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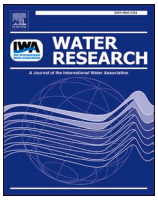

A comprehensive analysis of key factors influencing methane production from CO_2 using microbial methanogenesis cells

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ABSTRACT

With increasing attention on carbon capture and utilization (CCU) technologies for the conversion of CO_2 into chemical products, microbial methanogenesis cells (MMCs) have been extensively studied over the past few decades for biomethane production. Using rapidly accumulating data for MMCs with varying configurations and operating conditions, a comprehensive analysis was conducted here to investigate the critical factors that influence methane production rates (MPR) in these systems. A comparison of MPR and set potentials or current densities showed weak linear relationships ($R^2 < 0.6$, $p < 0.05$), indicating the significant contributions of other important factors impacting methane production. A non-quantitative analysis of these additional parameters indicated the potential importance of using metal catalysts for anode materials where oxygen evolution reaction occurs, while most previous MMC research focused more on cathode materials where the biocatalytic reaction occurs. The use of undefined mixed anaerobic cultures as inocula was found to be sufficient for producing high MPRs, as the electrochemical environment at the cathode provides a strong selective pressure to converge on desirable methanogenic cultures. Other operational parameters, such as catholyte pH control and CO_2 supply methods, were also important factors impacting MPR in MMCs, indicating the cumulative impact of these various factors will require careful consideration in future research.

1. Introduction

Globally, there is a growing focus on defossilizing industries and promoting a circular economy, which has led to increased interest in carbon capture and utilization (CCU) technologies. One of these technologies is microbial methanogenesis cells (MMCs), which is a bioelectrochemical process that converts CO_2 into methane, an energy-carrying gas. At the anode, current can be generated either by water splitting or by exoelectrogenic microorganisms using organic matter and voltage added to the circuit (Zhang et al., 2019). However, if chloride is present in the water, chlorine gas can also be generated from chloride ion oxidation (Baek et al., 2021b; Ghernaout et al., 2011). The reduction of CO_2 at the cathode by biofilms or suspended cells can occur through different mechanisms. The first is a direct electrotrophic mechanism by attached electroactive methanogens ($CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$), often called electromethanogenesis. The second is hydrogen-mediated methanogenesis with hydrogen formation ($2H^+ + 2e^- \rightarrow H_2$ or $2H_2O + 2e^- \rightarrow H_2 + 2OH^-$ depending on the solution pH) followed by its conversion to methane ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) (Baek et al., 2021a). A third route can include enzymes on the electrode surface that facilitate release of H_2 or other chemicals (Deutzmann et al., 2015; Lohner et al., 2014). Finally, it is also possible to produce methane following acetogenesis by other microorganisms on the cathode (Deutzmann et al., 2015).

In recent years, there has been increasing attention to research on MMCs aimed at improving their efficiency and exploring alternative configurations. Changes to the reactor architecture have been made to MMCs to reduce the internal resistance (Baek et al., 2022a; Chen et al., 2022), overpotential of the cathodes (Baek et al., 2022b; Gomez Vidales et al., 2021; Nwanebu et al., 2022), and improve biofilm formation on the cathode by using specially designed electrodes (Bian et al., 2022). In addition, various operating conditions in MMCs have been examined, such as improving CO_2 transport to the cathode biofilm (Dessì et al., 2023; Rojas et al., 2021), neutralizing the pH of the catholyte which is alkalinized continuously with operation (van Eerten-Jansen et al., 2015), or enhancing CO_2 reduction efficiency by using defined microbial cultures for desired reactions (Kracke et al., 2020; Mayer et al., 2019).

There are multiple factors that have been recognized to have impacts

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on the performance of MMCs such as inoculum, electrode material, membrane (if present), electrode surface area, and how densely packed the electrodes are in the electrolyte and reaction chamber (i.e., the surface area-to-volume ratio, S/V) (Fig. 1). For example, MMCs can be inoculated either by defined cultures (i.e., different pure methanogenic cultures) (Mayer et al., 2019), or undefined mixed cultures collected from anaerobic sludge (Baek et al., 2022b; 2017; Cerrillo et al., 2017) or effluents of previous bioelectrochemical systems (Siegert et al., 2015). Carbon- or graphite-based electrodes have been typically used as anodes or cathodes in MMCs as they provide compatible surfaces for biofilm formation, high electric conductivity, and they are relatively low cost materials (Pawar et al., 2022). Several MMCs have also used metal catalyst-based electrodes to reduce electrode overpotentials and thus boost electrochemical performance, for example by reducing the overpotential needed for hydrogen gas production (Rossi et al., 2023). Apart from the diverse range of system configuration factors, there are also some crucial operational factors that affect the performance of MMCs. For example, the method for CO_2 feeding or pH adjustment of catholyte which is continuously alkalinized as reactions occur that could affect the performance of MMCs, microbial activity, and thus long-term stability of these systems.

