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Towards the industrial application of microbial electrolysis cells for hydrogen production: a critical review on cell's components

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Abstract: During the last 20 years, Bioelectrochemical Systems (BES) have been studied as an environmentally friendly technology. These systems exploit the ability of peculiar microorganisms to interact with solid state electrodes by exchanging electrons. A type of BES is represented by a Microbial Electrolysis Cell (MEC), mostly used for hydrogen production while abating organic waste. This review underlines the steps needed to reach industrial readiness of MEC technology. Bioanodes, responsible for the electricity generation, suffer from low conductivity of the biocompatible and low cost materials. Even if steps were made to enhance the performance and, reaching a stable current density of 12 A/m^2 using real wastewater, the scalability and stability of bioanodes remain issues. Unfortunately, there are biological limits that cannot be overcome. However, there is still a lot of space for improvements. Cathodes are fundamental for hydrogen production. Research on cathodes has focused on affordable and durable materials like stainless steel, which in some configurations can reach the performance of platinum without its susceptibility to poisoning. Various strategies have improved hydrogen capture efficiency and reduced contamination by methanogens. Separators are essential for compartmentalizing the system, but are usually expensive and, as reported, prone to operational issues such as biofouling and pH split. We report new alternatives, including porous membranes or cellophane, which may help reduce costs and enhance performance. In our view, developing materials and conducting standardized tests under realistic conditions are critical steps toward making MECs commercially viable for sustainable hydrogen production. The scaling up step is still missing, even if in recent years some attempts were made by a few research groups reaching volumes of 1 m^3. Perhaps in the coming years MECs will support the circular economy, but at the current state of the art, they are still not cost-effective. On the other hand, we aim to help the scientific community remove the last barrier that separates MECs from industrial scale-up by reporting information and giving a rough but fair opinion on how this should be achieved.

Key words: Separators; Anode materials; Bioanodes; Wastewater; Biohydrogen

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1. Introduction

The growing demand for energy and the depletion of fossil fuel resources have intensified the search for more efficient and sustainable energy production methods. Hydrogen, in particular, has attracted significant attention due to its potential as a clean fuel, as its combustion produces solely water, making it an environmentally friendly alternative to fossil fuels if coming from renewable resources. Currently, around 96% of hydrogen is produced from fossil fuels like coal, natural gas, and oil through processes such as steam methane reforming, methane pyrolysis, and water-gas shift reactions [1]. Only 4% is produced via electrolysis, which is not considered green unless powered by renewable energy. Traditional electrolysis requires a potential difference of approximately 2 V, with a theoretical minimum of 1.23 V [2]. This means that, to produce 1 L of hydrogen in 1 day, 4.4 Wh of energy are required. An innovative approach to hydrogen production involves microbial electrolysis cells (MECs), which exploit electro-active microorganisms (EAMs) capable of oxidizing organic molecules such as acetate or glucose while using solid-state electrodes' surfaces, named anodes, as electron acceptors [3]. As depicted in Figure 1, this anodic electron transfer is facilitated by membrane proteins, conductive pili, or/and redox molecules capable of rapid electron exchange [4]. The potentials needed for these bioanodes to operate range between −0.3 and +0.2 V vs SHE (Standard Hydrogen Electrode) [5], significantly lower than the potential required for water oxidation [6]. This reduces the energy consumption required to produce hydrogen. So, to produce the same 1 L of hydrogen in 1 day, 2.2 Wh are needed.

Using bioanodes permits to oxidize the organic content of an aqueous stream, directing electron flow to the cathode, where protons are reduced to hydrogen. This offers the advantage of treating a waste stream while recovering energy in

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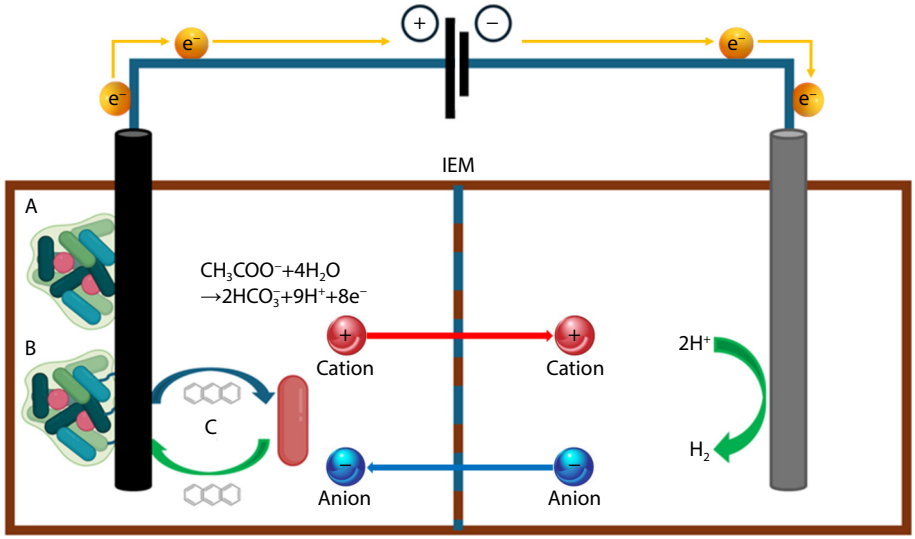

Fig. 1. Schematic representation of a microbial electrolysis cell for acetate bioelectrochemical oxidation and hydrogen production. Electroactive microorganisms are cultured in the anodic chamber (left). Acetate is oxidized producing bicarbonate, protons, and electrons. Electron transfer from the microorganisms to the anode can occur through membrane proteins (A), conductive pili (B), or natural redox mediators such as flavins or phenazines (C). The electrons travel through an external circuit to the cathodic compartment (right). A potentiostat is used to furnish energy to perform protons reduction into molecular hydrogen (H_2) on the cathode. The ion exchange membrane (IEM) separates the two chambers while allowing ionic balance.

form of hydrogen.

However, the current status of knowledge regarding the selection of cell components for microbial electrolysis cells operation with real substrates (i.e. anaerobic digestion effluents, wastewaters, etc.) is insufficient. Hence, the objective of this review is to critically evaluate and summarize the scientific literature present on the topic, underlining the main challenges and bottlenecks regarding cell components operation in real conditions, in the optics of scalable systems for real industrial implementation. Despite the increasing number of studies on microbial electrolysis cells, most existing reviews still focus on the general operating principles or lab-scale optimization. Several reviews have been published on microbial electrolysis cells (MECs) over the past decade, mainly focusing on fundamental aspects or laboratory-scale performances [7-10]. However, limited attention has been given to the critical evaluation of MEC components under real wastewater conditions and their potential for scale-up. This review aims to fill this gap by providing a systematic and comparative assessment of bioanodes, separators, and cathodes in terms of material selection, cost, durability, and operational challenges. The review further identifies key bottlenecks and future opportunities for the industrial application of MEC technology in sustainable hydrogen production.

2. Microbial electrolysis cells for hydrogen production

2.1. Principles and theory

The efficiency of bioelectrochemical reactions is governed by fundamental thermodynamic and kinetic principles. First, the total power or electrical work in a process is the product of total charge and the potential difference between electrodes, as represented by the equation (1):

$$W = nFE \text{ where } E = E_{cat} - E_{an} \quad (1)$$

In this equation, n represents the number of moles of electrons transferred, F is the Faraday constant (96485 C/mol_{e^-}), and E is the electromotive force, defined as the difference between the cathode electrical potential (E_{cat}) and the anode electrical potential (E_{an}), both expressed in volts (V). This means that the maximum power obtainable from a chemical reaction corresponds to the change in Gibbs free energy, expressed as equation (2):

$$W_{max} = -\Delta G = nFE_{max} \quad (2)$$

This relationship can be used to derive the Nernst equation (equation (3)), which describes the relationship between the redox potential of an electrochemical reaction (at a certain temperature) and the concentrations of the reactants and products, or in our case the potential of MEC:

$$\Delta E = \Delta E^{\circ} - \frac{RT}{nF} \ln K_{eq} \text{ where } K_{eq} = \frac{C^c D^d}{A^a B^b} \quad (3)$$

for the reaction (equation (4))

$$aA + bB \leftrightarrow cC + dD \quad (4)$$

Unfortunately, the real potential is affected by overpotentials and resistances, including ohmic resistances, which follow Ohm's law, the activation losses and the concentration losses. These resistances can be minimized by selecting appropriate materials, optimizing cell geometry and the turbulence[11], increasing the conductivity of the electrolytes and the concentration of the reagents like the organic substrates for the anodic chamber and the protons in the cathodic chamber. The MEC overpotential ($\Sigma\eta$), which represents the total potential losses, can be calculated by subtracting to the theoretical potential difference (ΔV_{Theo}) the measured potential difference (ΔV_{meas}) between the electrodes (equation (5)):

$$\sum \eta = \Delta V_{meas} - \Delta V_{theo} \quad (5)$$

It's possible to calculate the electrode overpotentials too. The later, are influenced by charge transfer resistance and mass transport limitations [12]. These can be calculated as follows (equation (6)):

$$\sum \eta_{Cat} = E_{Cat}^{meas} - E_{Cat}^{theo} \text{ and } \sum \eta_{An} = E_{An}^{meas} - E_{An}^{theo} \quad (6)$$

For example, the bio-oxidation of acetate occurs at the anode as (equation (7)):

$$(CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-) \quad (7)$$

Following the Nernst equation, the theoretical potential for this anodic semi reaction is (equation (8)):

$$E_{An}^{theo} = E^0 + \frac{RT}{8F} ln \frac{[HCO_3^-]^2 * [H^+]^9}{[CH_3COO^-]} \quad (8)$$

with E^0 = + 0.187 V vs SHE

Knowing the bicarbonate and acetate concentration and the pH it is possible to calculate the theoretical anodic potential, but for the previously mentioned reasons this potential won't match with the measured one. The pH and concentration gradient between the bulk and the electrode's surface will affect the potential. For this reason, is fundamental to have a turbulence in the chamber.

