



Exploring key operational factors for improving hydrogen production in a pilot-scale microbial electrolysis cell treating urban wastewater

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ABSTRACT

Bioelectrochemical systems (BES) are becoming popular technologies with a plethora of applications in the environmental field. However, research on the scale-up of these systems is scarce. To understand the limiting factors of hydrogen production in microbial electrolysis cell (MEC) at pilot-scale, a 135 L MEC was operated for six months under a wide range of operational conditions: applied potential [0.8–1.1 V], hydraulic residence time [1.1–3.9 d], and temperature [18–30 °C], using three types of wastewater; synthetic (900 mg CODs L⁻¹), raw urban wastewater (200 mg CODs L⁻¹) and urban wastewater amended with acetate (1000 mg CODs L⁻¹). The synthetic wastewater yielded the maximum current density (1.23 A m⁻²) and hydrogen production (0.1 m³ m⁻³ d⁻¹) ever reported in a pilot scale MEC, with a cathodic recovery of 70% and a coulombic efficiency of 27%. In contrast, the use of low COD urban wastewater limited the plant performance. Interestingly, it was possible to improve hydrogen production by reducing the hydraulic residence time, finding the optimal applied potential or increasing the temperature. Further, the pilot plant demonstrated a robust capacity to remove the organic matter present in the wastewater under different conditions, with removal efficiencies above 70%. This study shows improved results compared to similar MEC pilot plants treating domestic wastewater in terms of hydrogen production and treatment efficiency and also compares its performance against conventional activated sludge processes.

1. Introduction

The challenge of achieving sustainable wastewater treatment within the framework of addressing climate change is a critical challenge for modern society. Despite advancements, municipal wastewater treatment remains an energy-intensive process, mainly because of the large aeration requirements of the activated sludge process, which constitutes the primary operational cost of a standard wastewater treatment plant (WWTP) [1]. However, environmental engineering is undergoing a paradigm shift towards viewing wastewater as a resource rather than merely waste.

Focusing on energy recovery, wastewater contains many different organic compounds (from simple carbohydrates to more complex molecules) that contain valuable energy stored within their chemical bonds [2]. The quantification of the energy content of domestic wastewater is a complex and multifaceted challenge. Numerous studies have attempted to establish correlations between this energy content and widely used parameters such as biological oxygen demand (BOD) or chemical oxygen demand (COD). Combustion enthalpy of typical wastewater compounds can be calculated from reference data [3], giving values (kJ g⁻¹ COD) of 13.6 for acetic acid, 14.6 for glucose and 15.6 for glutamic acid. Then, a typical value around 15 kJ g⁻¹ COD seems to be a reasonable

Abbreviations: AD, Anaerobic digestion; AEM, Anion exchange membrane; ARB, Anode respiring bacteria; BES, Bioelectrochemical system; BOD, Biological oxygen demand; CAS, Conventional activated sludge; CE, Coulombic Efficiency; COD, Chemical oxygen demand; COD_s, Soluble chemical oxygen demand; E_c, Energy consumption; EE, Energy efficiency; *emf*, Electromotive force; E_p, Energy produced; HRAS, High-rate activated sludge; HRT, Hydraulic retention time; IEM, Ionic exchange membrane; *j*, Current density; MEC, Microbial electrolysis cell; MET, Microbial electrochemical technologies; NE_p, Net energy production; OLR, Organic loading rate; ORR, Organic removal rate; PTFE, Polytetrafluoroethylene; PVC, Polyvinyl chloride; R², R-squared; r_{CAT}, Cathodic recovery; E_E, Energy recovery; RSL, Return sludge liquor; SHE, Standard hydrogen electrode; SS, Stainless-steel; SWW, Synthetic wastewater; T, Temperature; TSS, Total suspended solids; UWW, Urban wastewater; UWW_A, Amended urban wastewater; VFA, Volatile fatty acid; VSS, Volatile suspended solids; WAS, Waste activated sludge; WW, Wastewater; WWTP, Wastewater treatment plant; ΔG_R, Gibbs free energy; ΔV, Applied potential; θ, Coefficient of temperature dependency; σ, Electrical conductivity.

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approach considering the inherent variability of urban wastewater fractionation, and it agrees with different reported values [4–6]. In a modern society, each population equivalent (PE) produces about 200–300 L of wastewater per day with an organic matter load about 80–120 g COD person⁻¹ d⁻¹ [7]. This means that, for example, in the case of Spain, with a population about 47 million people, an ideal maximum of $3 \cdot 10^{16}$ J per year could be recovered from wastewater, which is equivalent to the energy of burning around 735,000 tonnes of crude oil in a modern power station. This value is certainly overestimated, and a realistic goal may be to recover between 25 and 50% of this energy [8].

There are different processes for harnessing the energy contained in wastewater. The most common methodology is anaerobic digestion aiming at biogas production. Other promising alternatives aim at producing green hydrogen as an energy carrier. Hydrogen does not generate greenhouse gas emissions when is transformed into electricity, and its combustion enthalpy is higher than that of methane (122 kJ g⁻¹ vs 55 kJ g⁻¹). Nevertheless, the world's demand for hydrogen is nowadays mainly supplied by the steam reforming process [9], and therefore, a high environmental impact is associated with its production [10]. In this context, microbial electrochemical techniques (MET) have gained considerable attention as they can produce hydrogen from wastes in devices that combine electrochemistry with the metabolism of electroactive microorganisms. These systems, known as microbial electrolysis cells (MEC), can simultaneously treat wastewater and produce hydrogen [8]. For more details regarding the reactions occurring in MECs, please refer to the “Reaction Mechanisms” section in the [Supplementary Information](#).

At lab-scale, MEC configurations can be divided into single and double chamber depending on the presence of an ion exchange membrane (IEM) that separates the anodic and cathodic compartments [18]. Electroactive microorganisms grow as a biofilm on the anode whereas abiotic hydrogen evolution takes place at the cathode. These microorganisms, known as exoelectrogens or anode-respiring bacteria (ARB), can degrade organic matter using an insoluble electrode as the terminal electron acceptor [11]. ARB possess the unique ability of transferring the electrons extracellularly, which travel from the anode to the cathode through the electric circuit and reduce protons on the cathode, thereby forming hydrogen. Thermodynamics in an MEC show a positive Gibbs free energy (ΔG_R) because of a negative electromotive force (emf). For instance, for the case of acetate as electron donor, the theoretical reduction potential is -0.28 V vs standard hydrogen electrode (SHE) assuming standard conditions at a pH of 7. For the cathodic hydrogen evolution reaction, the theoretical reduction potential is -0.41 V vs SHE at standard conditions and pH of 7. Therefore, under these conditions, the emf is -0.13 V and ΔG_R is positive. Thus, the overall process is non-spontaneous, and a minimum applied potential (ΔV) of 0.13 V will be required to drive the reactions [8]. This value is one order of magnitude lower than the theoretical value for water electrolysis, 1.23 V [12] and is the main reason for the recent interest in this technology. However, the inherent voltage losses increase to a large extent the ΔV requirements. The common ΔV in MEC at lab-scale to drive hydrogen production is reported to be not lower than 0.4 V [13], being usually between 0.6 and 1.0 V [14,15].