In recent years a substantial amount of data has been gathered on MMCs having different configurations and operational conditions. Consequently, it is important to examine the factors that impact the efficiency of methane production in MMCs and determine the ideal operating conditions necessary to attain high-rate and high-efficiency systems. While several previous reviews on MMCs have provided useful summaries of the literature (Blasco-Gómez et al., 2017a; Geppert et al., 2016; Pawar et al., 2022), they have not provided a sufficiently critical analysis of the data in these studies to understand factors that improve performance. What is therefore needed is not only information on methane production rates achieved in different MMCs but also a quantitative examination of relationships between these methane generation rates and the various conditions and parameters that influence system performance. Without such quantitative analyses, it is difficult to make meaningful comparisons between different systems or draw conclusions about the factors that contribute to MMC operation.

In this analysis, we provide a comprehensive assessment of the key factors affecting the performance of MMCs for efficient CO_2 to methane conversion under optimized configuration and operating conditions. Through a critical analysis of the literature (37 datasets from previous MMC studies where key configuration and operating parameters are available), we provide a more nuanced understanding of the relationship between the different factors and methane production rates. In this article, we use the term MMC instead of microbial electrosynthesis (MES) cell. Our choice was driven by the fact that our investigation focused solely on datasets derived from bioelectrochemical systems with the specific objective of generating methane gas, rather than liquid chemicals such as volatile fatty acids (VFAs) as the end products. Nevertheless, the insights gained from our analyses hold the potential to enhance the refinement of MMCs and other MES systems that utilize analogous bioelectrochemistry principles as found in MMCs.

2. Analysis methods

2.1. Data collection and analysis

To analyze the factors that impacted methane production rates (MPR) in MMCs, data were compiled using 37 experimental results published in 19 different studies (Cheng et al., 2009; Fu et al., 2015; Gomez Vidales et al., 2019; Jiang et al., 2013; Kracke et al., 2020; Liu et al., 2018; 2017; Mao et al., 2021a, 2021b; Mayer et al., 2019; Ragab et al., 2019; Siegert et al., 2014; van Eerten-Jansen et al., 2015; Van Eerten-Jansen et al., 2013, 2012; Villano et al., 2010; Xu et al., 2017; Zhou et al., 2021, 2020). For all these studies an abiotic anode was used rather than a bioanode. The data encompassed information on distinct parameters, such as cathode projected surface area, cathode applied potential, inoculum type, membrane type, electrode materials, liquid volume of the reactor, current density, and methane production rate (Table S1). We excluded data points from MES designed to produce chemical products other than methane such as acetate. For a comparison among different studies, we normalized current density and methane production rate by the cathode surface area (m^2). The methane production rate can be also normalized by the liquid catholyte volume (L) as typically reported in previous datasets as well. All data analyzed with liquid volume based MPR are reported in the Supporting Information (Fig. S1, S2 and S3). The range of possible influencing factors analyzed in our study was: cathodic potential (–0.58 V to –2.5 V vs. Ag/AgCl), cathode projected surface area (4–361 cm^2), reactor liquid volume (28–650 mL), and cathode surface area-to-volume ratio (1.1–89.3 m^2/m^3). The range of output from MMCs was: methane production rate (0.1–205.4 L/m^2-d), current density (0.1–68.1 A/m^2), and cathodic methane recovery (18–99%) (Fig. 2).

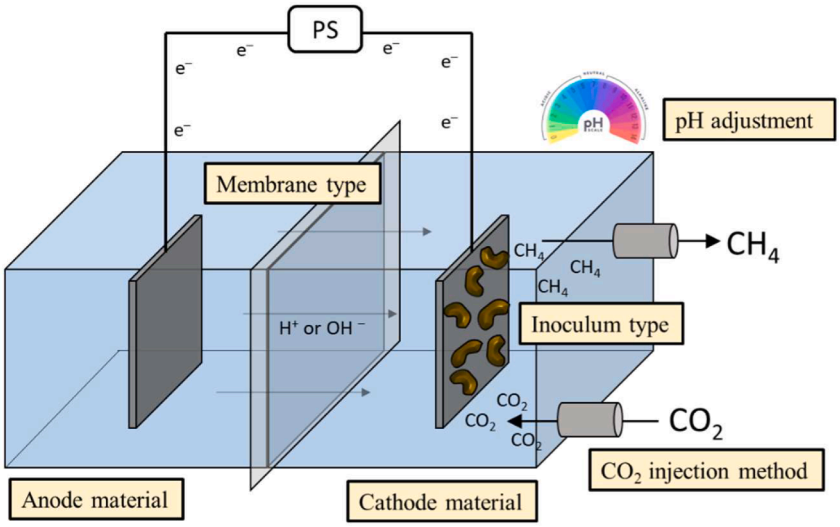

Fig. 1. Key configuration and operational factors influencing the performance of microbial methanogenesis cells.