The HER at the cathode proceeds through either the Volmer-Tafel mechanism or the Volmer-Heyrovsky mechanism. The Volmer reaction represents the adsorption of hydrogen atoms onto the catalyst surface (equation (9)):

$$H_3O^+ + e^- + * \rightarrow H^* + H_2O \quad (9)$$

In contrast, the Tafel equation (10)

$$2H^* \rightarrow H_2 + 2* \quad (10)$$

and Heyrovsky equation (11)

$$H^* + H_3O^+ + e^- \rightarrow H_2 + H_2O \quad (11)$$

involve the desorption of molecular hydrogen from the catalyst. In these equations, the symbol $*$ denotes an unoccupied active site on the catalyst, while H^* represents a hydrogen atom adsorbed onto the active site.

Recent research has revealed that in alkaline environments, hydrogen evolution may proceed through cooperative mechanisms that couple H_2O dissociation and H adsorption at adjacent sites on the electrode surface. In particular, Wang et al. [13] demonstrated that a local electric field can promote the Volmer step by accelerating H_2O dissociation, while electron localization at doped metal sites facilitates H adsorption and recombination to form H_2. This dual effect, defined as "cooperative alkaline hydrogen evolution mechanism", suggests that the interplay between nanometer-scale electric fields and atomic-scale charge localization significantly reduces the kinetic barriers for hydrogen evolution under alkaline conditions

The theoretical cathodic potential is given by equation 12:

$$E_{Cat}^{theo} = E^0 - \frac{RT}{2F} ln \frac{pH_2}{[H^+]^2} \quad (12)$$

with E^0 = 0 V vs SHE

Considering a HER at atmospheric pressure and a constant temperature, what's not stationary is the pH. As mentioned above, a good mixing inside the chamber is fundamental to avoid a concentration gradient, but, since there is a continuous consumption of protons (on the other hand, in the anodic chamber there is a production of protons), it is crucial to control the pH, ideally at low values.

To maintain electrical neutrality, electrodes are placed in the same solution or separated by ion-permeable separators, such as cation exchange membranes (CEM), anion exchange membranes (AEM), or bipolar membranes (BPM), which selectively allow passage of ions between the two compartments (as shown in Figure 2.)[13]. The membrane itself has an intrinsic overpotential but in MECs with membranes, additional factors such as pH gradients and differences in electrolyte composition contribute way more to the overall losses [14]. The pH split overpotential can be expressed as equation 13:

$$\eta_{pH} = \frac{RT}{F} \ln \left(10^{(pH_{Cat} - pH_{An})}\right) \quad (13)$$

showing how a higher pH difference between the cathodic and anodic chamber increases the pH overpotential decreasing the performance.

Additionally, the ionic overpotential is influenced by ionic resistance (equation (14)):

$$\eta_{ionic} = I_{ions} \left(\frac{1}{2} R_{An} + \frac{1}{2} R_{Cat}\right) = I_{ions} \left(\frac{d_{An}}{2A\sigma_{An}} + \frac{d_{Cat}}{2A\sigma_{Cat}}\right) \quad (14)$$

Here, I_{ions} represents the number of charges migrating through the membrane, which is equivalent to the electric current flowing in the system. The resistance (R) of the liquid phase can be calculated based on the distance (d_{An} and d_{Cat}) between the electrode and the membrane, the membrane area (A), and the conductivity of the liquid phases (σ). This equation demonstrates that lower conductivity and a greater distance between electrodes increase ionic overpotential, thereby reducing the system's overall performance.

2.2. Operational parameters

Effective management of pH [15], temperature [16], and electrical potential can significantly enhance MEC performance, making them more viable for large-scale applications in wastewater treatment and renewable energy production. Each operational parameter directly impacts microbial activity and the overall efficiency of the MEC system. Optimizing these parameters will reduce significantly the energy consumption in MECs. Electroactive microorganisms exhibit optimal activity within a pH range of 6.5 to 7.5 [17,18]. Deviations from this range reduce microbial growth and electron transfer efficiency, thereby decreasing the overall effectiveness of the bioanode in energy conversion processes [16,17]. For this reason, many synthetic media have high buffer capacity; however, this tactic is not available when using real wastewater. Moreover, the pH environment is important for ion transport, particularly proton migration within the biofilm and surrounding solution [18]. Effective proton transport helps maintain a stable electro-

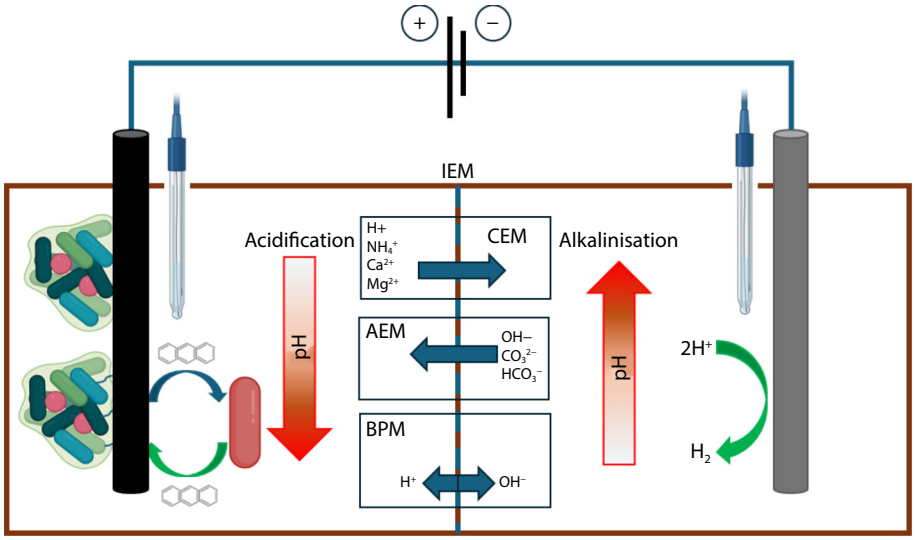

Fig. 2. Schematic representation of microbial electrolysis cells equipped with different ion exchange membranes illustrates their roles in selective ion transport and pH control. The anodic compartment (left) hosts electroactive microorganisms that oxidize organic substrates, generating electrons and protons. Electrons flow through an external circuit to the cathode, where protons are reduced to molecular hydrogen (H_2). pH split phenomenon occurs due to the migration of ions through the membrane different than H^+ and OH^-. Cation exchange membranes (CEM) selectively transport cations (e.g., H^+, NH_4^+, Ca^{2+}, Mg^{2+}), while anion exchange membranes (AEM) facilitate the transfer of anions (e.g., OH^-, CO_3^{2-}, HCO_3^-). In contrast, bipolar membranes (BPM) do not permit direct ion movement between compartments; instead, water splitting occurs at the membrane interface, producing H^+ and OH^-. These ions are transported to the cathode and anode, respectively, to maintain electrical neutrality within the system.

chemical environment, preventing harmful local pH changes that could negatively impact microbial activity [18]. The pH within the cathodic chamber is critical for hydrogen production [14]. As protons are reduced to hydrogen, the pH increases due to proton consumption. This rise in pH can hinder hydrogen evolution, increasing energy consumption if not properly managed. Therefore, maintaining an optimal pH is essential for maximizing proton reduction efficiency, often achieved by using bicarbonates or other buffering agents to stabilize the cathodic environment [19,20]. Temperature is another crucial factor affecting MEC performance, as microbial activity is highly temperature-dependent [21]. Mesophilic and thermophilic conditions (35°C–55°C) are most favorable for microbial activity, with lower temperatures slowing microbial metabolism and higher temperatures potentially inhibiting growth or disrupting electron transfer. Identifying the optimal temperature range for a given microbial community is vital for efficient energy conversion [15]. Furthermore, if one takes a close look at the pH split overpotential and the Nernst equations, the results clearly show that a higher temperature increases the theoretical reaction potential and the pH split overpotential. Electrical potential also plays a significant role in MEC operation [22]. The applied potential must be sufficient to permit the bioelectrochemical oxidation of organic substrates at the anode while driving proton reduction at the cathode, but not so high as to cause excessive energy loss or microbial inhibition [23]. The goal is to apply the minimum energy required to sustain microbial activity and hydrogen production, thereby achieving the highest performance. Considering this, it's possible to affirm that MECs operate at potentials significantly lower than those required for traditional water electrolysis, thereby enhancing their attractiveness. What is truly lacking, however, are the high current densities.