MECs have shown promising results at laboratory scale in terms of hydrogen production, usually attaining values between 1 – 10 m³ H₂ m⁻³ reactor d⁻¹ [16–18], with high cathodic recoveries (r_{CAT}) and high coulombic efficiencies (CE) [19,20]. However, despite the large number of manuscripts published about the fundamentals of bioelectrochemical hydrogen production, few studies are conducted at pilot scale. Jadhav et al. specified that less than 1% of the more than 20,000 studies about MET addressed the issue of scaling-up [21]. Even though lab-scale research is essential, it may put out of sight the challenging issues involved in the operation of MECs under real-scale conditions. MECs need to be operated under conditions like those of WWTPs to bridge the gap between lab and industrial adoption. Among the few experiences of

such systems at pilot scale, a general reduction in reactor performance is reported compared to bench-scale reactors [22,23]. Hydrogen production is usually poor, in the range 0.005 – 0.040 m³ m⁻³ d⁻¹ [24–27], with low CE (<50 %) and significant hydrogen leakage [28,29]. Also, the reported range of organic matter removal in these studies is very broad (between 10 and 60%) due to the different influent load and composition, with reported hydraulic retention times (HRTs) between 4 and 48 h.

Commonly organic loading rates (OLR) in systems for urban wastewater (UWW) treatment are in the range 0.04 – 1.6 g COD L⁻¹ d⁻¹ [30]. In this sense, MECs must operate at a short HRT to be competitive with other technologies (e.g., activated sludge and anaerobic digestion systems) [31]. Achieving high COD removal efficiencies under short HRTs is one of the recent objectives of WWTP designers aiming only at COD removal. However, poor anode-based oxidation rates are typically found in MECs due to low mixing [32], which is translated in higher volumes or higher HRTs. Conventional stirring or recycling pumps are implemented to improve turbulence in the reactor to avoid mass transfer limitations [27,29], but this may hinder the system energy balance.

The highest hydrogen production in a double-chamber MEC pilot plant (0.031 m³ m⁻³ d⁻¹) was reported in our previous study [27] and has been used as a reference to evaluate the technical feasibility of MECs [33], its environmental performance [34], and discussed in many other works [35–37]. Despite promising results were obtained with our reactor configuration, limited operational conditions were tested. Hence, it was considered appropriate to further study the reactor in other scenarios to understand the limiting factors of MEC operation at pilot scale. The 135 L double-chamber MEC was operated under a wide range of operational conditions and a critical assessment was performed to determine how hydrogen yield and energy efficiency can be improved by modifying the operational parameters. Finally, the experimental efficiency obtained in this study was used to evaluate the treatment of 1 m³ of UWW by an MEC and the results were compared with two existing technologies reported in the literature: conventional activated sludge (CAS) and high-rate activated sludge (HRAS).

2. Materials and methods

2.1. Reactor design

The pilot plant (Figs. S1–S3) consisted of a 220 L stainless-steel (SS) tank containing nine cassette-type cells, each of which working as a double-chamber MEC. The cells were placed into the reactor, forcing the wastewater to circulate on both sides of them. A similar cell configuration was used as reported in our previous study [27]. The reactor had a total anodic volume of 135 L once the nine cells were placed inside the reactor. The cells consisted of a central PVC frame (3 cm width \times 36 cm length \times 46 cm height) acting as a cathode chamber, containing pressed SS wool (Steel wool #2, Barlesa SL, Spain). The internal cathode section of each cell had a volume of 3.7 L. The cathode was separated from the anodes using an anion exchange membrane (AEM) on both sides (AMI-7001S, Membranes International Inc., United States). The anodes were made of carbon felt (PX35 Carbon Felt, Zoltek™, United States) and were pressed to the membranes on both sides of the cathode through a SS mesh. The anodes were thermally pre-treated (400 °C for 20 min) to improve biomass adhesion. Marine SS wire and SS connection strips (Maranges SA, Spain) were used for the electrical connections. The membranes and the anodes were located between the cathode compartment and two outer PVC frames (1 cm length \times 36 cm width \times 46 cm height in external dimensions with a 6 cm frame). The central frame was provided with an olive fitting at the top that allowed the gas collection via a tube (Marprene® Tubing 9.6 mm BORE, Watson Marlow, United Kingdom) connected to a 10 L gas sample bag (FlexFoil® Standard, SKC, United States). The pieces were held together by tightening 34 wing nuts onto bolts through 5 mm holes in the PVC frames. The projected anode surface was 0.163 m² per cell (i.e. a total of 1.47 m²

in the reactor), which yielded a total area/volume ratio of $10.9 \text{ m}^2 \text{ m}^{-3}$.

2.2. MEC operation

Each individual cell was powered by a separate power source (Programmable DC LAB Power Supply LABPS3005DN, Velleman Goup, Belgium). Both voltage and current were digitally monitored using the AddControl software developed in LabWindows CVI by the research group [38]. The current intensity for each cell was recorded every 5 min for calculating several performance parameters. pH and temperature were monitored in the anodic compartment using a pH probe (HACH pH electrode Crison5233, Spain) and a thermoresistance (Pt1000, Axiomatic, Spain). Conductivity was measured using a conductivity meter (COND 8, XS Instruments, Italy). The anodic temperature was controlled using the AddControl software and a silicone heating tape (HBSI 10 m, HORST GmbH, Germany) placed around the tank.

Three different types of wastewaters were used during the operation of the plant (Table 1). In the first period, synthetic wastewater with acetate as sole carbon source and macronutrients was used. It was prepared in a refrigerated 2000 L tank with tap water, sodium acetate (2335 g), NH_4Cl (305.67 g), K_2HPO_4 (56.19 g), KH_2PO_4 (43.91 g) and 50 mL of micronutrient solution [39], obtaining the concentrations reported in Table 1. In the second period, real UWW from a municipal WWTP (Manresa, Spain) after primary settling was used, refilling the 2000 L tank approximately every 10 days (see composition in Table 1). The levels of organic matter in the raw UWW after filtration ($0.22 \mu\text{m}$) were in the range of $200\text{--}300 \text{ mg COD L}^{-1}$. Also, the raw influent was amended with acetate during the temperature experiments to increase the organic concentration up to 1000 mg L^{-1} of COD. The catholyte was a 5 g L^{-1} NaCl solution. It was only renewed on day 30 before the continuous operation with UWW and its pH was measured weekly (it remained around 11–12 during the whole operation).

The pilot plant was started-up with nine non-colonised cells, using an anaerobic sludge inoculum from the same WWTP. The anodic chamber was filled with synthetic medium and operated in batch mode with an internal recycle of 150 L d^{-1} to improve mixing conditions, using a timed peristaltic pump (520 FAM/R2, Watson Marlow, United Kingdom). After 20 days of enrichment period, the plant was moved to continuous operation. During the whole operation, the influent was fed in the range $46\text{--}125 \text{ L d}^{-1}$ from the 2000 L supply tank and using 150 L d^{-1} of recirculation flowrate.

2.3. Analytical methods

Organic matter concentration was measured using commercial COD kits (LCK 514, Hach, United States) that covered the range of $100\text{--}2000 \text{ mg O}_2 \text{ L}^{-1}$ and a spectrophotometer (DR2800, Hach, United States). Hydrogen, oxygen, nitrogen, and methane were analysed with a gas chromatograph (7820-A, Agilent Technologies, United States). The total hydrogen or methane production was calculated using the Gas Bag Method presented by Ambler and Logan [40]. The detailed analytical procedure can be found in the [supplementary information](#).

Table 1
Average characteristics of the different wastewaters treated.