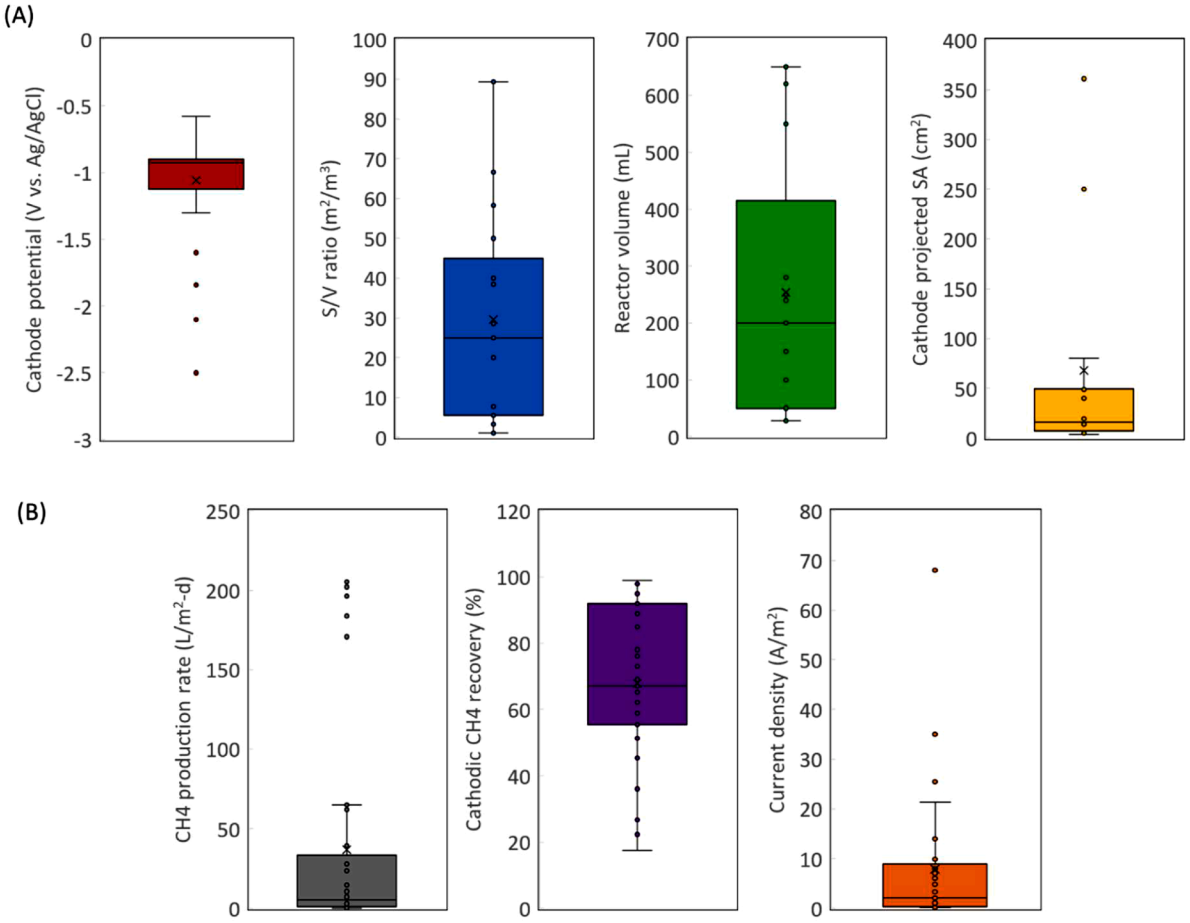

Fig. 2. Box plots showing the distribution of (A) key parameters influencing the performance of microbial methanogenesis cells (cathodic potential, cathode surface area-to-volume ratio, reactor volume, and cathode projected surface area) and (B) the performance of methane and current productions (CH_4 production rate, cathodic CH_4 recovery, and current density).

3. Results and discussion

3.1. Correlation between methane production and numerical operational factors

There was a low linear correlation between MPR and cathodic potential ($R^2 = 0.56$, $p<0.05$) and MPR and current density ($R^2 = 0.50$, $p<0.05$) (Fig. 3A and 3B). The trends observed in these analyses indicated that the highest MPRs required the highest current densities and the most negative cathode potentials. However, the lack of a very high correlation between MPR and these other two parameters indicated that other parameters also had a comprehensive impact on methane generation rates. For MMC operation, external energy must be applied to drive the endergonic reaction for the water oxidation and oxygen evolution reaction at the anode and the hydrogen evolution reaction at the cathode (which can lead to further conversion into methane). This energy input was typically achieving by setting the cathodic potential, rather adding an additional cell voltage between anode and cathode, in order to accurately control of cathodic potential where a desired reaction (i.e. electromethanogenesis) occurs. Based on our analysis, the average value of cathodic potential in MMCs was –1.06 ± 0.41 V vs. Ag/AgCl (Fig. 2A), although there was a large variation between –0.58 to –2.50 V vs. Ag/AgCl (Table S1). Higher MPRs could be achieved at more negative cathodic potential than the average potential because it could help to readily overcome the energy barrier of CO_2 reduction to methane and produce more current (Zhang et al., 2019).