3. Bioanodes for bioelectrochemical oxidation of organic compounds

3.1. Materials utilized as electrode for bioanodes

The core of the MEC is the bioanode, where the bioelectric-oxidation process take place. Real substrates bring several challenges, due to the usual presence of inhibitory substances for the electroactive microorganisms (EAM) such as ammonia, sulphates, etc. and mixed-microbial culture which will compete with EAM both for substrates (methanogens) and for the growth support (hydrolytic and fermentative biofilm-forming microorganisms) [23,24]. Carbon is the most used electrode material used for bioanodes in Microbial Fuel Cells (MFC) and MEC systems [25]. Various carbon-based materials have been utilized as bioanodes, including carbon cloth, carbon paper, carbon felt, graphite fiber, graphene, graphene oxide, activated carbon, graphite, and carbon nanotubes [26–30]. However, a significant disadvantage of carbon-based materials is their relatively low electrical conductivity, which is two to three orders of magnitude lower than that of most metals, such as stainless steel [31]. As reactor scales increase, the uniform distribution of current density becomes a decisive factor, with material conductivity and electrode architecture jointly determining the overall electrochemical performance. Moreover, there is substantial variability in the electrical performance of bioanodes made from different types of carbon materials.

Few comparative studies have systematically benchmarked these diverse carbon materials [26–28,32]. For instance, Roubaud *et al.* [27] compared five grades of industrial synthetic graphite. The graphite grade that achieved the highest bioanode performance was further benchmarked against carbon felt and carbon cloth, resulting in a 50% higher steady-state current density and the highest presence of

Geobacter species in the biofilm. Similarly, Zainab *et al.* [26] conducted a comparative assessment of carbon brush, carbon granules, and three types of carbon felt—one with improved conductivity, one with a higher active area, and one with greater thickness. The carbon brush and the carbon felt with an enhanced active area outperformed the other materials in terms of electricity generation and coulombic efficiency. Among the materials tested by Kipf *et al.* [25], graphite foil, activated carbon cloth and graphite felt obtained similar limiting current densities during polarization curves, surpassing the performance of a self-prepared buckypaper-based nanostructured electrode material.

For large-scale industrial applications, the long-term stability, cost-effectiveness, availability, and performance of anode materials are crucial factors. Several reviews have suggested that advanced anode materials and modifications of carbon materials could offer feasible solutions for MEC field applications [29,30,33–35]. Other proposed solutions include the addition of nanomaterials [35–37], quantum dots [38], and metal oxides [33–41] to the anode. Many reviews have also extensively covered the use of graphene-based materials (such as graphene oxide, reduced graphene oxide, and graphite) as anode materials for MFC [29,30,33,36]. This class of materials has been extensively investigated in the energy sector, for example has catalyst for oxygen evolution reaction [42], HER [43] and oxygen reduction reaction [44], exploring the possibility of doping graphene materials with many different compounds and atoms [45]. As clearly described by Wang *et al.* [44], there are an almost infinite ($> 2{\cdot}10^6$) ways to modify graphene, in order to increase its electrocatalytic performances.

Improvements in the performance of carbon materials primarily derive from enhancements in their electrical conductivity, achieved by adding more conductive elements such as graphene, metals, and metal oxides, and by increasing surface roughness through the addition of nanomaterials. Increased electrical conductivity leads to a decrease in the specific electrical resistivity of the material. In an electrochemical system like a MEC, any reduction in electrode resistivity decreases cell overpotential, and therefore, cell voltage. Furthermore, it has been reported that nanostructures favour biofilm formation [46,47]. Therefore, in line with the hypothesis of Wang *et al.* [44], it can be suggested that adding elements to enhance the electrical conductivity, nanostructure, and surface roughness of carbon materials will result in improved bioanode performance compared to bare carbon materials. However, these advanced chemical modifications often present challenges in scalability and have not been tested in realistic industrial conditions.

An alternative to carbon for anode materials are metal-based materials. The suitability of metal-based anode materials is debated. Several reviews suggest they are unsuitable as bioelectrodes due to issues with corrosion and poor biocompatibility [29,30,33]. However, experimental research has demonstrated that stainless steel-based materials often exhibit superior performance as bioanodes, frequently surpassing carbon-based alternatives [31,32,48–52].

In line with this, Vázquez *et al.* [31] tested six anode materials—stainless steel wool, carbon paper, graphite felt, graphite plate, graphite foil, and stainless steel mesh—and found that stainless steel wool achieved a limiting current density of 4.5 A m^{-2}, outperforming carbon-based materials like graphite felt without showing signs of corrosion. Similarly, Ketep *et al.* [49] compared high porosity stainless steel and carbon cloth as bioanodes and found that stainless steel foam bioanodes significantly outperformed carbon cloth bioanodes. The stainless-steel foam achieved current densities between 63 and 82 A m^{-2} at −0.2 V vs. SCE, approximately twice the current densities of the carbon cloth bioanodes.

3.2. Electroactive biofilms

The initiation of the bioelectrochemical process through the formation of an electro-active biofilm (EABf) on an anode surface requires the inoculation of biomass containing electroactive microorganisms (EAM). An EABf is a conductive, three-dimensional structure that develops on the surface of a conductive electrode material [4]. Within this biofilm, microbial cells are interconnected by a matrix of self-produced extracellular polymeric substances (EPS), which facilitate electron transfer between the cells and the electrode [52]. Among EAM, species of *Geobacter* and *Shewanella* have been extensively studied for BES [4], demonstrating superior performance when cultivated together as a multispecies exoelectrogenic community [53,54]. Electro-active biofilms (EABf) developed from mixed microbial communities are often dominated by *Geobacter* species, which are capable of generating high and stable current densities [4,55,56]. These biofilms are particularly preferred for use with real wastewaters and in industrial applications due to their robustness and efficiency [57]. Mixed microbial communities offer the potential to consume a variety of substrates and provide a cost-effective and scalable approach to biofilm development. However, these communities often contain an unknown and typically low concentration of EAM, which are not naturally acclimated to using polarized materials as electron acceptors. To enhance the performance of these biofilms, pre-treatment methods can be applied to enrich the EAM population, stimulate their exoelectrogenic activity, and inhibit competitive microorganisms [47]. This prevents EAM from being outcompeted by other biofilm-forming microorganisms for electrode space and by methanogens for substrates, which would otherwise result in lower current production [47]. In a study by Flayac *et al.* [58], carbon plates were inoculated with wastewater treatment plant sludge at a concentration of 10% v/v. The anolyte consisted of a mineral medium, with acetate, lactate, propionate, and butyrate each serving individually as the sole electron donor in the anodic compartment. Regardless of the substrate used, the resulting biofilms were dominated by members of the Geobacteraceae family, which accounted for 62.41% of the biofilm sequences. *Geobacter sulfurreducens* was the predominant species in biofilms fed with acetate, lactate, and butyrate. In a study by Wang *et al.* [59], 3D N-doped macroporous carbon foam anodes were inoculated with 6.5% (by anode volume) anaerobic sludge collected from a full-scale plant operating at 35°C and treating starch wastewater. These bioanodes, when operated in an acetate medium, were capable of producing current densities of up to 12 A m^{-2}. Kosuke and Freguia [60] explored different inoculation procedures for bioanodes derived from graphite granules. They mixed real hydrolyzed urine with thickened waste activated sludge from a municipal wastewater treatment plant at various volumetric ratios, ranging from 50% to

99% hydrolyzed urine. Current generation began within 24 hours in all cases, with the anodes inoculated with the lowest initial urine fraction (50%) exhibiting the highest current output, reaching a peak of 11 A m^{-2}. Using sequential EABf harvesting and reinoculation, Alvarez Esquivel *et al.* [62] developed bioanodes on self-synthesized graphite-cellulose paper, achieving a maximum current density of 3.1 A m^{-2} in an anolyte composed of commercial whey powder dissolved in a nitrogen-free carbonate buffer. The initial inoculum was a 50-50% mixture of soil extract from a commercially available gardening mix and wastewater from the effluent after the primary settlement tank of a wastewater treatment plant (WWTP). This initial inoculum was used to perform three cycles of biofilm harvesting from the best-performing bioanode and reinoculation into fresh reactors.

