the availability and transport of protons. Anode and cathode feed of both biotic and abiotic MEC consisted of nitrogen-purged synthetic medium with no added organic carbon sources, and had the following characteristics: 4 g L⁻¹ NaHCO₃, 10 mL L⁻¹ buffer (10 g L⁻¹ NH₄Cl, 60 g L⁻¹ Na₂HPO₄, 0.15 g L⁻¹ CaCl₂, 2.5 g L⁻¹ MgSO₄·7H₂O; 5 g L⁻¹ NaCl, 30 g L⁻¹ KH₂PO₄) and 0.1 mL L⁻¹ microelements solution (1 g L⁻¹ EDTA, 1 g L⁻¹ FeSO₄·7H₂O, 70 mg L⁻¹ ZnCl₂, 100 mg L⁻¹ MnCl₂·4H₂O, 6 mg L⁻¹ MgSO₄·7H₂O, 130 mg L⁻¹ CaCl₂·6H₂O, 2 mg L⁻¹ CuCl₂·2H₂O, 24 mg L⁻¹ NiCl₂·6H₂O, 36 mg L⁻¹ Na₂MoO₄·2H₂O, 238 mg L⁻¹ CoCl₂·6H₂O (adapted from Ref. [17])). The medium had a pH and conductivity around 8.0 and 5 mS cm⁻¹, respectively.

2.3. MEC start up and operation

The cathode of the biotic MEC was inoculated and operated in a recirculation loop for 4 days. The inoculum was a mixture of two different effluents coming from i) an urban wastewater treatment plant treating organic matter, nitrogen and phosphorus biologically, and ii) the effluent from a parent Microbial Fuel Cell (MFC) treating wastewater, with simultaneous nitrification-denitrification at the cathode. Microbial communities had complex diversity, including members of the Actinobacteriaceae, Bacteroidetes, Proteobacteria, Firmicutes, Chloroflexiaceae and Deinococcaeae groups, were present at samples used as inoculum (results not published).

During inoculation process a vigorous recirculation loop (150 L d⁻¹) was applied to generate stress conditions for the microorganisms and to force them to fix at the electrode surface. After inoculation, biotic MEC was started up in Open Circuit Voltage (OCV) and continuously fed with synthetic medium. When the biotic MEC reached a steady voltage value, different tests were done by gradually decreasing the cathode potential from -400 to -1800 mV. Once cathode potential was poised, samples were taken after the system reached the steady state. Steady state conditions were assumed when current demand and voltage were maintained at constant values, approximately 3–4 days after poising cathode potential. Based on NCC, HRT was about 6.24 and 7.65 h in biotic and abiotic MEC, respectively. Abiotic MEC was not inoculated, both anode and cathode compartments consisted only of previously treated graphite electrodes.

MECs were operated in three-electrode configuration, where Working Electrode (WE) was the cathode electrode, Reference Electrode (RE) was an Ag/AgCl (described before) placed in the cathode chamber and Counter Electrode (CE) was the anode electrode. Cathode potential was poised, and current demand was monitored with a potentiostat (BioLogic, Model SP50, France). All the experiments were duplicated.

2.4. Analyses and calculations

Samples for the determination of chemical oxygen demand (COD) were taken on each experiment and analyzed with standard wastewater methods according to [18]. Chromatographic techniques were used to analyze volatile organic compounds with a Varian CP-3800 equipped with FactorFour™ CP8860 column and a Flame Ionization Detector (FID) in order to detect Volatile Fatty Acids (VFA; Acetate, Propionate, Butyrate) and Alcohols (Ethanol, Methanol, Propanol and Butanol).

Produced gas was trapped in a methacrylate chamber and sampled with a glass syringe. Gas samples were analyzed to detect hydrogen, carbon dioxide, methane, oxygen and nitrogen (H₂, CO₂, CH₄, O₂, N₂) with an Agilent 7820A GC System equipped with Washed Molecular Sieve 5A and Porapak™ Q columns and a Thermal Conductivity Detector (TCD). Gas production calculations were given with respect to experimental conditions (Temperature 22 ± 1 °C; atmospheric pressure).