Wastewater	COD _S (mg L ⁻¹)	N-NH ₄ ⁺ (mg L ⁻¹)	P-PO ₄ ³⁻ (mg L ⁻¹)	σ (mS cm ⁻¹)	pH
SWW	903 ± 6	40 ± 2	10 ± 1	2.8 ± 0.1	6.9 ± 0.1
UWW	247 ± 34	51 ± 4	5 ± 1	1.1 ± 0.2	6.7 ± 0.2
UWW _A	1069 ± 39	51 ± 4	5 ± 1	3.2 ± 0.2	7.2 ± 0.1

σ: Electrical conductivity; SWW: Synthetic wastewater; UWW: Urban wastewater; UWW_A: Urban wastewater amended with acetate.

2.4. Key performance indices (KPIs)

Current density (j) in each cell was expressed as function of the projected anodic surface (A m^{-2}). Hydrogen production was expressed considering the total production in the plant (9 cells) and the anodic volume (135 L) ($\text{m}^3 \text{ m}^{-3} \text{ d}^{-1}$) or surface ($\text{m}^3 \text{ m}^{-2} \text{ d}^{-1}$).

The cathodic gas recovery (r_{CAT}) compares the moles of electrons required for hydrogen production with the moles of electrons arriving to the cathode as current. The r_{CAT} was calculated using Eq. (1):

$$r_{\text{CAT}} = \frac{V_{\text{H}_2} \cdot V_{\text{m,H}_2}^{-1} \cdot b_{\text{H}_2} \cdot F}{\int_{t_0}^{t_f} I dt} \quad (1)$$

Being V_{H_2} : Volume of hydrogen produced (L), $V_{\text{m,H}_2}$: Molar volume of hydrogen (24.06 L mol^{-1} at 1 atm, 20°C), b_{H_2} : Number of electrons transferred per mole of hydrogen ($2 \text{ mol e}^- \text{ mol}^{-1} \text{ H}_2$), F : Faraday's constant ($96485 \text{ C mol}^{-1} \text{ e}^-$), t_0 : Initial time of the cycle (s), t_f : Final time of the cycle (s), and I : Current intensity (A).

The energy recovery (r_E) refers to the ratio of the amount of energy recovered as hydrogen and the electrical input. The r_E was calculated using Eq. (2):

$$r_E = \frac{n_{\text{H}_2} \cdot \Delta H_{\text{H}_2}}{\int_{t_0}^{t_f} I \cdot \Delta V dt} \quad (2)$$

With n_{H_2} : Number of moles of hydrogen produced, ΔH_{H_2} : Heat enthalpy of hydrogen ($-285.8 \text{ kJ mol}^{-1}$), and ΔV : Applied potential (V).

The coulombic efficiency (CE) is the relation between the coulombs recovered as current and the coulombs that could be theoretically generated from the substrate oxidation. It was calculated using Eq. (3):

$$CE = \frac{\int_{t_0}^{t_f} I dt}{\Delta C \cdot V_L \cdot M_{\text{O}_2}^{-1} \cdot b_s \cdot F} \quad (3)$$

Being ΔC : Difference of substrate concentration between the inlet and the outlet of the reactor (g L^{-1}), V_L : Anodic volume (135 L), M_{O_2} : Molar mass of oxygen (32 g mol^{-1}), and b_s : Number of moles of electrons transferred per mol of COD ($4 \text{ mol e}^- \text{ mol}^{-1} \text{ COD}$).

2.5. Temperature effect study and response modelling

The effect of temperature on hydrogen production and current density was studied experimentally. The temperature range ($18\text{--}30^\circ\text{C}$) was selected based on typical wastewater temperatures in an urban WWTP, which commonly varies between 10 and 25°C depending on the season and geographic location, while temperatures above 30°C are highly unlikely [30]. To model the response, the Arrhenius equation (4) was used and fitted to the experimental data.

$$k(T) = k(20^\circ\text{C}) \cdot \theta^{(T-20)} \quad (4)$$

with $k(T)$ and $k(20^\circ\text{C})$ being hydrogen production rate or current density at temperature T ($^\circ\text{C}$) and at 20°C .

The model was fit to the experimental data by minimising the sum of the quadratic differences between the experimental and predicted data using the GRG nonlinear method of Excel Solver. The goodness of fit of the models was evaluated with the coefficient of determination R^2 calculated with Eq. (5).

$$R^2 = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \quad (5)$$

2.6. CAS/HRAS comparison

The MEC performance was compared to CAS and HRAS systems by calculating the energy requirements of each technology for treating 1 m^3 of UWW with $300 \text{ mg COD L}^{-1}$. As these aerobic technologies only treat wastewater and do not produce energy by themselves, methane

production by anaerobic digestion (AD) of the purged sludge in the CAS and HRAS was considered as a revenue. CAS and HRAS performances were assessed using data reported in the literature. The energy consumption was calculated considering the dominant energy requirements for municipal wastewater treatment [41]: aeration energy for the biological processes and heating for anaerobic digester. Pumping energy was discarded as it was assumed that all technologies would account for similar energy consumption. The energy needs of the biological processes were considered as the amount of oxygen consumed in the aerobic bioreactor, and were calculated by Eq. (6) as proposed in [42]:

$$\text{Aeration energy} = \frac{\text{Mineralized COD}}{\text{AE}} \frac{\text{SatDO}}{(\text{SatDO} - \text{DO})} \quad (6)$$

With Aeration energy: Energy requirements for aeration (kWh d^{-1}), Mineralized COD: The influent COD mineralized fraction COD (kg d^{-1}), AE: Aeration efficiency ($1.5 \text{ kg O}_2 \text{ kWh}^{-1}$), [30], SatDO: Dissolved oxygen concentration at saturation (10 mg L^{-1} at 15°C), [42], and DO: Dissolved oxygen concentration in the bioreactor (mg L^{-1}).

The energy requirements of the anaerobic digester were considered as the amount of energy needed to increase the temperature of the incoming waste activated sludge (WAS) to the digester. A conservative value of 0.12 kWh m^{-3} wastewater was assumed for the comparison [43], but the actual requirements were also calculated for each case by Eq. (7):

$$Q = m C_p \Delta T \quad (7)$$

Being Q: Heating energy requirements (kWh d^{-1}), m: Mass flow rate of WAS (kg d^{-1}), C_p : Specific heat of WAS (considered as water: $1.17 \times 10^{-3} \text{ kWh kg}^{-1} \text{ }^\circ\text{C}^{-1}$), and ΔT : Temperature difference between the digester and the incoming WAS ($^\circ\text{C}$).

The operational temperature in the anaerobic digester was assumed to be 35°C , and the temperature of the A-stage was considered for the incoming WAS (15°C). Heat losses through the surface of the tank were not considered.

The gas mass flow rates were estimated using the ideal gas law under normal conditions (20°C and 1 atm). Both the hydrogen produced by the MEC pilot plant, and the methane produced by AD were considered to be completely transformed into electricity. Thus, only the combustion enthalpy of each gas was used. The amount of WAS generated was calculated performing the COD balances. Methane production was estimated considering the volatile suspended solids (VSS) in the WAS

(no primary), using a yield of $205 \text{ L CH}_4 \text{ kg}^{-1} \text{ VSS}_{\text{added}}$ reported under similar conditions [44]. On the other hand, hydrogen production in the MEC was calculated considering the efficiencies obtained in this study during the continuous operation with raw UWW at 20°C .

