

Brewery Wastewater Treatment Coupled to Green Hydrogen Production through Microbial Electrolysis Cell

Angela Marchetti^a, Syrine Berhouma^{a,b}, Habib Chouchane^b, Marco Zeppilli^{a,*}

^aDepartment of Chemistry University of Rome Sapienza, Piazzale Aldo Moro 5 00185 Rome

^bUniversity Manouba, ISBST, BVBGR-LR11ES31, Biotechpole Sidi Thabet, Ariana, Tunisia

marco.zeppilli@uniroma1.it

Brewery wastewater represents a challenging effluent due to its high organic load and variable composition, but also a valuable substrate for resource recovery within circular economy strategies. In this context, here a three-chamber microbial electrolysis cell (MEC) system was operated in continuous flow using real brewery wastewater without any pretreatment, with a bioanode surrounded by two stainless-steel cathodes. The system was operated at an organic loading rate of 0.5 gCOD/(L·d) and with the anode polarized at +0.2 V vs. SHE. Stable hydrogen production was achieved, with an average hydrogen purity of $65.5 \pm 0.7\%$ and a cathodic coulombic efficiency of $50 \pm 2\%$. COD mass balance indicated that most of the removed organic matter ($69 \pm 1\%$) was converted into electrical current (around 47%), while methane production was negligible ($0.8 \pm 0.2\%$). The energy balance resulted in an overall energy efficiency of $43 \pm 2\%$. The results obtained clearly demonstrate the suitability of brewery wastewater as a promising substrate for direct treatment and valorization through biohydrogen production in MEC systems. Kinetic characterization of the bioanode activity as a function of anodic potential also confirmed a good electrocatalytic performance of the bioanode under the selected working potential.

1. Introduction

The transition towards a low-carbon energy system requires the development of sustainable technologies to produce renewable energy carriers (Marchetti and Stoller, 2019; Yu et al., 2021; Zainal et al., 2024). Hydrogen can play a key role in energy storage, sector coupling and decarbonization of hard-to-abate sectors such as industry and transport. However, conventional hydrogen production processes as water electrolysis typically require electrical energy inputs in the range of 4–5 kWh per Nm³ of hydrogen produced, thus limiting their large-scale sustainability (Shiva Kumar & Lim, 2022). In this context, increasing attention has been devoted to the development of integrated systems capable of coupling biofuels production with waste valorization, following the principles of circular economy and resource recovery. In particular, industrial wastewaters represent an attractive opportunity due to their high organic content and relatively stable composition. Among them, brewery wastewater (BWW) is generated in large volumes worldwide as a by-product of beer production processes (3–10 L of wastewater generated per liter of beer). BWW is typically characterized by high chemical oxygen demand (COD), ranging from 2 to 10 g/L, high biodegradability, low toxicity and variable pH, making it suitable for biological treatment (Liu et al., 2025). Conventional treatment technologies for BWW mainly rely on aerobic biological processes, effective in COD removal but intrinsically energy-intensive, characterized by high operational costs. Also, anaerobic digestion has been widely proposed, allowing biogas production and partial energy recovery. However, methane-based systems suffer from several limitations, including long hydraulic retention times, limited conversion efficiencies and sensitivity to operational conditions (Laiq Ur Rehman et al., 2019). In recent years, microbial electrolysis cells (MECs), have emerged as a promising alternative for the simultaneous treatment of organic waste streams and hydrogen production. The electroactive biofilm established on the anode can oxidize organic matter, releasing electrons and protons, subsequently converted into hydrogen at the cathode under the application of a small external voltage. MEC benefit from a significant reduction in energy input, since part of the electrical energy required for hydrogen evolution is supplied by the

biochemical oxidation of organic substrates. Hence, MECs have been extensively investigated for the treatment of a wide range of wastewaters, including municipal effluents, food-processing wastewaters, agricultural residues and synthetic substrates (Alcaraz-Gonzalez et al., 2023; Saravanan et al., 2020). Between the several challenges that still hinder the practical implementation of MECs, reactor configuration plays a crucial role in determining the overall performance of MECs. In this view, most studies reported in literature have focused on single-cathode two-chamber configurations, which are simple in design but often limited by high internal resistance and suboptimal hydrogen recovery (Bora et al., 2022; Sharma et al., 2024). To overcome these limitations, alternative reactor geometries have been proposed, including multi-electrode and multi-chamber configurations. In this context, the present study investigates the feasibility of a three-chamber MEC consisting of a central bioanode and two stainless-steel cathodes for the simultaneous treatment of real BWW and hydrogen production. This geometry represents a promising design strategy due to reduced electrode spacing, improved current distribution, enhanced hydrogen production rates and lower overall energy consumption. However, its application to real industrial wastewater remains largely under-investigated. Hence, the reactor was operated in continuous flow under controlled electrochemical conditions, and its performance was evaluated in terms of COD removal, hydrogen production, coulombic efficiency, gas purity and overall energy balance. Additionally, a kinetic characterization of the bioanode as a function of the applied potential was carried out to investigate the electrocatalytic behavior of the anodic biofilm. The results here obtained provide insights into the potential of BWW as a valuable substrate for bioelectrochemical hydrogen production and contribute to the development of more efficient and sustainable wastewater-based energy recovery systems.