Gas production performance was characterized by calculating volumetric hydrogen production rate (Qₜₜ).
m$^3$ H$_2$ m$^{-3}$ NCC d$^{-1}$) normalized to cathode liquid volume, which is given by the following equation:

$$Q_{\text{H}_2} = \int_{0}^{t} C_{\text{H}_2} V_{\text{gas}} \, dt$$

(2)

Where $V_{\text{gas}}$ is the gas volume (m$^3$) sampled over a period of time (days), $C_{\text{H}_2}$ is the concentration (%, v/v) of hydrogen in the gas, and $V_{\text{NCC}}$ is the net cathode compartment volume (m$^3$).

Cathodic hydrogen recovery ($r_{\text{cat}}$, %) was used to evaluate hydrogen production efficiency of MECs and calculated as previously described by Logan and coworkers [19]:

$$r_{\text{cat}} = \frac{n_{\text{H}_2}}{n_{\text{CE}}} \times 100$$

(3)

Where $n_{\text{H}_2}$ is the amount (moles) of hydrogen experimentally recovered at the cathode, and $n_{\text{CE}}$ is the amount that theoretically could have been produced based on the measured current. $n_{\text{CE}}$ is given by the following equation [19]:

$$n_{\text{CE}} = \frac{I dt}{2F}$$

(4)

Where $I$ is the measured intensity (A), $dt$ is the time interval over which data are collected; $F$ is Faraday’s constant (96,485 C mol$^{-1}$ of electrons); and 2 as conversion number of moles of electrons into hydrogen.

Economic feasibility of the MECs was evaluated in terms of hydrogen production versus energy consumed over time, based on measured voltage and intensity. For this purpose, estimated operational costs per kg of hydrogen produced were calculated in order to facilitate comparison with existing data and other authors. Hydrogen operational costs were calculated from energy consumed and they were based on average current prices of electric energy in the US [20].

Cyclic voltammetry (CV) were performed using a potentiostat (model SP50, BioLogic, France). A three-electrode configuration was used for CV tests, which was the same that it was operated. Four cycles were done from $+200$ mV to $-1800$ mV by imposing a linear scanning potential rate of 0.1 mV s$^{-1}$. To represent the results, the average of the four cycles was calculated. CV experiments were done to distinguish between biotic and abiotic MEC performances. An additional deionized water medium was prepared for biotic MEC to determine the effect of turnover (presence of substrate) and non-turnover (substrate depletion) conditions [21].

2.5 Biofilm characterization

To assess the composition of the cathode microbial community graphite granules samples were extracted from the cathode at the first and last days of operation. Biofilm was dislodged from graphite surface by an ultrasonic bath (P-Selecta, Spain) for one cycle of 1 min followed by 2 min of centrifugation at 4000 rpm. Pellets were mixed and pooled in a single sample. Nucleic acids were extracted using the Fast DNA$^\text{SPIN}$ Kit for soil (MP Biomedicals, US) according to the manufacturer’s instructions. The 16S rRNA gen was amplified by PCR using universal primers 357F [22] and 907R [23], PCR products were analyzed by denaturing gradient gel electrophoresis (DGGE) according to method described by Ref. [24]. A denaturing gradient of 35–65% of urea-formamide with 6% acrylamide at 60 °C and a voltage of 160 V was applied during 14 h. Analysis of gel images was done with the GelComparII v.6.1 software. Intense and differential DGGE bands were excised, purified and reamplified by PCR using the above mentioned primers and conditions. Reamplified bands were sequenced in reverse direction using the 907R primer (Magen- rogen, Holland).

Scanning Electron Microscopy (SEM) analyses were performed at the end of the operational period. Graphite samples from the biotic and abiotic MEC were extracted to compare the electrode surface. The samples were immersed in 2.5% (w/v) glutaraldehyde in a 0.1 M cacodylate buffer at pH 7.4 for a period of 4 h. Next, the samples were washed and dehydrated in an ethanol series. Washes were done with cacodylate buffer and with water, both per duplicate. Dehydration with graded ethanol followed temperature steps of 50, 75, 80, 90, 95 and 3 × 100 °C in periods of 20 min. The fixed samples were dried with a critical-point drier (model K-850 CPD, Emitech, Alemanya) and sputtered-coated with a 40 nm gold layer. The coated samples were examined with a SEM (model DSM-960; Zeiss, Germany) at 20 kV and images were captured digitally. Energy-dispersive X-ray spectroscopy (EDX, QUANTAX Microanalysis System) was also performed in the abiotic MEC graphite samples in order to identify the compounds deposited on the surface. Analyzed samples were not pretreated. Digital images of both SEM and EDX analysis were collected and processed by ESPRIT 1.9 BRUKER program (AXS Microanalysis GmbH, Berlin, Germany).