The net energy production (NE_p) was calculated as the difference between the energy consumed (E_c) and the energy produced (E_p), and the process energy efficiency (EE) was defined as the amount of energy recovered as hydrogen or methane with respect to E_c .

3. Results and discussion

3.1. Start-up of the pilot plant with synthetic wastewater

The cells were inoculated with anaerobic sludge and started in batch mode using synthetic medium for a period of 20 days. Once a stable current density was obtained (around 1.0 A m^{-2}), the plant was shifted to continuous mode with an inlet flowrate of 46 L d^{-1} and an OLR of $0.33 \text{ g L}^{-1} \text{ d}^{-1}$. The synthetic feeding was maintained for the first days under continuous conditions to assess the performance of the MEC pilot plant in an ideal scenario: i.e. readily biodegradable carbon source and a moderate conductive medium ($>2.5 \text{ mS cm}^{-1}$).

The performance of the plant was studied under different fixed intensities (Stage 1) in each cassette cell: 0.10 , 0.15 and 0.20 A cell^{-1} (or 0.61 , 0.92 and 1.22 A m^{-2} , respectively) (Figs. 1. and S4). The power sources were configured to maintain the current intensity through each cell by automatically modifying the applied voltage within a range of $[0.00\text{--}1.00 \text{ V}]$. The maximum total gas production of 14.74 L d^{-1} ($0.01 \text{ m}^3 \text{ m}^{-2} \text{ d}^{-1}$ or $0.11 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$) with a 90% of hydrogen purity was obtained at the highest current intensity tested (0.20 A cell^{-1}) and at an applied potential of 0.93 V . This is the highest hydrogen production reported at such a high MEC scale ($>100\text{L}$) and represents a significant advancement on the way towards reaching viable hydrogen efficiencies. Most of the studies on hydrogen production from UWW in cassette-type pilot-scale MECs report values in the range $0.005\text{--}0.031 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ (Table 2). However, the performance and efficiency parameters obtained with the synthetic medium should be treated with caution, and not exactly considered for future scaling-up processes, since the characteristics of real UWW hinder to obtain these high-performance values, as will be discussed below. It was observed that r_{CAT} varied as a function of the current intensity used and increased from $36 \pm 1\%$ with 0.10 A cell^{-1} to $68 \pm 2\%$ with 0.20 A cell^{-1} . The major reported cause of low

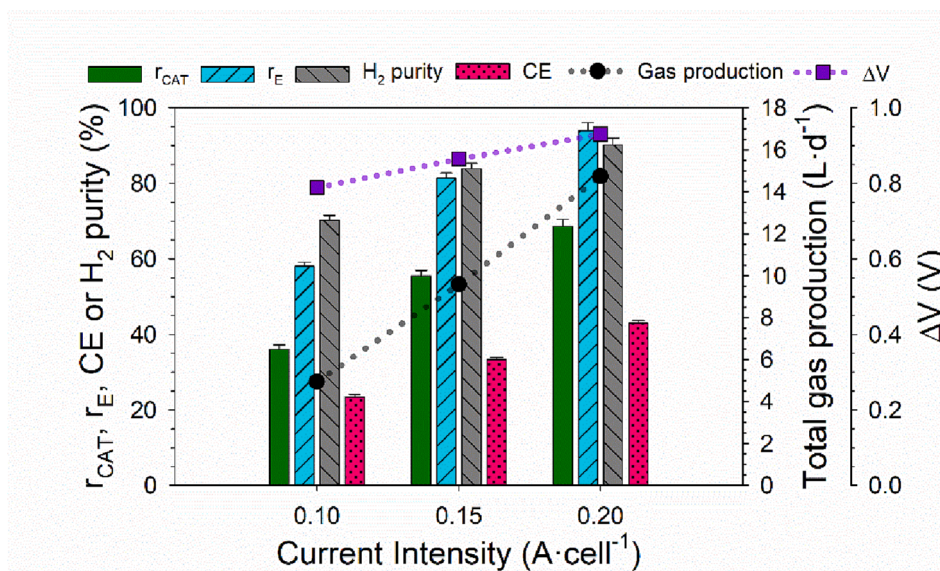


Fig. 1. Average key performance indices, applied potential and total gas production at different fixed intensities during the continuous operation with synthetic wastewater. Standard deviations are shown in the figure.

Table 2

Summary of the data reported with cassette-type MECs at pilot-scale for urban wastewater treatment.

Pilot plant	V (L)	WW (g COD L ⁻¹)	OLR (g L ⁻¹ d ⁻¹)	S/V (m ² m ⁻³)	HRT (h)	T (°C)	CODr (%)	r _{CAT} (%)	CE (%)	ΔV (V)	j _{max} (A m ⁻²)	H ₂ (m ³ m ⁻³ d ⁻¹)
Heidrich et al. [25]	120	UWW (0.45)	0.14	16.4	24	16	34	70	55	1.1	0.30	0.015
Heidrich et al. [28]	100	UWW (0.07–0.4)	0.54			1/22	33	49	41	0.7	0.25	0.007
Baeza et al. [27]	130	UWW (0.3–0.5)	0.5/0.25	12.6	24/48	22	6/25	82	28	1.0	0.30	0.031/0.020
Cotterill et al. [29]	175	UWW (0.3–0.5)	1.60	34	5	11.5	63	10	21	0.9	0.29	0.005
Leicester et al. [56]	36	RSL (2.2–4.3) ^a	4.4–8.6	20.2	0.36–432	10–20	52 ^b	1 ^b	6 ^b	–	1.11 ^b	0.011 ^b
This work	135	SWW (0.9)	0.33	10.9	65	18	64	70	26	0.9	1.23	0.099
		UWW (0.2–0.3)	0.2–0.07		26–94		30–77	77–66	37–23		0.42–0.27	0.038–0.022
		UWW _A (1.0)	0.40		65		63	88	17		0.34	0.040

V: Anodic working volume; S/V: Projected anodic surface to reactor volume ratio; CODr: COD removal; j_{max}: Maximum current density.^a Reactor fed with return sludge liquor (concentrated filtrate of anaerobic digestion sludge).^b Results reported at the optimal HRT found (0.5 d).

r_{CAT} are hydrogen leakages. It was determined that hydrogen leakages were constant in the range studied (6.23 ± 0.19 L H₂ d⁻¹). Hence, the higher the hydrogen production, the lower the effect of hydrogen losses due to leakages, causing r_{CAT} to increase with intensity.”

Hydrogen was the major compound in the gas collected, followed by methane. In all cases, the methane production remained constant (around 1.48 ± 0.05 L CH₄ d⁻¹). Methane could come from either the anodic or the cathodic chamber. In the first case, methane from anaerobic organic matter degradation would be transferred to the cathode through the AEM. This hypothesis implies that most of the methane is produced in the bulk liquid and, therefore, methanogenesis depends on the anolyte conditions rather than on the anodic biofilm. Under this hypothesis, the methane flux through the membrane should have been independent of the different applied potential, which seems to be in line with the observed results. Moreover, if methane had been produced in the anode, CE would have been very low, since part of the entering substrate would be diverted to methanogenesis rather than to exoelectrogenesis. The CE values obtained were below 50% in all cases, which seems to corroborate that part of the COD was not consumed by the exoelectrogens.