2. Material and methods

2.1 Microbial electrolysis cell configuration

The laboratory-scale MEC comprised three identical Plexiglas chambers (each with a volume of 0.86 L and internal dimensions of 17 cm × 17 cm × 3 cm). A CMI International cation exchange membrane (CEM) (Membrane International, USA) was installed between the bioanode and the cathodic chambers, sealed with butyl rubber gaskets, permitting the migration of protons. The cathode chambers consisted of two sheets of 316 stainless steel (RS components), each with a surface area of 176.46 cm², while granular graphite with a diameter < 4 mm (Faima srl, Milan) was used as filler for the bioanode. Notably, the graphite granules worked both as the electrode material and as a biocompatible substrate for microbial biofilm development. The microbial consortium used as inoculum consisted of an activated sludge composed of MMCs from a full-scale wastewater treatment plant. Before the bioanode inoculation, this sludge was washed down with a mineral medium, which composition is reported elsewhere (Marchetti et al., 2025). The anodic chamber was operated under continuous flow conditions with a hydraulic retention time (HRT) of 1.2 days and an organic loading rate (OLR) of 0.5 gCOD/(L·d). The feeding solution, consisting exclusively of wastewater obtained from beer production, was characterised by a pH of 8.5 and a conductivity of 2.83 mS/cm stored in a tedlar bag. On the other hand, the cathodic chambers were operated in batch mode and filled with a 0.5 M NaCl solution, with daily removal of the catholyte to offset the water electro-osmotic diffusion through the CEM. Additionally, a 0.30-L sampling glass chamber, equipped with sampling ports, was positioned above each chamber to facilitate the collection of both liquid and gaseous phases. Throughout the entire experimental period, the MEC temperature was maintained at 25 ± 1 °C. Finally, to monitor and control the potential of individual electrodes, an Ag/AgCl reference electrode was placed in each MEC chamber. To integrate the graphite granules into the electrical circuit, three graphite rods (serving as collectors) were immersed in the chambers and connected via titanium wires to a potentiostat (IVIUM Technologies, Eindhoven, The Netherlands). A potential of +0.2 V vs. SHE was applied to the anode, designating it as the working electrode.

2.2 Analytical procedures and bioelectrochemical characterization

The volumetric flow rate of the outlet gas was measured using a Ritter® milligas counter. Gas composition (O₂, H₂, CO₂, and CH₄) was analyzed by extracting a 50 µL sample with a gas-tight syringe and injecting it into a Dani Master GC gas chromatograph (Milan, Italy) equipped with a thermal conductivity detector (TCD). These values were then converted into COD equivalents using a conversion coefficient of 1.42 g COD/g, based on the full oxidation of heterotrophic biomass modeled with the empirical formula C₅H₇O₂N (APHA, 1995). The total and soluble COD were assessed by digesting unfiltered and filtered (0.2 µm) samples, respectively, at 150 °C for 2 hours using commercial Spectroquant test kits (Macherey-Nagel, Duren, Germany) and measuring absorbance at 605 nm with a Nanocolor Vario4 UV–Vis spectrophotometer (Macherey-Nagel, Duren, Germany). Conductivity and pH were determined using an SI Analytics HandyLab680 meter (Fisher Scientific, Wien, Austria) (Vuppala et al., 2019). Finally, by the continuous electrical current and total charge recording through the Iviium n-Stat potentiostat, the electrochemical characterization of the anodic biofilm was assessed. To this

aim, the anodic kinetics were assessed by varying the working electrode potential in the range from -0.4 to $+0.6$ V vs. SHE. For each applied potential, the average current was recorded over a minimum period of 2 hours.