3. Results and discussion

3.1 Influence of cathode potential on hydrogen production

The influence of cathode potential on the hydrogen production rate was assessed by setting cathode potential from $-400$ to $-1800$ mV. All experiments were done comparing both biotic and abiotic MECs. Table 2 shows hydrogen production rate and the current demand at the different cathode potentials. In both MECs, current demand was directly related to hydrogen production. During biotic experiments, current demand increased very slowly, compared with the higher increase in the abiotic MEC. The low increasing rate of current demand was in agreement with the low hydrogen production rate observed in the biotic MEC. When both systems were fixed at cathode potential between $-400$ and $-900$ mV no gas formation was observed. In this range no variations were observed on the pH and the conductivity of the effluent.

When biotic MEC was poised at cathode potentials lower than $-900$ mV, gas was produced. The produced gas was enriched in H$_2$ (83–87%) and N$_2$ (13–17%), no carbon dioxide and methane were detected. In contrast to results obtained by Rozendal et al. [10], methane was not detected in the produced gas although bicarbonate was the only carbon source. This fact could indicate that methanogens were not active.
Tables microorganisms in the MEC. Due to operational conditions, acetoclastic neither hydrogenotrophic methanogenesis were likely [25].

From cathode potentials between −900 and −1800 mV, the volumetric hydrogen production in the biotic MEC increased almost linearly \((R^2 = 0.957)\) from \(0.10 \pm 0.01 \text{ m}^3 \text{ H}_2 \text{ m}^{-3} \text{ NCC day}^{-1}\) to \(11.60 \pm 1.10 \text{ m}^3 \text{ H}_2 \text{ m}^{-3} \text{ NCC day}^{-1}\). Above −1400 mV, volumetric hydrogen production rate was below \(10.00 \text{ m}^3 \text{ H}_2 \text{ m}^{-3} \text{ NCC day}^{-1}\) which is the value estimated to be the minimum volumetric hydrogen production rate required for practical applications [9]. Below −1600 mV, volumetric hydrogen production rate increased to values above \(10.00 \text{ m}^3 \text{ H}_2 \text{ m}^{-3} \text{ NCC day}^{-1}\) (Table 2).

The abiotic MEC produced gas at cathode potentials below −1000 mV. At such potentials (from −1000 to −1800 mV) abiotic gas was also enriched in hydrogen (81–93% \(\text{H}_2\)) being nitrogen the remaining fraction. As observed in the biotic MEC, no other compounds were detected in the gas composition. Abiotic hydrogen production also increased linearly \((R^2 = 0.977)\) with cathode potential, from \(0.50 \pm 0.02 \text{ m}^3 \text{ H}_2 \text{ m}^{-3} \text{ NCC day}^{-1}\) at −1000 mV to \(57.50 \pm 4.00 \text{ m}^3 \text{ H}_2 \text{ m}^{-3} \text{ NCC day}^{-1}\) at −1800 mV. At cathode potentials equal or lower than −1200 mV, hydrogen production rate was above \(10.00 \text{ m}^3 \text{ H}_2 \text{ m}^{-3} \text{ NCC day}^{-1}\).

When gas production started to increase, pH and conductivity in both systems rose up too. In the cathode of the biotic MEC operating at −1800 mV, \(\text{pH}\) and conductivity reached values of 10.8 and 8.5 mS cm\(^{-1}\), respectively. A similar case was observed with abiotic MEC, with a resulting \(\text{pH}\) of 11.9 and a conductivity of 10.2 mS cm\(^{-1}\) at the same cathode potential. The increases on conductivity of the cathode were probably due to transport of ion species other than protons and hydroxyl ions (i.e. \(\text{Na}^+, \text{K}^+, \text{NH}_4^+\)) through the membrane [26], which was conﬁrmed by the anode conductivity decrease. No detectable variations on COD were observed and VFA and alcohols were not detected in the aqueous phase in any of the experiments.