On the other hand, cathodic methane production implies the presence of hydrogenotrophic methanogens in the cathode, which would occur if the cells were not completely sealed. In this scenario, the pH gradient would have been reduced by the direct contact between electrolytes and the cathode may have become biocompatible (otherwise, the theoretical cathodic pH in a double chamber MEC should be around 12). Additionally, methanogenesis would depend on the current intensity values as there would be different concentrations of hydrogen for each case, thus affecting the methane formation kinetics. The pH of the catholyte was over 12, and hence it can be concluded that most of the methane collected came from the anodic chamber. Thus, it is necessary to use IEM with lower permeability to prevent methane diffusion produced anaerobically in the anode.

The highest r_E attained ($94 \pm 2\%$) corresponded to the highest current density and hydrogen production, but energy neutrality was not attained yet. The applied potential required was much higher than the theoretical value of 0.13 V (calculated under standard conditions, at a pH of 7 and with acetate as sole electron donor) due to the potential losses of the system. As high current densities are required to obtain significant hydrogen production, reducing the energy loss is essential to achieve energy neutrality.

3.2. Operation in continuous mode of the pilot plant with UWW

After the period with synthetic wastewater, the feed was entirely

replaced by wastewater from a municipal WWTP after primary settling without any amendment. As expected, this real effluent showed reduced electrical conductivity (1.1 ± 0.2 mS cm⁻¹) and concentration of organic matter (COD_S = 247 ± 34 mg L⁻¹). The organic matter fractionation of real influents is very relevant because the substrate-utilization capabilities of most exoelectrogenic bacteria are limited to fermentation products, basically volatile fatty acids (VFA). Thus, fermentative bacteria are essential to provide the substrate required for current generation from the potential complex molecules present in domestic effluents. A very diverse microbial community needs to be developed in the reactor, including exoelectrogens, hydrolytic and fermentative bacteria [18,45]. These populations establish a syntrophic relation with positive interactions that favour an efficient anodic-based oxidation of the complex organic compounds, which is the first step to obtain hydrogen evolution at the cathode. However, the inherent variability of UWW properties challenges the ARB predominance in the reactor and competitors for substrate can appear, thereby reducing the CE. For this reason, a comprehensive analysis of the plant performance under different scenarios is essential to understand the opportunities of these systems under real conditions.

The plant was operated for >100 days under different operational conditions. In a first period (Stage 2), different applied potentials were tested to find the conditions with better energy efficiency (section 3.2.1). Afterwards (Stage 3), the reactor was operated for >40 days at the optimal applied potential of 0.90 V to understand the effect of temperature on the plant performance (section 3.2.2). Finally (Stage 4), the plant was operated under different HRT values to determine its effect on the COD removal efficiency (section 3.2.3). The most important operational parameters are shown in Table 3.

3.2.1. Effect of applied potential on the plant performance

Increasing the input voltage can enhance H₂ production and boost current density as shown by previous authors [46] at expenses of higher energy requirements. However, undesired electrochemical oxidation of the compounds in the wastewater and even water electrolysis may occur when the applied potential is too high, resulting in a decrease of exoelectrogenic activity [47]. The applied potential has a significant impact

Table 3

Key operational parameters of each experimental stage.

Period	ΔV (V)	Inlet flowrate (L d ⁻¹)	T (°C)
Stage 1	0.8–0.9	50	18
Stage 2	0.8–1.1	50	18
Stage 3	0.9	50	18–30
Stage 4	0.9	35–123	18

on energy efficiency since it is directly proportional to the power consumption. Thus, it is important to find its optimal value to improve MEC performance at the lowest applied potential possible.

Fig. 2 shows the average current density and gas production values obtained at different applied potentials and Fig. S5 shows the current density profiles. The applied potential was increased in 0.10 V intervals, from 0.50 V to 1.10 V, which is the typical range for the pilot-scale operation with UWW. The best results for this set of experiments were obtained with an ΔV of 0.90 V, showing a maximum current density (0.21 A m^{-2}) and hydrogen production of $0.016 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ ($1.5 \cdot 10^{-3} \text{ m}^3 \text{ m}^{-2} \text{ d}^{-1}$), with $r_{\text{CAT}} = 64 \pm 2\%$, $r_E = 91 \pm 2\%$, and $\text{CE} = 30 \pm 7\%$. Higher ΔV were detrimental as they had no significant effect on the performance and only decreased energy efficiency to $r_E = 68 \pm 2\%$. Due to the inherent characteristics of real wastewater, current density and hydrogen production were down to 6 times lower than the reported in section 3.1 using synthetic wastewater. The most likely reason is that the limiting step was the fermentation of complex organic matter to VFA rather than the exoelectrogenic activity. At ΔV above 0.90 V, the exoelectrogenic ability to consume VFA was likely higher than the VFA production by fermentation. The composition of the gas was also richer in methane (around 30%), and although r_E remained at similar values, r_{CAT} and CE were approximately 10% lower. The low COD strength of domestic effluents can limit MEC performance when compared to synthetic wastewater. This difference in performance can lead to undersized designs if the productions and efficiencies of studies carried out under unrealistic conditions are taken as a reference for future scale-up. Laboratory-scale research is essential but understanding the limitations of such systems at larger scales with real effluents is crucial too.

Any voltage below 0.8 V was not enough to drive hydrogen production. Again, the minimum ΔV to drive evolution was higher than the theoretical value (i.e. 0.13 V) due to overpotentials and ohmic losses of the system [14]. Increasing the electrode surface area, improving the electrode catalytic effect, increasing the temperature, or increasing the ARB-enrichment in the anodic biofilm can decrease the overpotential losses [14]. Furthermore, ohmic losses can generally be minimized by reducing the electrode spacing, using a membrane with low resistivity, and increasing the solution conductivity [11,15]. In real WWTPs, the use of externally enriched cultures is unfeasible considering the large microbial diversity that is entering in the reactor and the potential evolution of the population and increasing the load and the conductivity by dosing chemicals to the reactor is unfeasible [48]. Thus, research should

focus on improving the structural design of the cells, enhancing the physical, chemical, and spatial properties of the electrodes and the membrane, to reduce the overall internal resistance to current flow.

3.2.2. Effect of temperature on the plant performance

In this second experimental period, the effect of temperature was studied for the first time in a pilot-scale MEC using both raw and amended (acetate addition) UWW to decouple the fermentation step from exoelectrogenesis. Temperature was progressively increased from 18°C to 30°C in 39 days feeding raw UWW. Afterwards, the reverse operation was carried out until room temperature was reached again, using amended UWW. Fig. S6 shows all the experimental profiles obtained during this period. The reactor showed a robust capacity to remove the organic matter present in the wastewater under different temperatures, with COD removal efficiencies above 70% ($75 \pm 4\%$) in the case of raw UWW, and around 60% in the case of amended UWW ($63 \pm 5\%$). Fig. 3 displays the dependence of current density and hydrogen production on temperature for both raw and amended UWW. The current density increased at 30°C up to $0.36 \pm 0.02 \text{ A m}^{-2}$ with raw UWW which was 91 % higher than that obtained when operating at 18°C , while an increase of 40 % was observed when the influent was amended with acetate ($0.50 \pm 0.01 \text{ A m}^{-2}$).