2.3 Calculation

To calculate the removed COD (mg/Ld), the following equation was employed, where COD_{in} and COD_{out} (mg/L) represent the influent and effluent COD concentrations, Q_{in} and Q_{out} (L/d) denote the influent and the effluent flow rates, respectively. The result was then normalized by the bioanodic chamber volume: The bioanode coulombic efficiency, in which meq_i represents the cumulative charge that has passed in the circuit and meq_{COD} represents the theoretical cumulative charge which could have been generated by the oxidation of the removed COD, was determined according to the equation 3. The cathodic coulombic efficiency, was determined as the ratio among the cumulative H_2 produced expressed in meq and the cumulative H_2 that might be produced with the electric current flowing in the circuit: The overall energy efficiency was evaluated using the equation 4.

$$COD_{removed} \left(\frac{mg}{L \cdot d} \right) = \frac{Q_{in} * COD_{in} - Q_{out} * COD_{out}}{V} \quad (1) \quad COD_{converted}(\%) = \frac{COD_{removed} * 100}{COD_{in}} \quad (2)$$

$$CE(\%) = 100 * \frac{meq_i}{meq_{COD}} \quad (3) \quad \eta_E = \frac{|\Delta G^0_{H_2}| CCE}{2|\Delta V|F} \quad (4)$$

3. Results and discussion

3.1 Bioanode performances

Figure 1.A shows the overall total COD conversion balance at the bioanode during the operating period. On average, approximately 70% of the influent COD was converted, while the remaining 30% was not removed by the system. The converted fraction was distributed among different pathways, including current generation, methane production, biomass growth and an unidentified fraction. While, by measuring the total COD subtracted from the soluble component, it can be stated that this 30% not converted can be associated with the particulate matter characterizing the BWW solution. This latter component can be reasonably attributed to the presence of a refractory or slowly biodegradable fraction of organic matter, which is commonly found in complex industrial wastewaters, as for brewery and food-processing ones (Marchetti et al., 2025). Such compounds are not readily accessible to microbial metabolism within the investigated hydraulic retention time and therefore remain in the effluent of the anodic chamber. Overall, the highest fraction of COD conversion was associated with current generation, with an average value of approximately 47%, indicating that most of the organic substrate was effectively diverted towards electrochemical oxidation. During the initial days of operation, a small fraction of COD was converted into methane, suggesting the partial activation of methanogenic pathways. However, this contribution progressively decreased over time and became almost negligible from day 7 onward, reaching a minimum value of 0.2% on day 9. This trend indicates the establishment of stable operating conditions, with a strong shift of substrate utilization towards anodic oxidation and electron recovery for hydrogen production at the cathode. The fraction of COD converted into suspended biomass was relatively low, with an average value of about 0.5% on COD basis. This result is consistent with the nature of MEC systems, which are mainly characterized by attached biomass growth. Indeed, VSS were measured only in the effluent of the anodic chamber, while a significant portion of biomass was likely retained on the electrode surface. This contribution may be included in the $COD_{unknown}$ fraction, which accounted for approximately 20.4 ± 4.5 % of the total COD balance. In addition to biofilm growth, this fraction may also include other oxidation processes involving non-bioelectroactive compounds present in the influent, such as inorganic species or trace components (e.g., metals), which are not directly quantified through current or gas production. **Figure 1B** reports the electrochemical characterization of the bioanode activity as a function of the applied anodic potential. This approach allows the identification of the most favourable operating conditions from an energetic point of view, providing insight into the relationship between electrode polarization and bio-electrocatalytic activity. In the investigated potential range, the current increased progressively with increasing anodic potential, indicating an overall enhancement of electron transfer from the biofilm to the electrode. However, a clear stationary regime was not observed, as the current continued to increase. While a quasi-steady behavior could be identified around $+0.4$ V vs. SHE, a further increase in current was detected at $+0.5$ V and $+0.6$ V vs. SHE, suggesting a non-ideal convergence of the electrochemical response. This behaviour differs from that typically reported when MECs is fed with synthetic substrates, where a well-defined current plateau is often observed and associated with the microbial metabolic activity. In the present case, the absence of a clear plateau may indicate that part of the current generated at higher potentials is not exclusively related to biological oxidation processes but may also involve electrochemical oxidation of inorganic or non-biodegradable species present in the real wastewater matrix.