3.3. Operation of bioanodes in realistic conditions

The ideal bio-anode for microbial electrochemical systems would exhibit long-term stable performance, require minimal start-up time, utilize a variety of substrates, and crucially, achieve high current densities [3]. While mixed microbial communities naturally present in real substrates have demonstrated the ability to develop highly efficient bioanodes, there remains a significant discrepancy between the performance reported in synthetic media —where the majority of studies are conducted [25] —and that observed with real substrates. When bioanodes are operated under industrially relevant conditions —using complex, non-sterile media as the electrolyte and without supplementary carbon sources—the current densities and long-term stability are typically much lower compared to those reported for bioanodes operated in acetate media under sterile conditions [25,52,61,62]. Additionally, pre-enrichment techniques do not offer significant advantages when bioanodes are operated with real substrates, as the initial biofilm is often almost entirely replaced [63].

Madjarov *et al.* [63] developed a multispecies model biofilm using a defined microbial community composed of *Geobacter sulfurreducens*, *Geobacter metallireducens*, and *Shewanella oneidensis*. The resulting bioanodes, formed on activated carbon cloth, were capable of producing an average current density of 4.07 A m^{-2} in an acetate medium. However, this current density significantly dropped to 0.42 A m^{-2} when the anolyte was switched to real municipal wastewater. Bioanodes inoculated with either sewage sludge or plain wastewater achieved similar current densities. Additionally, barcoding analysis revealed that 99% of the initial biofilm detached from the anode after 20 days of operation. Christgen *et al.* [62] compared various bioanodes developed on carbon veil as the electrode material, using three different inoculum sources: activated sludge, river sediment, and MFC effluent. These sources were either directly inoculated in the final operating medium or pre-enriched in an acetate medium. Interestingly, regardless of the inoculum source, acetate pre-enrichment did not significantly enhance bioanode performance. In fact, all bioanodes showed a decrease in current densities, from 0.25-0.45 mA cm^{-2} in acetate media to approximately 0.1 mA cm^{-2} when the medium was switched to municipal wastewater. Notably, bioanodes inoculated with activated sludge achieved higher current densities in both acetate medium and municipal wastewater compared to those inoculated with MFC effluent. Similar results were reported by Salar-Garcia *et al.* [64], who found that inoculating carbon veil with sludge promoted a more electroactive biofilm than inoculation with MFC effluent, leading to higher power output. Yanuka-Golub *et al.* [65] investigated the effects of adding a defined electroactive consortium to carbon felt anodes in single-chamber MFC treating an acetate-supplemented medium. The study revealed that bioanodes inoculated with this electroactive consortium, compared to those inoculated solely with wastewater, demonstrated faster start-up times and higher coulombic efficiency, while achieving similar maximum current densities. Furthermore, co-inoculation with wastewater—which contains a natural mixed microbial community—and the electroactive consortium improved the bioanode start-up performance. However, this co-inoculation also resulted in the near-complete displacement of the introduced bioaugmented community. Kretzschmar *et al.* [66] observed a significant decline in the signal of a *Geobacter* biofilm in a microbial electrochemical sensor within a real anaerobic digester, with the signal dropping to almost zero within 9 days of operation. The causes behind the deactivation of pre-grown *Geobacter* biofilms were further explored by Ngoumelah *et al.* [23], who reported severe inhibition during the stepwise adaptation of these biofilms to the effluent of an anaerobic digester, even at concentrations as low as 50% V/V of the effluent. Successive batch cycles with 50% anaerobic digester effluents pre-treated with either 2-bromoethanesulfonate (2-BES) or 0.2 μm microfiltration showed no inhibition of the anodic biofilm, with current densities remaining constant and comparable across cycles. The authors concluded that the loss of activity in the electroactive biofilm was most likely due to the presence of antagonistic microorganisms, which were inhibited by 2-BES and physically removed by microfiltration.

Colantoni *et al.* [51] investigated the performance of carbon-based and stainless-steel-based materials as bioanodes under anaerobic digestion conditions, employing two distinct inoculation procedures: one with a defined co-culture of *Geobacter sulfurreducens* and *Shewanella oneidensis* and another with the natural mixed microbial community from an anaerobic digester. Among the materials tested, stainless steel wool (SSW) exhibited superior performance, achieving stable current densities of up to 0.4 mA cm^{-2} after 60 days of operation under real anaerobic digestion conditions. This represents a fourfold improvement over previously reported values, with SSW outperforming other electrode materials, including carbon nanofibers (CNF-ES300) and graphite felt, across all experimental conditions. The choice of feeding media significantly influenced bioanode performance. Porous electrodes like SSW generated more than double the current densities when fed with undigested corn silage compared to anaerobic digester effluent (ADE). For example, SSW's peak current density increased from 0.16 mA cm^{-2} during the ADE phase to 0.32 mA cm^{-2} during the corn silage phase. Corrosion analysis indicated that SSW's remarkable performance stemmed from bioelectrochemical activity rather than material degradation. Furthermore, inoculation with a defined mixed culture of *G. sulfurreducens* and *S. oneidensis* enhanced the performance of flat electrodes, however, porous electrodes like SSW exhibited similar performance regardless of the inoculation strategy. Interestingly, the composition of microbial communities on the bioan-

odes was largely determined by the electrode material rather than the inoculation method.

3.4. Requirements for developing industrial grade bioanodes

The development of high-performing bioanodes capable of maintaining long-term high current densities is crucial for scaling up MEC and their industrial applications [3]. For a commercial-scale plant to be viable, it must deliver stable performance using real substrates without significant fluctuations in operating parameters. Mixed microbial communities in industrial waste streams present a promising source of electroactive microorganisms (EAM) with high scalability potential, capable of producing efficient bioanodes. However, as summarized in Table 1, the use of real substrates significantly impairs bioanode performance compared to synthetic media. Furthermore, there is a huge overrepresentation of carbon-based anode materials, with a wide variety of carbon types being utilized. To advance the field, it is crucial to test and compare novel anode materials under application-oriented conditions, such as real wastewater or process streams, to rapidly assess their scalability and performance in realistic operational settings [67]. Additionally, there is a pressing need for greater standardization of state-of-the-art electrode materials, which should be commercially available or easily scalable for industrial operations. The higher conductivity of metal-based electrodes proves particularly advantageous in up-scaled bioelectrochemical systems, where maintaining low resistance across the entire electrode structure and under prolonged operational stress is crucial for sustained efficiency.

4. Separators for MEC

4.1. Choose of separator

Using a separator to segregate anolyte and catholyte prevents hydrogen consumption from the hydrogenotrophic microbes present in the anolyte and the hydrogen contamination with the produced CO_2 and methane. The separator thus emerges as a key component in MEC, often representing a significant portion of the overall cost [68,69,70] and introducing considerable additional electrical overpotentials to the system [13]. The ideal separator should be affordable, have low electrical conductivity, prevent short circuits between the electrodes, and have low ionic resistance, resulting in a lower membrane overpotential, which would decrease the cell voltage losses [3,71,72]. Moreover, it should prevent the pH imbalance between the two compartments while keeping the anolyte pH in a neutral region, and it should be (bio)chemically stable and resistant to degradation and biofouling under operating conditions.

4.2. Issue with ion exchange membranes

The proximity to electrochemical cells (EC) has led to the widespread use of polymeric ion exchange membranes (IEM) as separators in BES and, specifically, in MEC. Common examples include cation exchange membranes (CEM) and anion exchange membranes (AEM), which are considered state-of-the-art materials in EC applications [70,71,73,74]. Although MEC are similar from the electrochemical perspective to their abiotic counterparts, their process conditions are quite different. EC are operated with abiotic electrolytes with high conductivities and purities, alkaline or acid pH, and no solid content, reaching current densities of 1-3 A cm^{-2} [75]. On the other hand, MECs are aimed at treating non-axenic waste streams, with various degrees of organic and solid loading and usually low conductivities, reaching current densities usually in the range of 0.1-10 mA cm^{-2} [76]. Additionally, microorganisms typically function optimally at a neutral pH, where the concentrations of H^+ and OH^- are around 10^{-7} M which is 5-6 orders of magnitude lower than the concentrations of other ions present in the waste streams [77]. These low concentrations of H^+ and OH^- lead to non-selective transport of species across the IEM, resulting in a pH imbalance between the anode and cathode compartments. If this pH gradient becomes severe, it can lead to system failure by inhibiting microbial metabolic processes [78–80,13,81–83]. Bipolar membranes (BPM) are an alternative to CEM and AEM. Unlike these, BPMs do not allow direct transport of species between compartments; instead, they function through water ionization to balance ion movement, potentially solving the issue of pH imbalance [83–85]. However, BPMs require a minimum current density to operate efficiently due to co-ion leakage. Sun *et al.* [83] investigated a solar-driven water-splitting cell equipped with a BPM, using 0.5 M aqueous potassium borate as the anolyte and 1.0 M H_2SO_4 as the catholyte. They found that at a current density of 5 A m^{-2}, 70-90% of the current was attributed to water dissociation, with the remainder lost to co-ion crossover. At higher current densities, the relative contribution of ion crossover decreased, with over 92% of the current attributed to water dissociation at 40 A m^{-2}. Similarly, Vermaas *et al.* [84] demonstrated that at 10 A m^{-2}, transport through the BPM was primarily dominated by H^+ and OH^- ions, with minimal potassium co-ion leakage observed after four days of continuous operation. Conversely, Wang *et al.* [85] reported significant pH increases in a BPM-equipped MEC after nine days of operation at a maximum current density of 10 A m^{-2}, where the cathodic pH rose from 1.4 to 11, a shift of 9.6 pH units. Another BPM-MEC operated for the same duration at 4.5 A m^{-2} showed a much lower pH shift of 1.1, resulting in a final cathode pH of 2.5, which was attributed to ion leakage across the BPM. Vermaas *et al.* [86] further clarified that in water-splitting electrochemical devices using BPMs, cations contribute more to ion crossover than anions. Additionally, the salt concentration in the electrolyte significantly impacts ion crossover, with a ten-fold reduction in concentration leading to an approximately four-fold decrease in ion crossover.