Another factor that can impact the MPR is the electrode packing density, or the amount of electrode area per reactor volume (S/V). A previous analysis found that the S/V ratio was the most critical factor for improving methane production when electrodes, developed for microbial electrolysis cells, were inserted into anaerobic digesters (Baek et al., 2021a). In that analysis, increasing biomass concentration (through biofilm formation on the electrodes) and stimulation of electrical syntropy between electroactive microorganisms were suggested as two possible reasons for enhanced methane production (Baek et al., 2021a). However, in this present analysis on MMCs, we did not observe any meaningful relationship between MPR and S/V ratio (Fig. 3C), suggesting that methane production from MMCs was not significantly influenced by increased amount of electrode surface in the reactor in these past studies, although it is expected it will become more important as other operational factors are optimized.

3.2. Impact of electrode materials

In many different types of bioelectrochemical systems, the electrode material and presence of a catalyst are critical components that can facilitate biotic or abiotic electron transfer mechanisms at the anode and cathode. In MMCs, the transfer of electrons between microbial catalysts (i.e., electroactive microorganisms) and the electrode surface, as well as the production of the final product (i.e., methane gas), occur at the cathode, while oxygen evolution typically occurs at the anode as a counter-reaction (Zhang et al., 2019). Therefore, in general, researchers tended to focus on optimizing the cathode in MMCs, for example by using metal catalysts and increasing the specific surface area

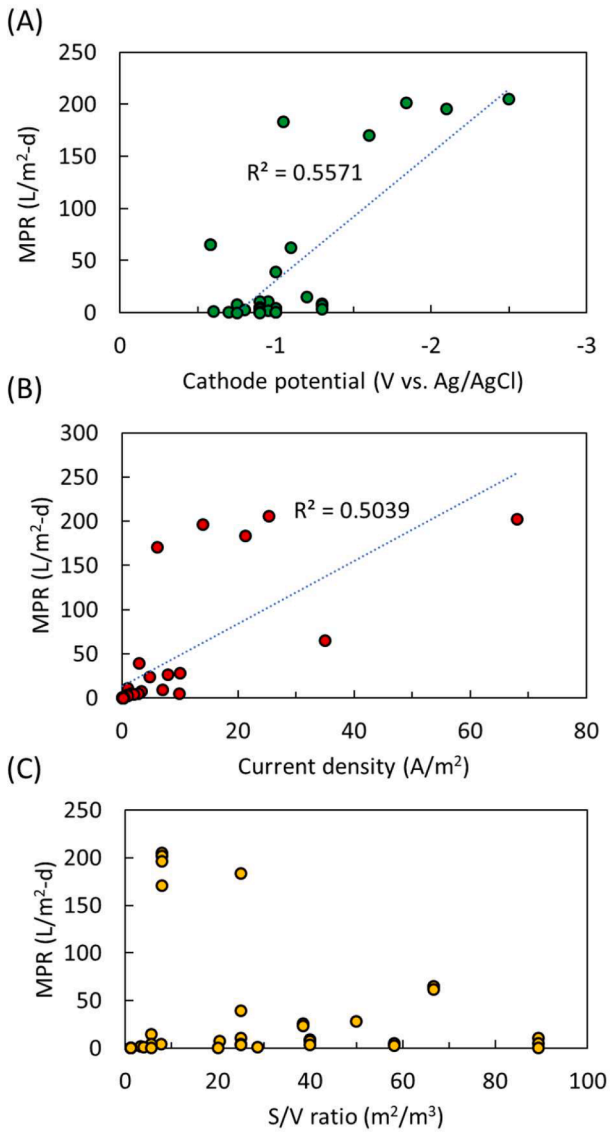

Fig. 3. Correlation between methane production rates and (A) cathode potential, (B) current density, and (C) cathode surface area-to-volume ratio in microbial methanogenesis cells.

(electroactive surface area per geometric area). Previous reviews of MMCs and MES systems have provided good summaries of the cathode materials used in these systems, and their impact on methane production (Blasco-Gómez et al., 2017b; Zhang et al., 2021, 2019). However, anode materials in MMCs have been rarely discussed. The anode in MMCs is important because a large amount of energy is consumed for the kinetically unfavorable oxygen evolution reaction at the anode unless the overpotential is reduced by choosing appropriate catalysts for the anode.