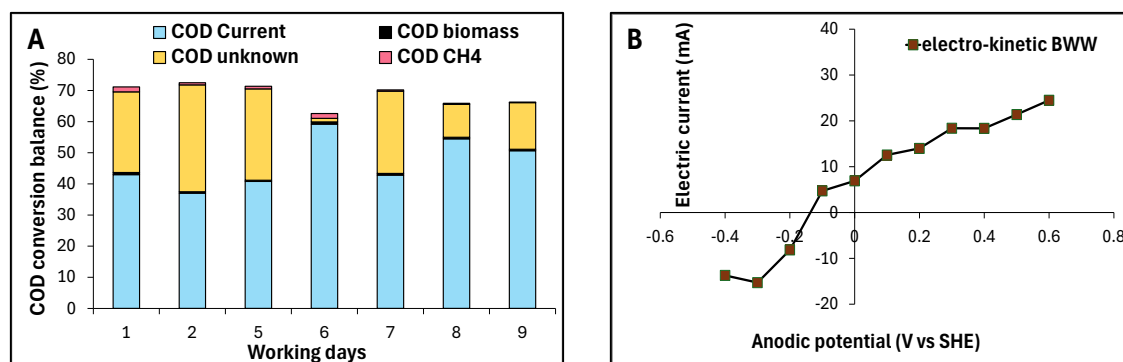


Figure 1: Bioanode performance during continuous operation - (A) COD conversion balance at the bioanode; (B) electrochemical characterization of the anodic biofilm as a function of applied potential.

This trend can be attributed to the lower energetic level imposed on the granular graphite electrode. Under such low-potential conditions, electron transfer from the microbial electron transport chain to the electrode becomes thermodynamically unfavourable, resulting in a progressive suppression of current production. Additionally, a reduction peak was observed around -0.3 V vs. SHE, which may be associated with redox reactions involving inorganic species present in the wastewater, in accordance with the oxidative deviation. For instance, iron compounds commonly found in industrial effluents are known to exhibit characteristic oxidation–reduction peaks and may contribute to non-biological faradaic processes (Carter, 1995). However, the results here obtained clearly show that at the imposed working potential of $+0.2$ V vs SHE the bioanode was electrochemically active.

3.2 Cathodic performances

Figure 2 shows the cathodic performances of the MEC during continuous operation in terms of CCE, η_E cathodic current (2A) and hydrogen production and degree of purity (2B). The cathodic current remained relatively stable over the entire experimental period, with an average value of 15 ± 0.3 mA, indicating steady electron availability at the cathode. This behaviour was reflected in a stable hydrogen production rate, with average values of approximately 2.0 ± 0.1 mmol H₂ d⁻¹, and a CCE ranging between 50 and 60%. The relatively constant CCE suggests that a significant fraction of the electrons delivered to the cathode were effectively recovered as hydrogen. However, the fact that CCE values did not reach 100% indicates that part of the electrical current was consumed by competing processes. Possible causes for incomplete coulombic recovery include reactions at the cathode surface (i.e., reduction of nitrates and metals). Additionally, hydrogen may have been partially lost due to mass transfer limitations or gas crossover through tubing and seals, which are commonly reported in laboratory-scale MEC systems (Marchetti et al., 2025). This gas losses by mixing phenomena within the cathodic chamber and gas collection system, is also confirmed by the hydrogen purity, remained around 60%, suggesting the presence of other gaseous components, likely associated with nitrogen entrained in the system. Overall, these results led to an average η_E of 43% which, although not optimal, demonstrates the technical feasibility of hydrogen recovery from real BWW under continuous operating conditions.

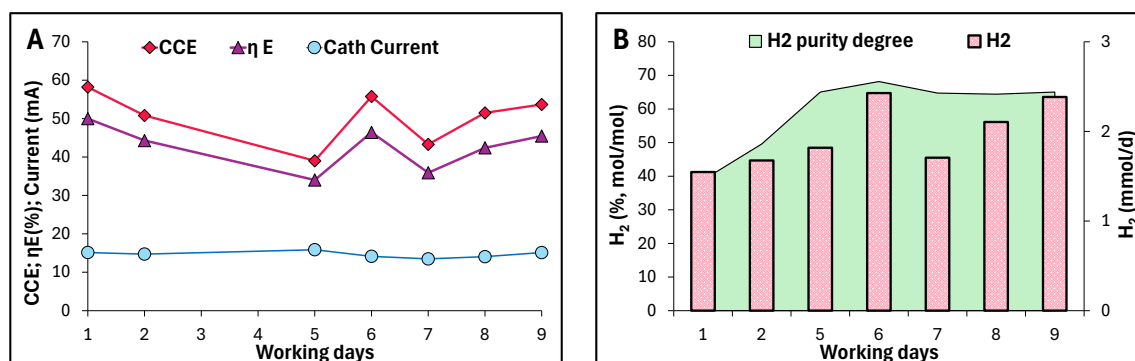


Figure 2: Cathodic performance during continuous operation in terms of (A) CCE, η_E and Current and (B) H₂ purity degree and production.