In real field applications, MEC separators are typically operated in amendment-free wastewater or effluents from industrial bioprocesses, such as those from anaerobic digestion. These environments are usually rich in solids, organics, microbes, and salts [77], which can lead to biofouling and, in the case of biodegradable separators, biochemical degradation. Biofouling of BES separators has been extensively reviewed [87–91]. In summary, biofouling occurs when the separator becomes covered by a biofilm, which is composed of microbes, biological substances like extracellular polymeric substances (EPS), organic substrates, and salt deposits. This biofilm can significantly decrease the performance of separators by physically obstructing mass diffusion through the separator pores [92–96]. EPS and bacterial adhesion can be reduced by decreasing surface roughness and using

Table 1. Comparison of bioanodes used in microbial fuel cells (MFC) and microbial electrolysis cells (MEC), detailing the type of anode material, electrical operation conditions, inoculum type and procedure, anolyte composition, and the resulting bioanode current densities.

Type of BES	*Anode material*	*Electrical operation*	*Inoculum type*	*Inoculation procedure*	*Anolyte*	*Maximum Bioanode current density (A m^{-2})*	*Reference*
MFC	Activated carbon cloth	Bioanodes polarized at 0 V vs. SHE	multispecies model biofilm with Geobacter sulfurreducens, Geobacter metallireducens and Shewanella oneidensis	Bioanodes pre incubation for 7 days at 241 mV vs. NHE in carbonate buffer	real municipal wastewater	0.42, average on wastewater, 4.07 average on acetate medium	[64]
			Non inoculated			0.56-0.65, average on wastewater	
			mixture of activated sludge and anaerobic sludge in the volume ratio 1:4 inoculated at 4% V/V			0.56 average on wastewater	
MEC	graphite granules, using ruthenium/iridium oxide-coated titanium plates as current collectors	Bioanode polarized at 0 V vs. SHE	Mixture of hydrolysed urine and thickened waste activated sludge		Hydrolysed urine	11	[61]
MFC	3D N-doped macroporous carbon foam (NMCF)	external loading resistance of 1000 Ω	anaerobic sludge collected from a full plant, operated at 35°C treating starch wastewater inoculated at 6.5% anode chamber volume		Acetate medium	12	[60]
MEC	Graphite-cellulose paper	Bioanode polarized at 0 V vs. SHE	sequences of electroactive biofilm generation and reinoculation, starting from initial inoculum (50-50% mixture of soil extract and wastewater)		Commercial whey powder dissolved in a N-free carbonate buffer	3.1	[62]
MFC	Carbon Veil	external loading resistance of 100 Ω	Activated Sludge	-	Acetate	4.5	[63]
				Bare	synthetic wastewater	0.7	
				Pre-enriched in acetate		1.1	
				Bare	municipal wastewater	1.2	
				Pre-enriched in acetate		1.1	
			Tyne River sediment	-	Acetate	2.6	
				Bare	synthetic wastewater	0.7	
				Pre-enriched in acetate		0.9	
				Bare	municipal wastewater	0.8	
				Pre-enriched in acetate		0.8	
			effluent from acetate-fed MFC	-	Acetate	3.5	
				Bare	synthetic wastewater	0.8	
				Pre-enriched in acetate		1.1	
				Bare	municipal wastewater	0.7	
				Pre-enriched in acetate		0.6	
MFC	Graphite plates	external loading resistance of 1000 Ω	MFC effluent	No sheer-stress	PBS medium with acetate	2.37	[68]
				Sheer-stress applied by flowing 10 mL min^{-1} of N_2 through the anode chamber		3.48	

Table 1. (Continued)

Type of BES	Anode material	Electrical operation	Inoculum type	Inoculation procedure	Anolyte	Maximum Bioanode current density (A m^{-2})	Reference
MEC	Graphite plates	Bioanode polarized at 0.4 V vs. SHE		Sheer-stress applied by flowing 40 mL min^{-1} of N_2 through the anode chamber		4	[55]
				Sheer-stress applied by flowing 80 mL min^{-1} of N_2 through the anode chamber		4.05	
			G. sulfurreducens	inoculated at 0.1 OD600	Acetate medium	3.9	
			S. oneidensis	inoculated at 0.1 OD600	Lactate medium	0.34	
			G. sulfurreducens and S. oneidensis	Inoculated at an optical density (600 nm) of 0.1 for each organism	Acetate + lactate medium	5.4	
MEC	Graphite plate	Bioanode polarized at 0 V vs. SHE	G. sulfurreducens PCA inoculation at an OD600 of 0.060 + real wastewater from the effluent of a local brewery inoculated at 1% V/V		semisynthetic brewery wastewater	2.9	[32]
	Carbon paper					4	
	Graphite foil					1.4	
	Graphite felt					3.5	
	Stainless-steel wool					6.4	
	Stainless-steel mesh					0.9	
MEC	Graphite plate	Bioanode polarized at 0 V vs. SHE	Mixed culture of *G. sulfurreducens* inoculated at an OD_{600} of 0.01 and *S. oneidensis* inoculated at an OD_{600} of 0.09		Anaerobic digester effluent	0.8	[52]
	Carbon nanofibers					2	
	Stainless-steel plate					0.3	
	Graphite felt					1.2	
	Stainless-steel wool					2.2	
	Stainless-steel mesh					1.1	
	Graphite plate		Mixed microbial community from an 8 L laboratory-scale anaerobic digester digesting corn silage			0.3	
	Carbon nanofibers					1.9	
	Stainless-steel plate					0	
	Graphite felt					2.3	
	Stainless-steel wool					1.3	
	Stainless-steel mesh					0	
	Graphite plate					1.1	
	Carbon nanofibers					2.6	
	Stainless-steel plate		Mixed culture of *G. sulfurreducens* inoculated at an OD_{600} of 0.01 and *S. oneidensis* inoculated at an OD_{600} of 0.09		undigested corn silage	0.3	
	Graphite felt					2.6	
	Stainless-steel wool					4	
	Stainless-steel mesh					1	
	Graphite plate					0.3	
	Carbon nanofibers		Mixed microbial community from an 8 L laboratory-scale anaerobic digester digesting corn silage			2.3	
	Stainless-steel plate					0	
	Graphite felt					2.8	
	Stainless-steel wool					3.6	
	Stainless-steel mesh					0	

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hydrophilic membranes, or by modifying membranes to make them more hydrophilic. Such modifications prevent biofilm formation on the membranes, maintaining stable ionic migration and resistance. Zhao *et al.* [97] strategically modified a PVDF (polyvinylidene fluoride) membrane by incorporating PDA (polydopamine) to increase hydrophilicity, thereby creating a steric barrier to bacterial adhesion. Additionally, they added polyethyleneimine (PEI) as a stabilizer and to introduce amine groups that electrostatically repel bacteria. Furthermore, the inclusion of Ag nanoparticles reduced biofouling due to their antimicrobial properties. As a result, the membrane resistance decreased to 1.01 mΩ m^2 and proton migration was enhanced, leading to an H_2 production of 2.9 $m^3/m^3/d$. This synergistic effect represents a significant advance in addressing biofouling, a key challenge in H_2 production through MECs.

The most common post-mortem analysis used to evaluate separator biofouling is Fourier Transform Infrared (FTIR) spectroscopy. Monitoring the evolution of FTIR spectra during operation can help identify changes in the chemical composition of a separator due to biofouling and/or biochemical degradation. Table 2 provides an overview of the FTIR peaks associated with biofouling in separators used in BES.