3.2. Electrochemical characterization

Table 2 - Measurement of hydrogen production rate and current demand of biotic and abiotic MEC based on cathode poised potential.

<table>
<thead>
<tr>
<th>Cathode potential (mV vs SHE)</th>
<th>Biotic MEC</th>
<th>Abiotic MEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen production rate (m^3 H_2 m^-3 NCC day^-1)</td>
<td>Current demand (A m^-2)</td>
<td>Hydrogen production rate (m^3 H_2 m^-3 NCC day^-1)</td>
</tr>
<tr>
<td>−400</td>
<td>0.00 ± 0.00</td>
<td>0</td>
</tr>
<tr>
<td>−600</td>
<td>0.00 ± 0.00</td>
<td>0</td>
</tr>
<tr>
<td>−800</td>
<td>0.00 ± 0.00</td>
<td>0</td>
</tr>
<tr>
<td>−900</td>
<td>0.10 ± 0.01</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>−1000</td>
<td>0.90 ± 0.10</td>
<td>47 ± 5</td>
</tr>
<tr>
<td>−1100</td>
<td>1.40 ± 0.30</td>
<td>132 ± 23</td>
</tr>
<tr>
<td>−1200</td>
<td>5.10 ± 1.10</td>
<td>501 ± 99</td>
</tr>
<tr>
<td>−1400</td>
<td>6.60 ± 2.60</td>
<td>455 ± 137</td>
</tr>
<tr>
<td>−1600</td>
<td>10.30 ± 3.50</td>
<td>731 ± 95</td>
</tr>
<tr>
<td>−1800</td>
<td>11.60 ± 1.10</td>
<td>963 ± 128</td>
</tr>
</tbody>
</table>

Fig. 1 shows CV tests of biotic and abiotic MEC. CVs corroborated what was observed previously in Table 2. Hydrogen production was directly related to current demand, which increased much higher in the abiotic MEC than in the biotic at cathode potentials below −1000 mV. At poised cathode potentials lower than −1000 mV, intensity demand of the abiotic MEC was greater than the biotic MEC. In both biotic and abiotic CVs an oxidation peak could be observed at −530 and −560 mV vs SHE, respectively. According to Nernst equation, the shift on cathode potentials could be caused by small differences on pH between biotic and abiotic MEC. These oxidation peaks are associated to hydrogen oxidation [27]. In the case of abiotic MEC, hydrogen oxidation peak was much higher than in the biotic MEC because a higher quantity of hydrogen was produced. Under non-turnover conditions, no oxidation peak was observed due to low conductivity of the water, which limits electron transfer through the medium [16]. Only the hydrogen produced at the electrode surface could be detected. The high conductivity of the medium favored electron transfer through the medium and allowed high hydrogen production rates.

3.3. Identification of cathode microbial community

The composition of the microbial community in the biofilm of the biotic MEC after 45 days of operation was rather simple according to results obtained by PCR-DGGE (Figure S1).
DGGE band pattern clearly differentiated from that of the parent MFC used as inoculum (Figure S1), thus indicating that microbial species were specifically enriched during the operational period. Three main bands of different intensities were detected. Obtained sequences showed the highest similarity to Hoeflea sp. (97%), Aquiflexum sp. (92%) and an unknown member of the Actinobacteria (Table S1). Aquiflexum sp. and Hoeflea sp. are both bacteria frequently observed in marine habitats with a broad tolerance to differences in salinity and alkalinity. Aquiflexum is described as a fermentative bacteria, which could produce hydrogen through fermentation [28,29]. According to this general description, it remains unclear whether the observed Aquiflexum phylotype could participate in H2 production in the biocathode at the used conditions, since no organic matter was added and neither organic acids nor alcohols were produced. Hoeflea sp. has no described relationship with hydrogen production, but high conductivities measured on the cathode are optimal for their growth [30]. Finally, Actinobacteriaceae are heterotrophic bacteria playing an important role in the decomposition of organic matter or decaying biomass.