3.3 Overall performance and literature comparison

Table 1 summarizes the main performance indicators obtained in this study and compares them with those reported by (Zeppilli et al., 2017), who investigated a MEC characterized by the same reactor geometry and bioanode materials. The main difference between the two systems lies in the cathodic configuration, consisting of a single cathodic chamber in the study by Zeppilli et al. (2017) and a double cathodic chamber in the present work, as well as in the nature of the real substrates employed. In the work by Zeppilli et al. (2017), the MEC was fed with a real substrate obtained from a two-stage anaerobic digestion treatment plant (TP-AD), consisting of an acidogenic effluent diluted with a digestate stream used as inert matrix, called “*Real Mix*”. As a result, the influent was characterized by a high content of volatile fatty acids (VFAs), accounting for approximately 80% of the soluble COD, mainly in the form of acetate and propionate, which are well-known to be highly suitable substrates for electroactive microorganisms (Logan et al., 2019). In contrast, in the present study the MEC was directly fed with untreated BWW, without any preliminary acidification or pre-treatment step. Overall, the system investigated by Zeppilli et al. (2017) showed higher performance, which can be reasonably attributed to the higher quality and bioavailability of the organic matter supplied to the bioanode. The presence of readily biodegradable VFAs likely enhanced microbial electron transfer processes and promoted higher bio-electrocatalytic activity. Despite this, the COD conversion achieved in the present study (69%) was significantly higher than that reported by Zeppilli et al. (2017) (28%). This difference may be attributed to the presence of suspended solids and recalcitrant compounds in the digestate used as diluent in the real mixture adopted by Zeppilli et al. (2017), which may have partially inhibited the microbial activity or limited substrate accessibility at the bioanode. Regarding the lower current values observed in the present work can also be explained by the different OLRs applied. In particular, the OLR adopted by Zeppilli et al. (2017) was approximately three times higher than that applied in this study. Assuming a quasi-linear relationship between current generation and applied OLR, with some deviation due to different HRTs, the current measured in the present system would be expected to increase up to approximately 45 mA under similar loading conditions. Zeppilli et al. (2017). reported a CE exceeding 100% which were attributed, by the authors themselves, to the hydrolysis of particulate organic matter, leading to the release of additional soluble COD not included in the initial CE calculation. In contrast, in the present study the CE remained below 100%, indicating a more conservative estimation of electron recovery. Nevertheless, the CCE obtained in the two studies were remarkably similar, with values of 50% and 51% for the present work and Zeppilli et al. (2017), respectively, confirming a comparable effectiveness of electron conversion into hydrogen. While, the higher energy efficiency reported by Zeppilli et al. (2017), can be mainly attributed to the higher electrical conductivity of anaerobic digestion effluents, typically around 14 mS cm^{-1} for the high salts and mineral content, compared to the conductivity measured in brewery wastewater in the present study (2.83 mS cm^{-1}). The lower conductivity resulted in higher internal resistance and larger potential losses between electrodes, thus negatively affecting the overall energetic performance of the system. However, the results obtained in the present study can be considered highly promising, since a high degree of COD removal/conversion has been achieved in the reactor, but also a hydrogen production was obtained using a real industrial effluent without any pre-treatment. These findings suggest that the integration of an upstream acidification process, such as dark fermentation, could significantly enhance the quality of the substrate supplied to the MEC, leading to improved electrochemical performance and higher energy recovery efficiencies in future applications.

Table 1: Comparison of MEC performance with literature data based on key operational parameters.

Ref. Study	C-source	OLR (gCOD/Ld)	HRT (d)	Current (mA)	COD _{converted} (%)	CE (%)	CCE (%)	η E (%)
This study	BWW	0.5	1.2	15 ± 0.3	69 ± 1	47 ± 3	50 ± 2	43 ± 2
Zeppilli et al., 2017	Real Mix	1.5	0.6	60 ± 4	28 ± 3	119 ± 28	51 ± 1	57 ± 6

4. Conclusions

This study evaluated the treatment and valorization of real brewery wastewater (BWW) by using a two-cathodic MEC reactor. At the bioanode side, the system achieved a high COD_{removal}, with a conversion efficiency of 69% of the total COD being diverted into different products, mainly electrical current and subsequent hydrogen production at the cathode. The cathodic output remained stable throughout the operational period, indicating that the system rapidly reached steady-state conditions. Moreover, the double cathodic chamber design showed discrete CCE (50%) and η E (43%), despite the use of a real substrate without any pre-treatment to enhance COD quality. Compared to single-cathode MECs fed with real VFA-rich effluent, the obtained performances

suggest that this configuration is promising but still open to further optimization. Overall, these results highlight the potential of the proposed MEC architecture for the valorization of complex substrates and wastewaters through hydrogen production, while indicating that future improvements should focus on substrate pre-treatment, thus improving COD quality and enhancing the overall system energy recovery.

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