4.3. Alternative separators

To address the challenges associated with ionic membranes and to lower overall technology costs, some studies have explored the use of alternative separators to the IEM in BES, such as nonwoven fabrics [72,92], textile fabrics [92], cellulose esters [92], polystyrene [69], ionic liquid membranes [98,99], ceramics [100], and compostable bags [100]. Kondaveetia *et al.* [91] evaluated low-cost separators in MFC fed with real domestic wastewaters, which proved to be beneficial for power generation, compared to a commercial CEM. Winfield *et al.* [99] tested a biodegradable material based on starch and compostable polyester as separator for MFC operated in synthetic medium. The material performance in the short-term was comparable with a commercial, and far more expensive, CEM. However, the biodegradable material was degraded and failed after 8 months of operation. Zhao *et al.* [71] showed that hydrophilic porous materials can outperform CEM in MECs operate in synthetic anode medium. The use of porous membranes improved hydrogen production, and reduced separator resistance, pH gradients and conductivity shifts. Moqsud *et al.* [100] compared filter paper, cellophane, and PEM (Nafion, N-115, Dupont Japan) in a MFC fed with a water-based blend of grass and leaf mold over the course of 40 hours. The MFC equipped with Cellophane exhibited the best performances, achieving a maximum power output of 42.5 mW m^{-2}. Koók *et al.* [101] tested novel types of CEM and AEM in MFC, using as anolyte phosphate buffer supplemented with acetate. The AEM presented lower internal resistance and 2–5 times higher energy yields than CEM, which instead exhibited similar performance as a commercial proton exchange membrane [102,103,104]. Cardeña *et al.* [70] applied the EXPROM-2 decision-making method to evaluate and rank novel materials and commercial CEM and AEM for MEC, using synthetic acetate

Table 2. FTiR peaks associated to biofouling reported in literature for separators tested in BES

Wavelength(cm^{-1})	*Peaks assignment*	*Separators presenting the peak*	*References*
3297 – 3292	N-H stretching associated with proteins	Nafion-117 (PEM)	[94]
3022	aromatic C-H stretching	AMI-7001 (AEM)	[95]
3285	Organic acids and halogenated carbon chains, generated by bacterial metabolism	Polypropylene non-woven fabric	[92]
2963 – 2961	C-H asymmetric stretching from CH_3 groups in fatty acids	Nafion-117 (PEM)	[94]
2980 – 2851	aliphatic C-H stretching	AMI-7001 (AEM)	[96]
2928 – 2850	Stretching vibration of CH_2 from carbon chain compounds in biofilm	Nafion-117 (PEM)	[93,94,97]
2917		Polypropylene non-woven fabric	[97]
2124	C=C or C ≡ C stretching related to alkynes or alkenes (i.e. fatty acids) produced by bacteria	Nafion (PEM)	[92]
1737	Stretching vibration of C=O from carboxylic acids	Nafion-117 (PEM)	[93]
1673 – 1622	C=C stretching	AMI-7001 (AEM) CMI-7000 (CEM)	[96]
1660	C=C stretching from proteins	Polypropylene non-woven fabric	[97]
1651		Nafion-117 (PEM)	
1652 – 1642	C=O and C-N stretching from amide I groups, related to the presence of proteins originated from microbial activity	Nafion-117 (PEM) Polypropylene non-woven fabric	[93,94]
1630	Presence of proteins originated from bacteria	Nafion (PEM)	[92]
1550	N-O asymmetric stretching from organic nitro compounds	Polypropylene non-woven fabric	
1548	N-H bending and C-N stretching from amide II groups, suggesting the presence of proteins originated from microbial activity	Nafion-117 (PEM)	[93,94]
1456	C=C stretching from carbon chains in the biofilm	Polypropylene non-woven fabric	[92]
1455 – 1454	C=O stretching from aminoacids	Nafion-117 (PEM)	[94]
1450	Deformation vibration of CH_2 from carbon chain compounds in biofilm		[93]
1238 – 1243	P=O stretching from phospholipid		
1054 – 1034	Stretching vibration of C-O-C in polysaccharides indicative of bacterial activity.		[94]
841	unsaturated aliphatic deformation	Polypropylene non-woven fabric	[97]
520	Organic acids and halogenated carbon chains, generated by bacterial metabolism	Polypropylene non-woven fabric	[92]

and volatile fatty acids media as anolyte. The statistical material ranking indicated that both CEM and AEM are suitable separators for MEC applications with synthetic anolytes, without a clear difference between novel and commercial materials.

Colantoni *et al.* [105] evaluated the performance and biostability of commercial AEM, CEM, cellophane and a novel Cellophane material modified with polydimethylsiloxane (PDMS) under anaerobic digester conditions. When tested at current densities of 0.4 and 1 mA cm^{-2}, the AEM and Cellophane maintained stable anode pH levels between 7 and 7.5, whereas the CEM caused significant pH drops, reaching below pH 3. This pH imbalance led to a substantial increase in overpotentials for the CEM, exceeding 6 V at the higher current density, while the AEM and Cellophane materials showed lower overpotentials, ranging between 0.4 and 1.4 V depending on current density. The biostability tests revealed that unmodified Cellophane degraded completely within one month in anaerobic digester effluent, while modification with PDMS extended its lifespan to two months. Biofouling was significant for all materials, with PDMS-modified Cellophane showing reduced but still substantial fouling. Organic acid crossover rates were measured, with Cellophane exhibiting rates of 0.16–0.2 mg m^{-2} s^{-1}, slightly higher than those observed for the AEM and CEM, which ranged from 0.04 to 0.1 mg m^{-2} s^{-1}. The economic evaluation highlighted the cost-effectiveness of Cellophane (5–10 EUR/m^2) and PDMS-modified Cellophane (50–70 EUR/m^2) compared to the substantially higher costs of CEM and AEM (500–1000 EUR/m^2). While these findings showcase the potential of Cellophane-based materials as low-cost separators, their durability and resistance to biofouling require further improvement for long-term applications in anaerobic digester systems.

4.4. Further research needs

As summarized in Table 3, this literature analysis suggests that separators designed for abiotic EC are not optimal when applied to MEC. Instead, more experimental studies are needed to compare different separators under application-relevant conditions to determine the most suitable separator for specific processes. A significant limitation in the current literature is that most studies are conducted using synthetic media and short-term experiments. Thus, the suitability of separators for MEC should be evaluated under the real process conditions in which they will be used. Furthermore, most studies on separators in MEC focus on reporting process performance indicators such as hydrogen production rate, yield, current density, coulombic efficiency, and bioanode microbial community [25,103]. Although these parameters are influenced by separator properties, to effectively compare separators, it is essential to report their intrinsic properties directly, rather than focusing on their indirect effects on MEC performance.

Table 3. Comparative analysis of various separators used in microbial electrochemical cells highlighting their performance metrics, including average current density, resistance per area unit, and shifts in pH for both anolyte and catholyte.

Separator	*Anolyte*	*Catholyte*	*Average current density (A m^{-2})*	*Resistance per area unit (mΩ m^2)*	*Anolyte pH shift*	*Catholyte pH shift*	*Final pH imbalance*	*Reference*
AEM-AMI-7001	synthetic dark fermentation effluent	125 mM NaCl solution	7.1	1.33	8 → 6.1	-	6.9	[102]
AEM-AF49R27			9.4	0.18	8 → 6.2	-	6.6	
novel AEM-PSEBS CM DBC, functionalized with 1,4-diazabicyclo-octane			6.4	0.33	8 → 6.3	-	6.2	
Novel CEM-PSEBS SU	50 mM phosphate buffer solution (PBS)	50 mM phosphate buffer solution	0.8-1.6	-	7.2 → 6.2	7.2 → 9.1	2.9	[103]
Novel CEM-CF22R14				-	7.2 → 6.7	7.2 → 9.2	2.5	
CEM-Fumasep FKE				-	7.2 → 6.5	7.2 → 8.8	2.3	
Novel PEM-sulfonated poly(arylene ether sulfone) (SPAES)/polyimide nanofiber (PIN) composite	nutrient mineral buffer with 3mM acetate as only substrate	phosphate-buffered solution	-	-	7 → 6.23	7 → 7.71	1.48	[104]
PEM-Nafion-211			-	-	7 → 5.52	7 → 8.44	2.92	
CEM-PSEBS SU22	synthetic dark fermentation effluent	125 mM NaCl solution	7.2	-	8 → 6.3	6 → 12.8	6.5	[71]
CEM-CF22 R14			6.8	-	8 → 6	6 → 13	7	
BPM-FBM			7.4	-	8 → 7.1	6 → 12.4	5.3	
Non-woven cloth	Acetate mineral medium	-	-	0.9	7 → 6.8	-	0.4	[72]
Polyvinylidenedifluoride (PVDF)			-	1.5	7 → 6.6	-	0.7	
PEM-Nafion 117			-	3.4	7 → 6.26	-	2.82	
CEM	Anaerobic digester effluent	Carbonate buffer	4	59 ± 32	7 → 2.2	9.2 → 8.1	5.9	
AEM				6 ± 2	7.25 → 7.25	9.2 → 8.21	0.85	
Cellophane				9 ± 2	7.2 → 7.14	9.2 → 8	0.86	
Novel Cellophane+PDMS				22 ± 4	7.2 → 7.25	9.2 → 8	0.75	
CEM			10	176 ± 160	7 → 2.2	9.2 → 8.1	5.9	
AEM				17 ± 3	7.25 → 7.25	9.2 → 8.21	0.85	
Cellophane				20 ± 6	7.2 → 6.95	9.2 → 8	1.05	
Novel Cellophane+PDMS				11 ± 3	7 → 6.63	9.2 → 8	1.37	