Microbial population had been reduced to Actinobacteriaceae, Bacteroidetes and Proteobacteria at biotic MEC cathode, but no correspondence was obtained for any of the observed bands. These changes on the microbial community were selected from the system, which had restricted operational conditions. No organic matter was present in the MEC influent, the only carbon source was bicarbonate and the cathode was poised at very low cathode potentials. Conductivity and pH at the cathode were always higher than 5 mS cm⁻¹ and 8.0, respectively. Although these results provide the first attempt to characterize the microbial community structure of a hydrogen producing biocathode, further analyses, such as culture-dependent methods or additional molecular methods, are required to verify microbial identification and determine H2 producing activity by microorganisms.

3.4. Biotic and abiotic cathode morphology

At the end of the experimental period, graphite granules samples from the cathodes of both biotic and abiotic MEC were extracted and analyzed by Scanning Electron Microscopy (SEM). In Fig. 3 the obtained SEM images of abiotic (A) and biotic (B) cathodes are shown.

In the image of the abiotic MEC a large quantity of crystals over the graphite surface could be observed. The elemental composition analysis showed that mostly oxygen, calcium and carbon, but also sodium, phosphorus, magnesium and aluminum were present in different proportions in the abiotic graphite surface.

On the other hand, large quantities of microorganisms were attached to the cathode of the biotic MEC forming a biofilm. Most microorganisms were forming large aggregations. The dominant morphology among the observed cells was large rods, although some spirochetes were also observed. The surface of the biotic cathode was covered by different substances: (1) mineral precipitation coming from the feed components (with high concentration of sodium bicarbonate) and (2) Exopolymeric Substances (EPS), secreted by some microorganisms to remain attached at the electrode.

The mineral precipitation over the graphite surface in both biotic and abiotic MEC could lead to higher energy consumption due to overpotentials of the system.
3.5. Energy recovery

In most tests, energy required to achieve cathodic potentials in the abiotic MEC was higher than that required for biotic MEC. Hydrogen production rate versus energy consumed is represented in Fig. 2. For both biotic and abiotic MEC, the relationship between both parameters was found to be linear, obtaining an R2 of 0.982 and 0.977 for biotic and abiotic MEC, respectively. Net cathode compartment volume was applied to correct the linear relation obtained, the slope observed for biotic and abiotic MEC was 0.116 m3 H2 kWh−1 and 0.064 m3 H2 kWh−1, respectively. By using microorganisms as cathode catalyst, the biotic MEC achieved hydrogen production values which represent almost half consumption of energy. Although hydrogen production rates in the abiotic MEC were higher than that in the biotic MEC (Table 2), energy consumption in the biotic MEC was much lower.

Hydrogen was produced in a more efficiently way in the biotic MEC, obtaining productions as high as 0.365 m3 H2 kWh−1 at −1000 mV, while the highest production observed in the abiotic MEC was 0.071 m3 H2 kWh−1 at −1200 mV. The pH difference between the anode and the cathode of abiotic MEC was higher than biotic MEC. As shown by the Nernst equation, the resulting membrane pH gradient causes a potential loss of ~60 mV per pH unit. Therefore, the energy demand of the abiotic MEC was higher than the biotic MEC to overcome this loss.

The results of the present study, in terms of energy recovery and efficiency are shown in Table 3, and compared to other existing technologies for hydrogen production [31]. Efficiency of the biotic and abiotic MEC was calculated from cathodic hydrogen recovery, applied current was the only electron source.

The average efficiency (fer) obtained was 113% and 67% in the biotic and abiotic MEC, respectively. However maximum values observed were of 175% and 96% at −1000 mV and −1800 mV in the biotic and abiotic MEC, respectively. Those high efficiencies could only be explained by parallel reactions in the MECs. In general, energy requirements for hydrogen production in the biotic MEC were considerably lower compared to the abiotic. As other researchers already demonstrated, electrons were utilized more efficiently in the biotic MEC [10].

At best operation conditions, energy requirements of the biotic MEC (33.2 kWh kg−1) are below that of partial oxidation of heavy oil, coal gasification and grid electrolysis of water, and close to Steam methane reforming (22.4 kWh kg−1), which is a well-established hydrogen production technology.