The simplified Arrhenius equations model fit are also presented in Fig. 3, showing a good agreement (R^2 up to 0.972) between experimental data and model prediction. The highest deviation appears for the current density of UWW at the higher temperature tested ($T = 30^\circ\text{C}$). Typical activated sludge models [49] categorize biological processes based on their temperature dependency θ : none (1.00), low (1.04), medium (1.07), and high (1.12). In this study, the operation with UWW leads to a medium value ($\theta = 1.065$), while operating with VFA-amended UWW resulted in a low temperature dependency ($\theta = 1.031$). These results suggest that the operation with raw UWW was more sensitive to temperature changes than the operation with VFA-amended UWW. This observation agrees with fermentation being the limiting step, and that temperature has a strong effect on the rate of the fermentation processes required to transform complex carbon sources into VFA. When increasing the temperature with raw UWW, the limitation due to fermentation was mitigated and VFA production increased. In contrast, when the pilot plant was fed with acetate in excess, fermentative bacteria did not play a limiting role because there was already enough substrate for exoelectrogens and the effect of

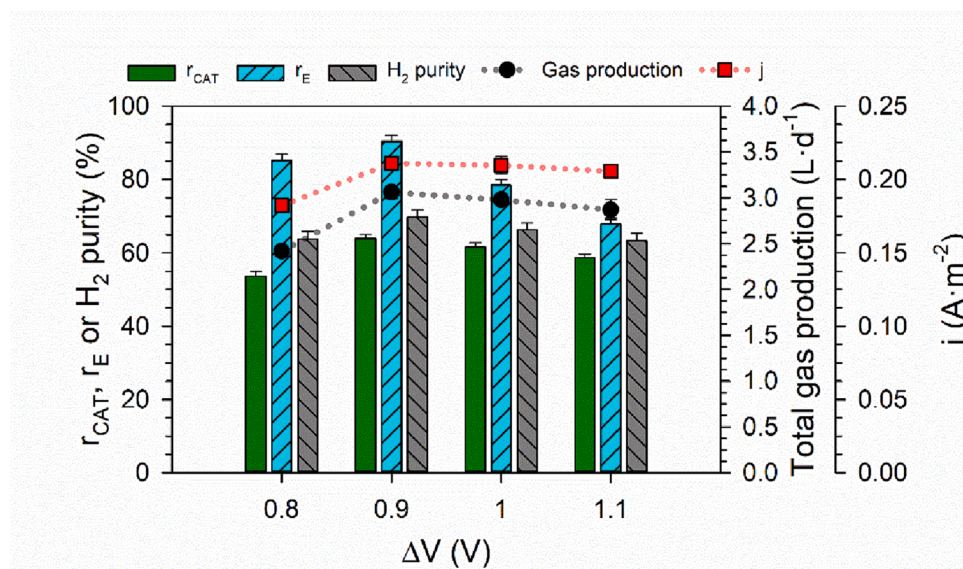


Fig. 2. Average key performance indices, current density and total gas production at different applied potential during the continuous operation with urban wastewater. Results for $\Delta V < 0.8 \text{ V}$ are not shown in the figure. Standard deviations are shown in the figure.

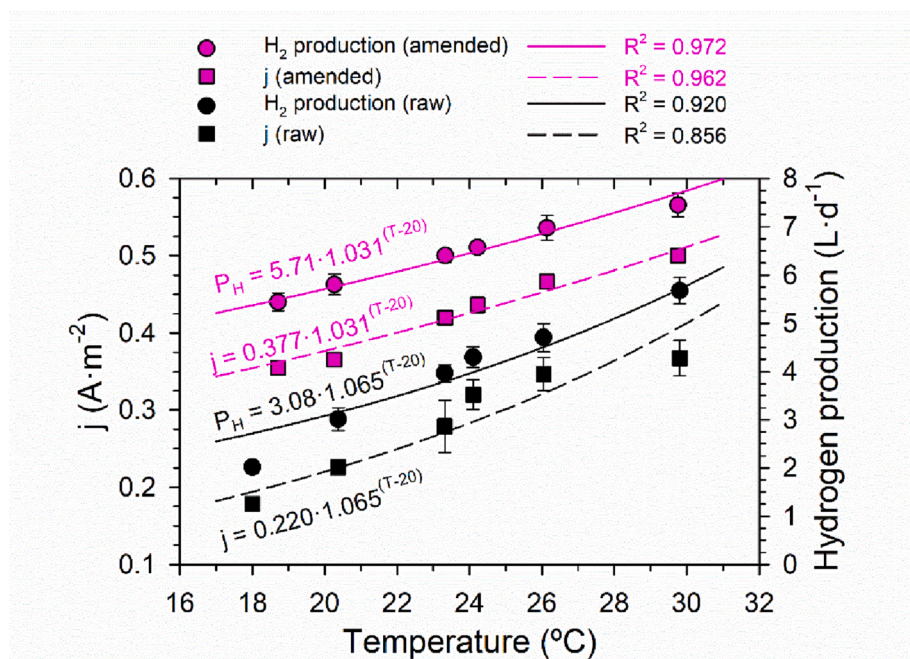


Fig. 3. Average current density and hydrogen production at different temperatures during the continuous operation with raw and amended urban wastewater. Experimental data are presented with standard deviation. Lines represent the model predictions (equation (4)).

temperature on fermentation was not observed in the experimental results.

The value of r_{CAT} (Fig. S6) increased progressively with temperature to a value of $80 \pm 2\%$ for raw UWW, and in the case of amended, up to $85 \pm 3\%$. Methane production was also present in this scenario for both substrates, but it was not significantly affected by temperature. As a result, the gas produced had a higher percentage of hydrogen. CE was also improved by increasing the temperature with raw UWW. On one hand, the organic removal rate (ORR) was not substantially influenced by temperature because it appeared to be limited by the low COD concentration in the reactor. These low COD concentrations did not have much room to be reduced. On the other hand, current density did increase with temperature because COD was being fermented faster and VFAs availability for ARBs improved. These results suggest that ARBs uptake VFAs faster than methanogens. Nevertheless, a large variability in this parameter can be observed because of the variable influent concentration. CE decreased drastically below $15 \pm 2\%$ when the OLR was increased from day 39 onwards (Fig. S6, VFA-amended UWW period), indicating an increase of the substrate consumed by other planktonic microorganisms present in the anode chamber, causing the reactor to show lower current density and gas production than that with only synthetic wastewater, despite the OLR was increased to $0.40 \text{ g L}^{-1} \text{ d}^{-1}$.

Some studies also showed a reduction in the MEC performance under low temperatures [50]. Heidrich et al. [28] operated a MEC pilot plant fed with domestic wastewater at room temperature for 12 months of operation, but the general low levels of performance and high variance in the data masked any temperature trend that may have occurred. Moreover, the influence of temperature on the composition and production of VFAs in AD has been investigated by several authors. These systems share some of the hydrolytic and acetogenic steps with MECs and are limited by low temperatures [51]. For instance, significant changes on the performance while increasing the temperature from 10°C to 35°C under mesophilic conditions have been reported [52,53]. Thus, it can be stated that temperature plays an important role in MEC operated with UWW as the metabolism of the entire microbial community is altered. Considering the daily and seasonal temperature fluctuations, this system will provide variable hydrogen production if

temperature is not controlled.

3.2.3. Effect of HRT on the plant performance

HRT is a critical parameter for MEC performance. Low HRTs imply an increase of the organic matter load at expenses of not having enough time for an efficient substrate conversion to hydrogen. In our previous work, the HRT was increased from 1 to 2 days to tackle an unsuccessful organic matter removal [27]. In fact, batch experiments showed that 10 days were needed to reach 72% of organic matter removal. To the best of our knowledge, there are no reports on the effect of HRT on a double-chamber pilot-scale MEC treating real UWW, but some dynamic models are being used to study its effect [54,55]. In any case, reducing the HRT remains a critical factor in making MEC technology feasible.