5. Cathodes for hydrogen production

The hydrogen evolution reaction (HER) increases the pH in the cathodic chamber as protons are depleted [106]. Without sufficient proton replenishment, the energy demand for hydrogen production rises. Therefore, controlling the pH in the cathodic chamber is crucial to minimizing energy consumption. Molecules like phosphates and bicarbonates, which donate protons even at high pH, play a key role in maintaining hydrogen production efficiency [19,20]. Continuous sparging of an acid gas, such as CO_2, could lower the cathodic potential while also abating a waste gas. This option is one of the most common in the literature, but a real game changer could be the cathodic material.

Among catalysts for the HER, platinum is considered the most effective and widely used [107]. However, its high cost and susceptibility to poisoning by anionic species, such as sulphide or phosphate [107], as well as organic molecules transported from the anode [108], limit its practicality. To address these limitations, alternative materials have been explored, including stainless-steel [18], cobalt [109], molybdenum-based compounds [110–111] and nickel-based materials [112]. While the use of nanomaterials and advanced material modifications can enhance hydrogen production rates, their scalability to industrial applications is limited by high costs and complexity. Consequently, commercially available materials like stainless steel or nickel are more viable options, with stainless steel being approximately 100 times less expensive than nickel [113]. Notably, stainless steel has demonstrated performance comparable to platinum when used with carbonate buffers [18,114–115], which are significantly cheaper than phosphate buffers commonly utilized as catholyte for the HER in MEC [107,114]. Additionally, bicarbonate ions are naturally generated at the anode through the bioelectrochemical oxidation of organic molecules. This process enriches the anodic gas with CO_2, offering an inexpensive and readily available source of buffer for the catholyte. Furthermore, CO_2 is a common byproduct in industrial processes such as fermentation and anaerobic digestion, providing another accessible source. Thus, the combination of stainless steel and a carbonate buffer emerges as a promising and economically feasible cathode-catholyte system for scalable hydrogen production in MECs. Real wastewaters are highly variable and can differ significantly depending on season, origin, and location. This variability affects temperature, conductivity, pH and the amount of pH buffer present. Since it is unrealistic to expect constant inlet parameters, it is more practical to implement an online monitoring system to control and adjust several process settings. Low pH can enhance H_2 productivity but may also inhibit microbial activity. Low conductivity inevitably increases the overpotential, while low buffer concentration causes strong pH fluctuations. High temperature could increase the efficiency but it would increase the operational costs and may inhibit, if too high, some microorganisms. In addition, the presence of chemicals that are toxic to the cathodic material may reduce the active electrode surface, eventually leading to complete inactivation. Separators not only allow the use of a cathodic solution different from the anolyte but, more importantly, protect the cathode from poisoning. Zhao *et al.* [7] designed a “sandwich” cathode which consists in a hydrophilic PVDF membrane placed on one side of the cathode and a second membrane in PTFE (Polytetrafluoroethylene, so hydrophobic) to reduce the H_2 crossover and limit it depletion by homoacetogens and methanogens. This study showed high electric current densities (515 A/m^3) and a constant H_2 production for over 30 days ($\approx$ 5 m^3/m^3/d) without using any inhibitors to stop H_2 oxidation. This innovative design combines an efficient separation with simplicity and easy scalability, reducing the cost in comparison of a standard MEC equipped with a Nafion membrane and enhancing H_2 production and purity. The removal of H_2 is crucial not only because of the presence of microorganisms that can use it as an energy source, but also in light of Le Chatelier’s principle: by removing the product, the chemical equilibrium is shifted further towards product formation. Finally, the accumulation of H_2 bubbles on the electrode surface can create areas of theoretically infinite resistance, thereby increasing the overall energy consumption of the system. For this reason, equipping MECs with a vacuum pump could be beneficial, especially considering the volatility and fugacity of H_2.

5.1. Strategies to Improve Hydrogen Production and Purity in single-chamber MECs without a separator

Membrane-less MEC are a common configuration, capable of reducing the overall cost and complexity of the system [25,47]. One of the main challenges for the scaling-up and commercialization of single-chambered MEC systems is the low cathodic capture efficiency and the low purity of the produced hydrogen caused by the growth at the cathode or in the nearby bulk liquid of hydrogenotrophic microorganisms, such as hydrogenotrophic methanogens [116]. These microbes can utilize hydrogen as an electron source for their metabolism, producing gaseous products as methane and carbon dioxide which will contaminate the produced hydrogen. Various pretreatment to suppress the methanogens activity has been reported in literature, such has additions of chemicals [117–118], O_2 shock [119–120], heat treatment [55], UV irradiation [121], and ultrasonication [58]. The main drawback was that this can also damage the electroactive microorganisms. Some solutions based on active H_2 harvesting from the cathode chamber [122-126] showed promising results in inhibiting the activity of hydrogenotrophic microorganisms, without posing risks for the electroactive microbial community.

Lu *et al.* [122] achieved a hydrogen capture efficiency ranging from 66% to 85%, based on the stoichiometric 3.5 mol H_2/mol acetate, using an underpressurized "hydrogen breathing cathode." This cathode was composed of carbon felt decorated with Pt as the catalyst and a hydrophobic PTFE membrane, designed to harvest the evolving hydrogen away from the liquid phase. Notably, the hydrogen obtained using this configuration was free from methane contamination, although it did not prevent the growth of hydrogenophilic microorganisms. Additionally, no electrolyte leakage or salt deposition was reported, even after 51 days of operation. However, this study was conducted in a small batch system with a working volume of 25 mL, using 1 g L^{-1} sodium acetate in a 50 mM phosphate buffer solution as the feed for the MEC. This setup leaves room for further optimization, such as testing the configuration with more complex substrates, larger reactor volumes, and/or in continuous operation mode. Despite these limita-

tions, the results provide a solid proof-of-concept for enhancing the coulombic capture efficiency (CCE%) without disrupting the microbial communities within the system. This approach presents itself as a promising solution for implementation in anaerobic digestion-microbial electrolysis cell (AD-MEC) integrated systems, where maintaining balance and avoiding stress in the anaerobic community is crucial to prevent process failures or the accumulation of inhibitory compounds, such as volatile fatty acids [123–124].

Tartakovsky *et al.* [123] were the first to employ a gas-diffusion cathode in a MEC. The catalyst used was a carbon-supported Pd/Pt alloy, with the support consisting of Toray carbon fiber paper impregnated with 10% PTFE. The resulting gas-diffusion electrode (GDE) was hot-pressed onto a Nafion 117 membrane, forming a Membrane-Electrode Assembly (MEA). In their study, they reported a significant loss of up to 50% of the hydrogen produced at the cathode, primarily due to the activity of hydrogenotrophic methanogens. Post-mortem analysis of the MEA revealed the detachment of the Nafion membrane from the GDE, leading to electrolyte leakage and the presence of biofilm on both the Nafion membrane and the GDE.

This MEC configuration was improved in a subsequent publication [124], where the authors compared a proton exchange membrane (PEM)-separated MEC and a membrane-less MEC at different cell voltages. Both MECs were fed with acetate and inoculated with anaerobic sludge. The anode consisted of 5-mm thick carbon felt, while the cathode was a GDE with a Pt load of 0.5 mg cm^{-2}. The anode and cathode were separated by a piece of J-cloth, resulting in an electrode distance of 0.3 mm. Each voltage was maintained for 1-5 days, and only the hydrogen production from the last 12 hours of each test was used for calculations. At an applied voltage of 1 V, both MECs achieved their highest performances in terms of hydrogen production and recovery. The membrane-less MEC produced 6.32 $L_{stp}\ L_A^{-1}\ d^{-1}$ of hydrogen with a recovery efficiency of 90% of the theoretical mol H_2/mol acetate. In contrast, at the same voltage, the PEM-separated MEC achieved only 1.22 $L_{stp}\ L_A^{-1}\ d^{-1}$ of hydrogen production and a 60% hydrogen recovery efficiency. It was hypothesized that this disparity was due to lower ohmic losses and the absence of pH imbalance between the compartments in the membrane-less MEC, which resulted in higher power density. This higher power density likely favored the growth of electroactive microorganisms capable of competing with the methanogen population. Additionally, it is possible that the absence of reference electrodes in the setup, coupled with the lower internal resistance due to the lack of a membrane, resulted in lower electrode overpotentials. Consequently, the membrane-less MEC may have experienced higher cathodic potential compared to the PEM-MEC at the same applied cell voltage, leading to a higher electrochemical production rate of hydrogen.