To better understand the impact of the anodes on MMC performance in terms of energy consumption, we separated the MMC data into two categories depending on the type of electrode (anode or cathode) materials used: plain carbon-based electrodes (such as carbon or graphite felt, cloth, brush, and paper), and metal catalyst-based electrodes (Fig. 4). Metals such as iridium- or platinum-doped titanium electrodes have been used on the anode to catalyze the oxygen evolution reaction (OER). For cathodes, platinum- or nickel-based electrodes are often added to catalyze the hydrogen evolution reaction (HER) (Son et al., 2021). Based on the results of our analysis, the use of metal catalysts on cathodes in MMCs had a positive impact on MPR according to the distribution of data points, despite the significant variation observed within each group (35 ± 45 L/m^2-d for carbon materials and 99 ± 102 L/m^2-d for metal catalysts) (Fig. 4B). This finding supports the use of metal catalysts as cathode materials in MMCs to improve methane production. It is important to note that the anode material also had a significant impact on MPR as MMCs produced higher MPR when metal catalyst-based anode materials were used (82 ± 79 L/m^2-d) than carbon materials were used (3 ± 4 L/m^2-d) (Fig. 4A). This finding of higher MPRs with catalyzed anodes might simply result from reducing the anode overpotential, enabling more energy directed to driving the methane production reaction at the cathode due to the higher current densities based on using similar amounts of input energy (i.e., similar applied voltages). This analysis suggests that the use of superior catalytic materials on the anode has a more pronounced effect on MMC performance than the use of such materials on the cathode. The highest MPR (205 L/m^2-d) was obtained from the MMCs which adopted the titanium with IrO_2 as the anode and titanium with platinum as cathode materials. Indeed, the corrosion of carbon-based anodes at high anodic potential (the standard potential of carbon oxidation reaction is 0.207 V vs. reverse hydrogen electrode) has been reported in previous MMC studies based on the observation of the development of a dark colored anolyte (Baek et al., 2022a, 2022b; Yi et al., 2017). Replacing the carbon cloth anode with a platinized titanium mesh can resolve the issue of corrosion in MES (Baek et al., 2022a). Therefore, based on our analysis results, using anode materials with high catalytic activity is as critical a factor as the cathode materials for overall performance.

3.3. Impact of inoculum

In several MMC studies, pure cultures have been used as inocula to improve the specificity of methane versus other products, and also avoid poorly understood functionality of different microorganisms in mixed-culture communities (Kracke et al., 2020; Mayer et al., 2019). Generally, using pure or defined cultures can be advantageous as the function and interactions may be either known or easier to study (Rosenbaum et al., 2019). However, our analysis showed that high MPRs were obtained from MMCs inoculated with mixed cultures, except for a single study datum, (Fig. 5A). The average MPR values presented in Fig. 5A were 81 ± 80 L/m^2-d for mixed cultures and 4 ± 11 L/m^2-d for defined cultures. This analysis indicated that using a defined methanogenic culture as an inoculum did not necessarily impact the methane production in MMCs but using a well-acclimated mixed culture medium (usually originated from anaerobic sludge) could be a better approach than pure cultures.

At the electrodes in bioelectrochemical systems including MMCs, the set electrode potential greatly influences the extracellular electron transfer pathways and the composition of microbial community and therefore it exerts a selective pressure for enrichment of different species (Chatterjee et al., 2019). Due to this strong selective pressure, it is commonly found after repeated operational cycles that microbial communities of anodes in microbial fuel cells (MFCs) fed acetate as a substrate converged to *Geobacter*-enriched cultures regardless of the initial inoculum sources (Jung and Regan, 2007; Yates et al., 2012). Similarly, biocathodic microbial communities after several repeated batch cycles have become enriched with *Methanobacterium* based on reports in multiple MMC studies when potentials are set in a range to avoid excessive hydrogen gas production (Baek et al., 2017; Cheng et al., 2009; Siegert et al., 2015; Zhou et al., 2021). For example, all 16S rRNA gene sequences derived from the biocathode of MMCs initially inoculated with anaerobic sludge were assigned to a single methanogenic genus, *Methanobacterium* (Baek et al., 2017). Similarly, *Methanobacterium*-dominated biocathodic microbial community has been reported from another MMC study where various types of electrode materials including carbon-based and metal catalyst-based materials were tested as cathodes. The initial inoculum was an anaerobic digester sludge and the archaeal communities of all cathodes except only one with platinum