In the last experimental period (from day 60 onwards), different volumetric flow rates were tested to study the effect of HRT on the reactor performance. Surprisingly, the plant showed $23 \pm 2\%$ increase in current density compared to Stage 2, although the same operational conditions were maintained ($\text{HRT} = 2.9 \text{ d}$; $\Delta V = 0.9 \text{ V}$ and $T = 18^\circ\text{C}$), probably caused by a change in the biofilm populations after the period with amended UWW. As a result, the pilot plant achieved energy neutrality with all volumetric flow rates tested.

Fig. 4 shows the average results for each HRT during the continuous operation with raw UWW. Decreasing the HRT from 4 to 1 d caused an increase of 86% in current density, reaching 0.42 A m^{-2} , with hydrogen production following the same trend and a cathodic recovery of $77 \pm 2\%$. Specifically, hydrogen production increased from 0.024 to $0.038 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ when the HRT decreased from 4 to 1 d (i.e. 52% increase). This boost in current density or hydrogen production through the inlet flowrate can be attributed to two factors: i) increasing the OLR increases both the COD concentration in the reactor and the exoelectrogenic kinetics and ii) it also decreases the residence time of the planktonic biomass in the system, favouring the washout of methanogens (i.e. less anodic methane production and higher CE). On the other hand, higher flowrates increase the velocity of the liquid in the anode chamber, increasing the turbulence and improving external mass transfer from the bulk to the biofilm surface. However, the quality of the treated water was drastically reduced at low HRT. The OLR was increased from $63 \pm 6 \text{ mg COD L}^{-1} \text{ d}^{-1}$ at $\text{HRT} = 4 \text{ d}$ to $200 \pm 15 \text{ mg COD L}^{-1} \text{ d}^{-1}$ at $\text{HRT} = 1$

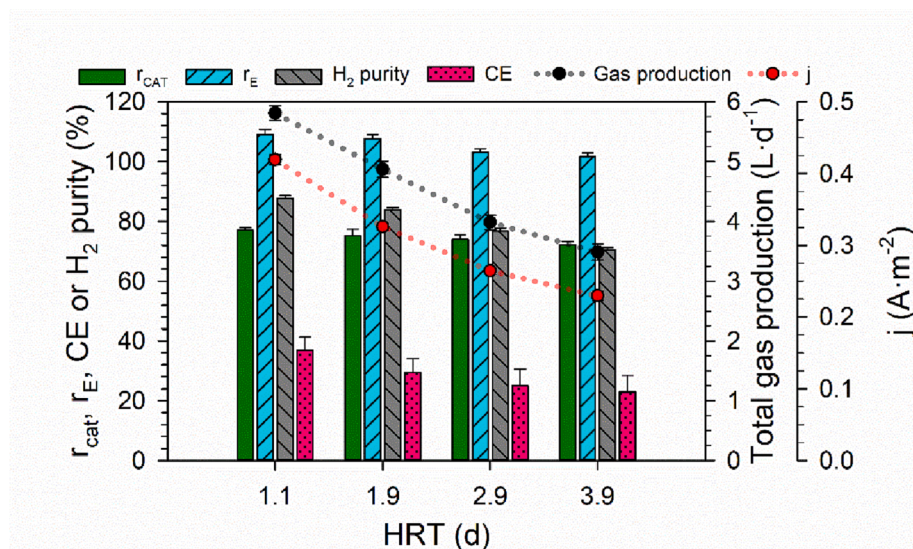


Fig. 4. Average key performance indices, current density, and total gas production at different hydraulic residence times during the continuous operation with urban wastewater. Standard deviations are shown in the figure.

d, but the maximum ORR achieved was around $70 \pm 9 \text{ mg COD L}^{-1} \text{ d}^{-1}$ at $\text{HRT} = 1 \text{ d}$, leading to a significant reduction in the percentage of COD removed (from $75 \pm 4\%$ to $30 \pm 3\%$ (Fig. S7)). Despite the higher water treatment efficiencies at high HRTs, CEs were still lower due to biological organic matter degradation pathways different from exoelectrogenesis. In fact, an increase in CE from 23 to 37% can be observed as the HRT decreases to 1 d. Despite the percentage of COD removed decreased at lower HRT, the energy recovery was higher. For the same reason, the amount of methane produced and transferred from the anode was lower at low HRTs: $1.14 \pm 0.22 \text{ L CH}_4 \text{ d}^{-1}$ at $\text{HRT} = 4 \text{ d}$ whereas $0.72 \pm 0.11 \text{ L CH}_4 \text{ d}^{-1}$ were recovered at $\text{HRT} = 1 \text{ d}$. Thus, there is a trade-off between effluent quality and hydrogen production in the choice of the optimal HRT. High HRT will result in higher percentage of COD removal, whereas low HRT will result in higher hydrogen production. Low HRT configurations would require higher anodic surface to volume ratios ($\text{m}^2_{\text{anode}} \text{ m}^{-3}_{\text{reactor}}$) to promote the exoelectrogenic substrate consumption over other biological processes. However, the changes in the biofilm structure due to these new hydraulic conditions could play an important role and deserve more study.

3.3. Comparison with previous cassette-type MEC pilot plants

The implementation of MECs in current WWTPs has the potential to transform organic pollutants into energy and valuable products as well as reducing the current treatment costs with environmental benefits. However, there is a wide gap between theoretical and practical performance. As indicated in Table 2, the current densities and hydrogen production rates reported with pilot-scale cassette MECs are far from those obtained in lab-scale systems. Current densities reported in pilot plants usually range between 0.25 and 0.30 A m^{-2} ; only the plant presented by Leicester et al. [56] reported a value of 1.11 A m^{-2} but working with a smaller sized plant with anodic volume of 36L, and treating return sludge liquor with higher organic matter concentration. On the other hand, lab-scale MECs can yield up to 2 A m^{-2} [57,58]. Hydrogen production in double-chamber cassette-type MEC pilot reactors is usually in the range $0.005\text{--}0.031 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$. This is much lower than $1\text{--}1.3 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ reported in bench-scale [16,19]. In this study, a maximum hydrogen production of $0.099 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ was obtained with synthetic wastewater. Furthermore, $0.038 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ of hydrogen were obtained feeding UWW, which is 23% higher than the maximum value reported previously under similar conditions [27]. This is equal to a 60% increase in the specific hydrogen production ($\text{m}^3 \text{ m}^{-2}$

d^{-1}). We attribute this improvement in performance to different upgrades implemented in the pilot plant: i) the replacement of the current and voltage monitoring system, removing the external resistance to determine the intensity in the cells, ii) the enhancement of the electrical wiring, replacing the crocodile clips with SS connection strips to avoid rusting and iii) the replacement of PTFE tubing with Marprene® in the gas collection system. Furthermore, the microbial populations established in the anodic compartment could have played a critical role.

The organic matter removal efficiencies were similar to those obtained in other studies, and $\text{HRT} > 3$ days was required to achieve organic matter removal of 70%. The only exception appears in Cotterill et al. [29], who proved at pilot-scale that increasing the anode surface to volume ratio can result in a significant reduction of the required HRT, obtaining a good quality effluent. However, the lack of pilot-scale MEC experiences available in the literature does not allow a reliable comparison. More research is needed in the field.