In a subsequent study, Manuel *et al.* [125] investigated various Nickel alloy-based GDE using the same setup as the previous studies. The MECs were operated without a separation membrane at a cell voltage of 1.0 V, and the same bioanode was used for testing all the different cathodes. The study proposed a direct correlation between the hydrogen production rate, cathodic recovery, and the Nickel content of the catalyst. The Coulombic capture efficiency (CCE%) ranged from 70% to 100%, indicating that the GDE exhibited good hydrogen harvesting performance. However, the differences in current achieved and the voltage scan results may not solely be attributable to the cathode performance, as suggested in the paper. Instead, these differences could be due to varying anodic polarization resulting from applying the same cell voltages with different electrodes. It is important to note that in MEC, the "bottleneck" for current generation is typically the activity of the bioanode, rather than the abiotic HER at the cathode. Thus, the performance variations observed might be more closely related to the bioanode's behavior under different operational conditions rather than the specific properties of the cathodes.

In a later study, Gil-Carrera *et al.* [126] explored the scaling-up of MEC equipped with GDE. They tested three different anodic volumes—50 mL, 855 mL, and 10 L—across a cell voltage range of 0.5-1 V, using various synthetic media, with the 10 L system also being tested with raw municipal wastewater. The GDEs were composed of electrodeposited Nickel on carbon paper, separated from the anode (carbon felt) by a 0.7 mm polyester cloth. The results indicated a decline in hydrogen yield (mol H_2 $mol^{-1}_{acetate}$) and an increase in energy consumption (Wh $L^{-1}H_2$) as the system scaled up. Notably, the hydrogen yield obtained from the 50 mL MEC fed with synthetic wastewater was up to three times lower than that reported in previous studies [126-128] using acetate-based media. This lower cathodic efficiency is likely due to the increased proliferation of hydrogenotrophic methanogens in synthetic media and scaled-up systems, as well as the flooding of the GDE. To address this issue, a cathode condensate collector was necessary for the two larger MECs. These findings underscore the challenges associated with scaling up MECs, particularly the impact of methanogens and GDE flooding on hydrogen production efficiency. The study highlights the importance of optimizing operational conditions and system design when transitioning from laboratory-scale to larger systems, especially when using complex or real wastewater as feedstock.

Feng *et al.* [129] applied an under-pressure of −600 mbar to a single-chamber MEC with a 0.5 L working volume, achieving over 80% electron recovery as hydrogen consistently over 30 days. In contrast, the same MEC operated at normal pressure (0 mbar) showed a decline in electron recovery to hydrogen, starting at 40% and dropping to just 10% by the end of the experiment. In another experiment, when a cell initially operated under −600 mbar pressure for the first 10 days was switched to normal pressure, methane production rapidly increased at the expense of hydrogen, with electron recovery decreasing from 60% to 10%. Reapplying the under-pressure was unable to inhibit the methanogens effectively. The negative pressure likely reduces the solubility of hydrogen in water, facilitating its faster harvesting. Since the inoculum was taken from MEC amended with 2-bromoethanesulfonate (a methanogenesis inhibitor [130]), the methanogens were already inhibited at the start-up, and the lack of available hydrogen prevented their growth. However, once the negative pressure was stopped, hydrogen became available again, allowing methanogens to proliferate and rapidly consume hydrogen, even when the negative pressure was reestablished.

In a recent follow-up study [131], higher removal of

organic compounds was observed in the AD-MEC system compared to the anaerobic digester alone. However, it is important to note that the anaerobic digester was inoculated with inhibited methanogens, and the experimental duration was relatively short (35 days), which is insufficient for achieving an efficient anaerobic digestion process. Thus, this study may be better considered as an evaluation of MEC performance in digesting food waste rather than as an assessment of a fully integrated AD-MEC system. These findings emphasize the critical role of pressure in controlling hydrogen recovery and the challenges in maintaining methanogen inhibition, particularly when transitioning from under-pressure to normal conditions. The studies also highlight the complexities involved in integrating MECs with anaerobic digestion systems, especially concerning methanogen management and the efficiency of organic compound removal.

6. Overall conclusions and future outlook for MEC Systems

As global demand for renewable energy grows, scalable and cost-effective solutions like MEC systems are becoming increasingly vital. While MEC systems offer numerous benefits, such as energy recovery, nutrient recycling, and waste reduction, several challenges must be addressed before these technologies can be widely implemented. One of the main challenges is scalability. Scaling up MEC technology from laboratory experiments to industrial applications requires further research. Reactor design, process optimization, and the durability of electrode materials are some of the key areas that need to be addressed to make MEC systems economically viable on a larger scale.

Bioanodes have seen improvements in performance through material innovation and biofilm optimization. However, their efficiency under real wastewater conditions, where low conductivity, inhibitory substances and microbial competition are prevalent, still lags behind that achieved with synthetic media. Moving from batch to continuous flow allows constant product removal and substrate delivery while helping in controlling the system pH. Separators, while essential for compartmentalization, introduce additional costs and operational inefficiencies such as biofouling and pH imbalance. Exploring alternative materials and optimized configurations holds promise for mitigating these limitations. Working in a single chamber configuration drastically reduces ohmic resistance, boosting current density. The challenge at that point would be controlling gas crossover (H_2 to the anode) and microbial cross-consumption, which would reduce the production of H_2. Careful hydrodynamics design and gas collection systems are needed. Cathodes, particularly those using cost-effective materials like stainless steel, offer a viable path for reducing production costs while maintaining efficiency. Nonetheless, scalability challenges and contamination by hydrogenotrophic methanogens must be addressed for long-term stability and performance. Another significant challenge is the high initial cost, particularly for electrodes and membranes. Ongoing research is focused on developing more affordable and sustainable materials to reduce these costs without compromising efficiency. Additionally, the performance of MEC systems relies heavily on maintaining stable and efficient microbial communities. Managing these microbial populations in industrial settings, especially under varying operational conditions, presents a challenge that requires further investigation. For this reason, we believe that more efficient collaboration among scientists from different fields is needed. Bioelectrochemical systems are complex, and achieving industrial application requires input from all disciplines. Microbiology, materials science, process engineering, and physical chemistry are very different fields, and no single person can ever fully master all of them. Therefore, broad understanding and, above all, collaboration are essential. Standardized testing protocols are not just desirable but essential; without common benchmarks, comparisons across materials and systems lose their scientific value. Future research on MEC systems will likely focus on several key areas. One of the main areas of interest is the development of cheaper, more durable electrode materials. This innovation is essential to reduce system costs and enhance long-term performance. Moreover, optimizing operational parameters such as pH, temperature, and electrical potential will be crucial for scaling MEC systems and improving their overall efficiency. Last but not least, the current densities reported in literature are not comparable to those of traditional electrolyzers. On one hand, it is important to remember that MECs are not designed to compete with these systems but to enhance waste management, as well as nutrient and energy recovery. On the other hand, there is a biokinetic limitation that will always keep the current densities lower than those in abiotic systems. Moreover, it is important to remember that the higher the current, the higher the overpotentials. This happens because at higher currents, the system requires more energy to overcome internal resistance, causing an increase in potential losses during the electrochemical reactions. It is important to remember that, as the current increases the system power losses will increase quadratically ($W = V^*i = i^2R$). In addition, a faster reaction means also higher activation and concentration losses. To keep under control these losses a dynamic control of the applied potential is not only necessary but also fundamental. This control, maybe optimized by real-time sensors and an AI, could adjust the voltage based on substrate concentration; during low load period a higher focus could be given to efficiency and to high production rate, during high load periods. Future studies should also include comprehensive life cycle and techno-economic assessments as larger-scale and long-term MEC data become available. Such analyses will be crucial to evaluate the overall sustainability and economic feasibility of industrial MEC systems. By addressing these challenges (cheaper materials with a good balance between energy efficiency, current densities, high surface area with optimized hydrodynamics and cost) and accepting their limits (mostly biological), MEC systems have the potential to become a key technology in sustainable waste management and energy production.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CRediT author contributions

Simone Colantoni: Conceptualization, methodology, validation, formal analysis, investigation, writing—original draft, writing—review and editing, visualization.
Angela Marchetti: writing—review and editing
Marco Zeppilli: Conceptualization, methodology, validation, writing—review and editing, visualization
Lorenzo Cristiani: Conceptualization, Methodology, formal analysis, writing—review and editing, visualization.

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