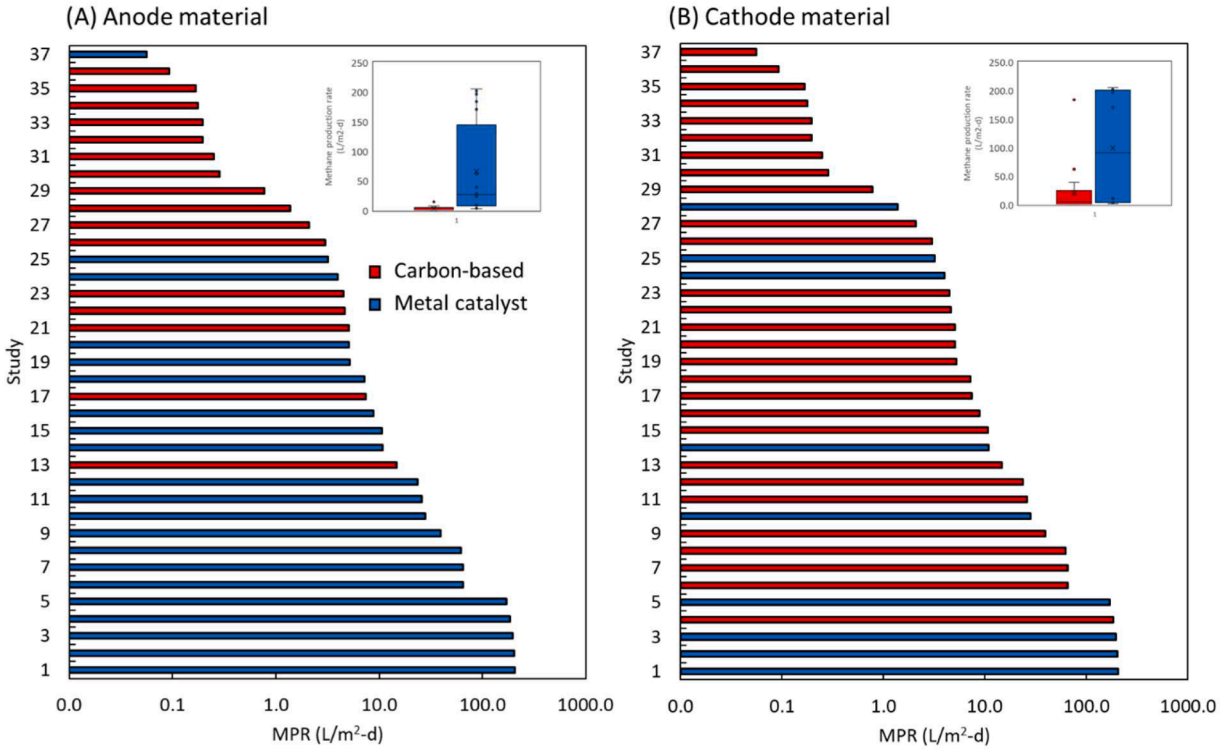

Fig. 4. Methane production rates yielded from each study and the (A) anode and (B) cathode materials applied. Box plots shows the distribution of methane production rates obtained from each data point that used carbon-based material (red) and metal catalyst (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

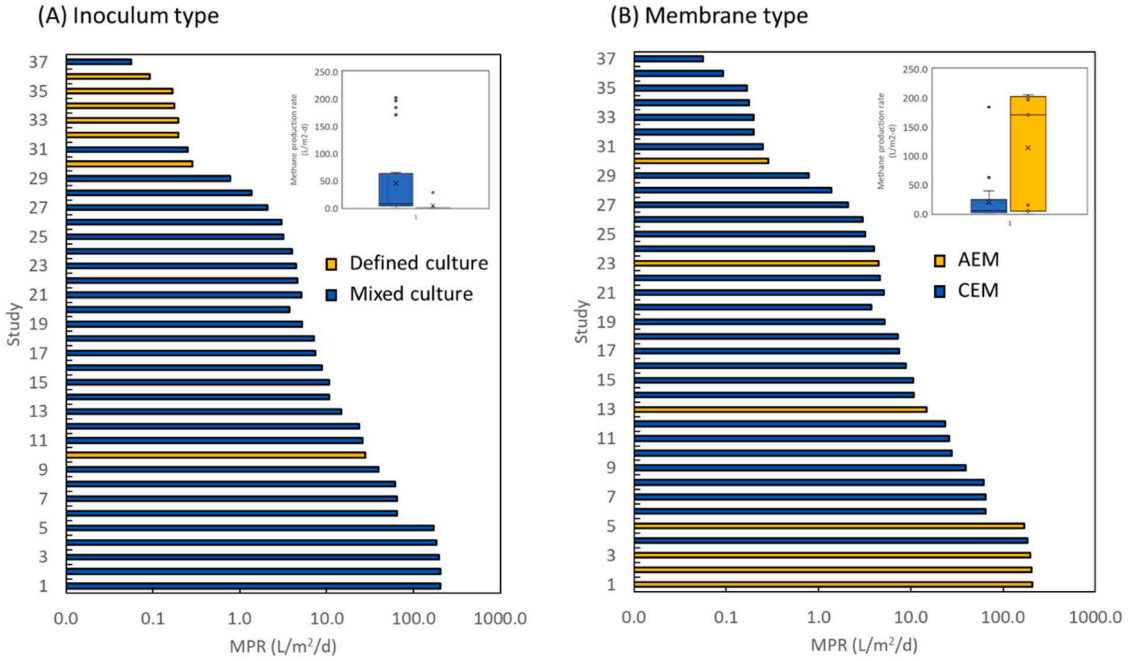

Fig. 5. Methane production rates yielded from each study with the (A) inoculum type and (B) membrane type applied. Box plots shows the distribution of methane production rates obtained from each data point.

showed a converged community with *Methanobacterium* (median of 97% in abundance of all archaea) (Siegert et al., 2015). Methanogenic communities can shift to different species under conditions where a catalyst such as Pt is used or highly negative cathode potentials are set that ensure efficient hydrogen gas production (resulting in a large percentage of hydrogen gas accumulation in the product gas) (Baek et al., 2022a; Li et al., 2020; Siegert et al., 2015; Werner et al., 2016). In cases where catalysts are used to produce high current densities and enhance H_2 gas production, the biocathodes become predominantly enriched with *Methanobrevibacter* rather than *Methanobacterium*. In addition, it has been reported that a long-term power interruption could be significantly impact the microbial community on the cathode, leading to a shift from

acetogenic bacteria to methanogens (Mateos et al., 2020).