3.4. Comparing MECs versus conventional wastewater treatment technologies

MECs need to achieve high COD removal efficiencies with short HRT to compete with other existing technologies for municipal wastewater treatment, such as the A/B configuration [59,60]. A/B systems consist of an A-stage with short HRT and a B-stage where autotrophic nitrogen removal occurs. The A-stage aims at converting the influent particulate, colloidal, and soluble COD into a single solid stream with minimum mineralisation and low energy consumption by the stimulation of sludge production, bacterial storage, and bioflocculation [61]. The surplus sludge of the A-stage is diverted to an energy recovery process such as anaerobic digestion, while the supernatant is treated in the B-stage.

The costs and possible revenues of the treatment of 1 m^3 of UWW with a COD concentration of 300 mg L^{-1} by different systems (CAS + AD, HRAS + AD and a MEC) were compared (Table 4). Wastewater treatment was successful in CAS with 85% of COD removal, but the EE was the lowest (59 %) of all technologies investigated and the HRT required was higher than in a HRAS. Rey-Martínez et al. [62] reported the highest E_c of all HRAS studied due to a higher COD mineralization, even though the maximum COD removal was achieved. On the other hand, the best results were obtained in the HRAS published by Kinyua et al. [63], with a NE_p of $-0.140 \text{ kWh kg}^{-1} \text{ COD degraded}$ and a COD removal of 77%. Negative values of NE_p indicated that the methane produced contained more energy than the required by the system.

Table 4

Comparison of conventional and high-rate activated sludge versus microbial electrolysis cell.

	Guven et al. 2019 [64]	Kinyua et al. 2017 [63]	Guven et al. 2019 [64]	Rey-Martínez et al. 2021 [62]	This study	
Technology	CAS	HRAS	HRAS	HRAS	MEC	
T (°C)	20	20	20	18–22	18	
DO (mg L ⁻¹)	2	1	0.5	1	–	
HRT (h)	6	0.5	1	2.5	70	26
SRT (h)	96	13.4	9.6	28.8	–	
CODr (%)	86	77	59	88	74	30
H ₂ or CH ₄ (m ³ d ⁻¹)	0.015	0.023	0.017	0.016	0.066	0.041
H ₂ or CH ₄ (m ³ kg ⁻¹ COD degraded)	0.057	0.098	0.098	0.059	0.325	0.455
E _C (kWh d ⁻¹)	0.238	0.168	0.160	0.234	0.198	0.107
E _P (kWh d ⁻¹)	0.139	0.200	0.145	0.144	0.188	0.116
E _C (kWh kg ⁻¹ COD degraded)	0.924	0.727	0.904	0.886	0.924	1.189
E _P (kWh kg ⁻¹ COD degraded)	0.541	0.867	0.819	0.545	0.892	1.293
E _P COD (%)	14	22	21	14	24	33
NE _P (kWh/kg COD degraded)	0.383	–0.140	0.085	0.341	0.031	–0.104
EE (%)	59	119	91	62	95	109

DO: Dissolved oxygen; SRT: Solids retention time; E_C: Energy consumed; E_P: Energy produced; E_PCOD: Energy recovered from removed COD from influent; NE_P: Net energy production; EE: Energy efficiency.

Guven et al. [64] reported lower percentages of mineralized and purged COD (operating at HRT < 1 h and SRT < 14 h) than those of Kinyua et al. [63], which resulted in a 23% reduction in methane production, decreasing EE to 91%.

Considering the results of our MEC pilot plant, 0.066 m³ d⁻¹ of hydrogen could be produced with a NE_P of –0.031 kWh kg⁻¹ COD degraded at HRT = 70 h, while an average of 0.019 ± 0.004 m³ d⁻¹ of methane would be generated with the HRAS + AD (average NE_P = 0.095 ± 0.024 kWh kg⁻¹ COD degraded). Additionally, a NE_P of –0.104 kWh kg⁻¹ COD degraded could be achieved with an EE of 109%, if HRT in the MEC was decreased to 26 h. Nonetheless, the MEC E_C at HRT = 26 h was the highest of all technologies compared (1.19 kWh kg⁻¹ COD degraded). Therefore, the applied potential in the MEC must be decreased to make it less energy-intensive, since the energy consumption is directly proportional to the ΔV.

MECs have the potential to become a realistic alternative to CAS or HRAS in terms of energy recovery because they recover similar or even more energy from the organic compounds in wastewater compared to CAS/HRAS + AD. Additionally, the sludge post-treatment requirements appear to be considerably lower considering the total suspended solids (TSS) in the outlet of the MEC pilot plant (<20 mg L⁻¹). Also, during the operation with the MEC pilot plant, an average of 15 ± 3% of N-NH₄⁺ and 8 ± 1% of P-PO₄³⁻ removal was observed (Fig. S8), possibly to cover the nutrient requirements of ARB and other microorganisms that might have activity. By contrast, in a HRAS, N-NH₄⁺ and P-PO₄³⁻ removal can account up to a 30% and 12%, respectively [62]. Thus, it appears to be more feasible to avoid nitrification in an MEC than in a HRAS (if it is intended to be used as A-stage in an A/B system). However, the HRT required in an MEC to achieve a similar COD removal to that of a HRAS may be several times higher. The successful implementation of such technology on a larger scale is currently hindered by the important differences in volumetric treatment capacity between MECs and

conventional wastewater treatment processes. Hence, future designs should be aimed at: i) maximizing the anode surface to reactor volume ratio, making the exoelectrogenic activity in the reactor prevalent to achieve high COD removal efficiencies at low HRT, and ii) optimizing the cassette design to reduce the electrical resistance to current flow in the cells.

4. Conclusions

- A MEC pilot plant was run under different operational conditions. The best results were obtained with synthetic wastewater, yielding the maximum current density (1.23 A m⁻²) and hydrogen production (13.40 L d⁻¹, 0.10 m³ m⁻³ d⁻¹ and 1.0·10⁻² m³ m⁻² d⁻¹) ever reported in a pilot-scale MEC.
- Low COD concentration in the UWW limited the plant performance. Specifically, an average hydrogen production of 5.1 ± 0.2 L d⁻¹ (0.038 m³ m⁻³ d⁻¹ or 3.9·10⁻³ m³ m⁻² d⁻¹) were obtained at HRT = 1 d. These are much better results than similar MEC pilot plants treating domestic wastewater.
- High COD removal efficiencies were obtained with UWW under a wide range of operational conditions.
- The effectiveness of double-chamber configuration in achieving viable current densities and recovering most of the hydrogen produced was proved (r_{CAT} > 80%).
- The optimum applied potential for this configuration was found to be 0.9 V, far from the theoretical 0.13 V.
- Low-moderate temperatures (<20 °C) resulted in poor anode-based oxidation but when temperature was increased (>30 °C), the fermentation step was improved, and hydrogen production increased from 2.0 to 5.7 L d⁻¹.
- The plant was able to produce 5.1 L H₂ d⁻¹ at HRT = 1 d, which was 52% more than that obtained operating at HRT = 4 d but followed by a significant reduction in the percentage of COD removed (from 77 ± 1% to 30 ± 3%).
- The MEC performance was compared to CAS and HRAS. MEC have the potential to become an alternative to conventional wastewater treatment in terms of energy recovery, but the limited volumetric treatment capacity hinders its full-scale implementation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2023.144001>.

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