3.6. Perspectives of hydrogen production in a biotic cathode

Although the MEC systems were not optimized for hydrogen production, an analysis of the results obtained can be made to assess the viability of its use and compared with other studies using MECs.

Although hydrogen production rate in the biotic MEC was lower than that obtained for the abiotic, the first one shown a better performance in terms of energy requirements. Best conditions in terms of energy requirement and therefore operational costs are found at a cathode potential of −1000 mV for the biotic MEC and −1200 mV for the abiotic, with estimated production costs of 3.20 USD kg−1 H2 and 16.44 USD kg−1 H2 respectively.

Estimated operational production costs of hydrogen on the biotic MEC was below estimated market value of hydrogen (6 USD kg−1 H2) [32] and also in the range of the US Department of Energy threshold cost of hydrogen for 2020, which was estimated in 2–4 USD kg−1 H2 [33], while the estimated production cost with abiotic MEC was much higher.

The lowest operational costs for hydrogen production of this study was slightly higher than 3.01 USD kg−1 H2 found by Cusick et al. [34] for a MEC treating domestic wastewater at the anode and producing hydrogen at the cathode using a Pt catalyst.

In this study conditions for methanogenic development were avoided. Organic matter was not present on the system so acetoclastic methanogenesis cannot occur. Although bicarbonate was used in the cathode, the pH was always higher than 8, avoiding the presence of CO2 and therefore hydrogenotrophic methanogenesis.

Finally, other studies suggest that other modifications on parameters of the biotic MEC can be made to optimize the system [34,35]. This could lead to its potential economic viability in future implementation. By introducing wastewater treatment at the anode chamber, the degradation of organic matter could reduce operational costs by reducing electric consumption of the system. In a recent study, Villano et al. [35] demonstrated the feasibility of a MEC in which degradation of low strength wastewaters could occur at the anode chamber. 600 mg L−1 of acetate were consumed by microorganisms present at the anode, generating a current of 110 mA. This current was used at the cathode chamber to biologically produce hydrogen and methane. In the biotic system of the present study a current of 18 mA was necessary to produce hydrogen at −1000 mV. If organic matter could be used in the anode chamber to avoid electric consumption, operational...

Table 3 – Key parameters of the results obtained in the present study and other existing hydrogen producing technologies.

<table>
<thead>
<tr>
<th></th>
<th>Biotic MEC (this study)</th>
<th>Abiotic MEC (this study)</th>
<th>Steam methane reforming</th>
<th>Partial oxidation of heavy oil</th>
<th>Coal gasification</th>
<th>Grid electrolysis of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency, based on energy input (%)</td>
<td>53–175</td>
<td>7–96</td>
<td>70–80</td>
<td>70</td>
<td>60</td>
<td>27</td>
</tr>
<tr>
<td>Energy consumption (kWh kg⁻¹ H₂)</td>
<td>33.2–117.0</td>
<td>170.1–995.5</td>
<td>22.4*</td>
<td>54.9*</td>
<td>96.3*</td>
<td>54.9*</td>
</tr>
</tbody>
</table>

* Data calculated from Ref. [28].

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cost would be considerably reduced. Even higher hydrogen production rates could be achieved without increasing operational costs. Further investigations would be necessary to determine the operational conditions and the viability of this experimental modification.

4. Conclusions

Hydrogen had a linear relationship with cathode potential in biotic and abiotic MECs within a range of −900 to −1800 mV. CV tests corroborated that higher hydrogen production rates could be achieved by decreasing cathode potential. At poised cathode potential of −1600 mV hydrogen production rate rose to values above 10 m³ H₂ m⁻³ NCC d⁻¹, which is estimated to be the minimum production for practical applications. Microorganisms present in the cathode of the biotic MEC were identified as Hoeflea sp. and Aquiflexum sp.

The results of the present study point out that biotic MEC shown a better performance in terms of hydrogen production per kWh consumed and therefore, lower estimated operational costs, which are below hydrogen market value and hydrogen threshold cost for 2020.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijhydene.2013.11.017.

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