In summary, to achieve high methane production in MMCs, a specific methanogenic culture is not always required as the electrochemical environment can exert selective pressure and enrich certain microbial communities. However, the available data points for MMCs using defined cultures are limited and may not yield a statistically significant relationship with MPR (Fig. 5A). A greater focus is needed on how operational conditions, such as the background rates of hydrogen gas production, could lead to greater clarity on the formation of different methanogens and MPRs.

3.4. Impact of membrane type

Two-chamber MMCs have two chambers that are separated using an ion exchange membrane, which comes in two major types: cation exchange membrane (CEM) and anion exchange membrane (AEM). Despite some drawbacks using ion exchange membranes in bioelectrochemical systems, such as high cost, pH gradient, and membrane fouling issues, two-chamber MMCs have been extensively used primarily to avoid crossover of chemicals. Methanogenic microbes can be shielded from any possible inhibitory chemicals originating from the anode, such as oxygen when there is water splitting at the anode, and the cathodic reaction can be studied independently of the anode reaction. (Zhang et al., 2019). Based on comparison of performance using CEMs versus AEMs (Fig. 5B) there is no apparent advantage of using a CEM over AEM in MMCs where there are gaps between the electrodes and the membrane. However, due to the prevalence of CEM usage in MMCs, it is challenging to draw conclusions on the effect of membrane type on MPR in these systems, suggesting that additional research might help to better determine the impact of AEMs on performance. It is worth noting that the present analysis did not consider studies on membraneless bioelectrochemical systems, such as AD-MECs, as these systems involve direct methane generation from organic matter fed to the anode.

3.5. Other critical factors influencing MMC performance

One of major challenges in typical two-chamber bioelectrochemical systems including MMCs is a large pH gradient between anolyte and catholyte due to a preferential transport of cations other than protons (when CEM is used) or anions other than hydroxyl ions (when AEM is used) (Rozendal et al., 2006). For example of using CEM in MMCs, Na^+ or K^+ ions in the anolyte will be transported through the CEM instead of H^+ ions due to their higher concentration in the buffer solution, resulting in a gradual decrease in the anolyte pH and accumulation of hydroxide ions in the catholyte pH (Baek et al., 2022a). If strategies are not taken to address pH imbalances in MMCs, there will be an increase in catholyte pH typically in the range of 7.7–10.1 depending on operational conditions, creating alkaline conditions that are not conducive to optimal growth of methanogens (Zhou et al., 2021, 2020). Several methods have been used to maintain catholye pH in a neutral range. One simple and most frequently used ways to control pH is to use a controller that adds acid to reach pre-selected pH range. For example, a target pH range (7.5–8.5 or 6.9–7.1) was maintained in previous studies by addition of 0.2, 0.5, or 1.0 M of HCl (Gomez Vidales et al., 2019; van Eerten-Jansen et al., 2015; Van Eerten-Jansen et al., 2012). However, a manual method of mitigating pH imbalance in MMCs by adding strong acid chemicals continuously is not practical due to cost issues and accumulation of other ions (such as Cl^- from HCl addition) in the electrolyte.

Several alternative approaches have been proposed for controlling pH swings in MES systems. One involves a vapor-fed anode that eliminates the need for a liquid anolyte and helps to alleviate the pH gradient (Baek et al., 2022a). In this system, a nitrogen gas is fed to the anode for the water oxidation reaction, and the produced H^+ is transported through the CEM, which is pressed against the anode to reduce the distance between the electrodes where H^+ and OH^- ions are produced and consumed through water ion association. This approach helps to maintain a near-neutral catholyte pH (6.6–7.2) and has resulted in a high MPR (2.9 ± 1.2 L/L-d), indicating the critical role of pH gradient control in achieving high performance in MMCs. Another approach was to continuously add CO_2 gas directly to the cathode biofilm using specialized systems, such as a ceramic hollow fiber wrapped with catalysts, to avoid the addition of liquid chemicals (Bian et al., 2021). This approach succeeded in maintaining a slightly acidic catholyte (6.33–6.91) due to CO_2 gas dissolving in the liquid and lowering the pH.

Another factor that impacts MMC performance is how CO_2 is delivered to the cathode biofilm for CO_2 fixation. Previous studies have often utilized continuous gas bubbling to provide CO_2 to the catholyte solution. However, the biofilm's CO_2 uptake may be limited due to the low solubility of CO_2 in water (0.04 mol/L under standard pressure and temperature) and, as a result, a low mass transfer rate of CO_2 into the aqueous medium (Bajracharya et al., 2016; Wu et al., 2022). In several MMC studies sodium bicarbonate ($NaHCO_3$) has been used to supply CO_2, as this approach helped to circumvent the issue of poor solubility of CO_2 in water (Mayer et al., 2019; Van Eerten-Jansen et al., 2013). However, to use MMC technologies for CO_2 utilization from flue gas or other industrial waste gasses, it is necessary to supply the CO_2 gas directly instead of as a bicarbonate. In several MES studies, CO_2 transfer efficiency was improved by using a porous hollow fiber electrode as a cathode with a bubbleless CO_2 supply (Bian et al., 2018; Fu et al., 2022). The authors reported that acetate production from MES systems using direct CO_2 delivery through pores of hollow fiber electrode was significantly higher than that from MES systems using CO_2 bubbling in the medium (Bian et al., 2018). Using a gas diffusion electrode (GDE) can be another approach to overcome low CO_2 transfer rates to biofilms. GDEs in bioelectrochemical systems enables to improving mass transfer between gas-phase reactant and liquid-phase reactant or solid electrode. Three-phase interfaces (gas-liquid-solid) can be achieved by combining hydrophobic gas diffusion layers and hydrophilic microporous electrode materials (Bajracharya et al., 2016). Several studies have reported the enhanced mass transfer rate and MES efficiency by using GDEs in their reactor configuration (Bian et al., 2018; Dessì et al., 2023).

4. Suggestions for future MMC studies

Our review and analysis of MMC studies revealed that the anode material is quite important for MMCs despite of the fact that cathode material is often the main subject of interest because that is where the biofilm is formed. For the efficient oxygen evolution reaction at the anode, the earth-abundant transition metals or noble metal-based materials can be used, including Ir, Ru, Pt, Co, Ni, and their alloys and oxides, to enhance overall electrochemical performance (Tahir et al., 2017). The overpotential, which is the extra energy required to drive an electrochemical reaction compared to its thermodynamic potential, can be reduced by using those metal catalyst-based anodes compared to carbon-based electrodes. Therefore, these catalysts which have been verified as effective to reduce the overpotential of OER in water electrolyzer can be used for the same purpose in MMCs unless biologically driven reactions by exoelectrogenic bacteria are used to produce current.

Another finding of our analysis is that defined cultures are not needed for achieving high-rate methane production in MMCs. Despite the use of different inocula the biocathode communities tend to become dominated by hydrogenotrophic methanogens (Baek et al., 2017; Cheng et al., 2009; Siegert et al., 2015; Zhou et al., 2021). The use of a diverse cathode community might also help to maintain a strict anaerobic environment needed by methanogens when oxygen is produced at the anode. Therefore, it is more important to focus on maintaining well-acclimated methanogenic activity over time without inhibition due to environmental factors such as pH imbalances or oxygen leakage into the cathode chamber. As previously discussed, pH imbalances are inevitable in most two-chamber MMCs when using an ion exchange

membrane due to the narrow pH range of 6.5–7.8 needed by most methanogens (Fang et al., 2014). Maintaining a neutral pH range and removing any potential inhibitory substances from the buffer solution or wastewater are important for the successful acclimation and stabilization of methanogenic cultures on MMC cathodes. Unlike other bioelectrochemical systems that obtain all chemical reactants for anodic biofilms from the liquid solution, like MFCs or MECs, MMCs require CO_2 for cathodic biofilm growth. The use of a GDE for supplying CO_2 from a gas stream to the MMC cathodes could benefit the biofilm structure in terms of substrate uptake rates. Nutrients and protons (if a CEM is used) would be provided from the liquid side, while CO_2 gas would be provided from the opposite side, potentially enabling a counter-diffusion pathway and promoting the growth of an active biofilm within the interior of the biofilm. (Rojas et al., 2021). Therefore, it may be more effective to control key parameters, such as pH neutralization and CO_2 supply methods, for acclimating healthy methanogenic cultures in cathodic biofilms rather than using defined cultures.

5. Conclusions

A comprehensive and quantitative analysis was conducted of the various configurations and operational factors that affect methane production in MMCs. There were weak correlations between the MPR and both cathodic potential ($R^2 = 0.56$) and current density ($R^2 = 0.50$), indicating the existence of other critical factors that impact MMC performance. Previous studies have mainly focused on cathode materials, but most of MMCs which reported high MPR have been equipped with metal catalysts rather than plain carbon-based materials for anodes. In addition, the use of a defined methanogenic culture was not necessary for achieving high MPR as the electrochemical niche can provide a selective pressure to converge a desired methanogenic community even when using an undefined mixed culture. The CO_2 supply method and pH adjustment of catholyte were also suggested as crucial factors that can improve MMC performance.

Declaration of Competing Interest

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

Data availability

The data that has been used is confidential.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2023.120657.

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