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Impact of surface area and current generation of microbial electrolysis cell electrodes inserted into anaerobic digesters

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ABSTRACT

The integration of microbial electrolysis cells (MECs) and anaerobic digestion (AD) has recently been pursued to improve methane production through a combined system (AD-MEC). Analysis of 24 different experimental results published in 13 different studies showed that the only significant correlation between increased methane generation rate and other parameters was the surface area provided by the electrodes per volume of reactor, or the S/V ratio ($R^2 = 0.63$, p -value < 0.01). Other possible relationships between hydrogen production in MECs, and methane enhancement in AD-MECs, were examined as a function of applied voltage and current density using data from previous studies. For the MECs, there was a high correlation between current density and hydrogen production rate. There was no significant relationship between methane production rate and current density in AD-MECs. A comparison of other factors between MECs and AD-MECs showed large differences in substrate concentration, substrate type (single substrates or wastewaters), and volumetric current densities. Adding electrodes increased methane production on average by 65%, but surface area was a more important contributing factor in AD-MECs than current generation. Based on our analysis of the results in these previous studies, increased area and conductive surfaces provided by the electrodes can have beneficial effects for methane production other than hydrogen gas production by increasing biomass retention and stimulating electrical syntrophy between microorganisms.

1. Introduction

Anaerobic digestion (AD) is a useful technology for bioenergy production that has been used extensively for many decades to achieve microbial oxidation of organic matter and biomethane production. Microbial electrolysis cells (MECs) have been more recently explored as a method to produce biogas using electrodes [1]. Hydrogen can be generated at the cathode by applying a small amount of voltage (>0.2 V) to a circuit with electroactive bacteria on the anode generating current and producing an additional voltage through the oxidation of organic matter. A new configuration has been used to improve methane production that consists of adding electrodes into the AD reactor so that they function as MECs. This combined system, referred to as an AD-MEC, has recently been extensively studied by many researchers to improve gas production rates and stability over time [2–6]. The operation of the MEC in ADs can increase the rate of organics oxidation and therefore also the rate of methane production [2–6]. Methane production at the cathode can occur by different electrochemical mechanisms: direct

electromethanogenesis, where microorganisms consume electrons and protons directly from the cathode or production of intermediates such as H_2 , formate and acetate followed by consumption by methanogens [7,8]. A similar range of applied voltages (E_{ap}) in MECs have been used in AD-MECs (0.2–1.0 V) [9]. Therefore, the specific applied voltage application could be a factor in performance for both MECs and AD-MECs [10].

The presence of the electrodes in AD-MECs can have beneficial effects other than current generation as the electrodes provide additional surface area for microbial adhesion and retention [11,12]. For example, the addition of a single high surface area carbon fiber brush with no current enhanced methane production to a greater extent than two smaller brush electrodes with current generation at an applied voltage of 0.8 V [11]. In another study, there was no measurable impact of current generation (applied voltages of 0.5 and 1.0 V) on methane production compared to a control reactor operated with an open circuit [12]. However, when the electrodes were removed from the control reactors methane production significantly decreased, indicating that a key factor

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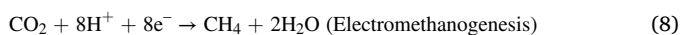
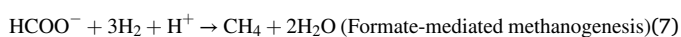
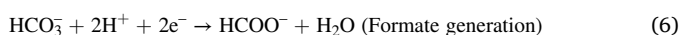
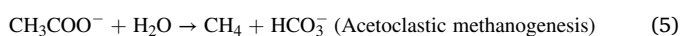
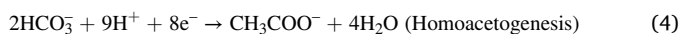
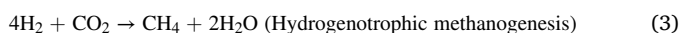
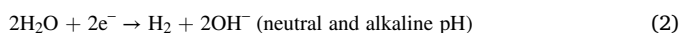
in the AD-MEC configuration was the biomass retention in the electrodes [12]. Adding electrically conductive materials can also stimulate direct interspecies electron transfer (DIET) by enriching electroactive microorganisms [13,14]. Although there are many studies looking at enhanced AD efficiency by adding electrically conductive materials, the separate roles of applied voltage, biomass retention, and other factors have not been adequately addressed.

Considering various factors that could possibly impact AD-MEC performance, it is important to identify the specific factors related to electrode addition to determine the best approaches for increasing methane generation rates in AD-MECs. While there have been several recent reviews on adding electrodes into AD, the focus was on summarizing the literature rather than using data in previous studies to provide a quantitative analysis of the impacts of the electrodes such as surface area [15–18]. Although those review papers mentioned that electrode surface area and the properties of electrodes should be further explored in AD-MEC studies, there was no direct examination of the relationship between electrode surface area and AD performance.

In order to identify the electrode-specific factors that impact performance of AD-MECs, we compiled data from many previous MEC and AD-MEC studies to quantify relationships between performance and reactor conditions. The performance of the AD-MECs was examined by considering both open and closed circuit results to investigate the effect of surface area separate from that due to current generation, a relationship which was not highlighted in previous reviews [15–18]. We first compared the operational conditions in MECs and AD-MECs, and then compiled and analyzed data from many previous MEC and AD-MEC studies to explore possible relationships between biogas production rate and applied voltage, current density, and surface area-to-volume ratio of the electrodes. For MECs, we examined hydrogen gas production, while for AD-MECs we looked at methane production and whether hydrogen production by MECs played a significant role in AD-MECs. Finally, we analyzed possible reasons for different patterns observed in MECs and AD-MECs with several reactor design and operating conditions. The goal of this analysis was to identify the importance of current generation relative to other operational conditions in AD-MECs such as electrode surface area for improving performance.

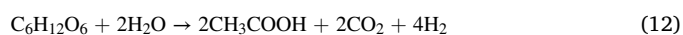
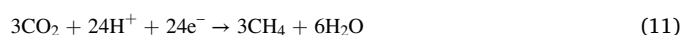
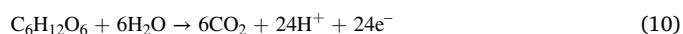
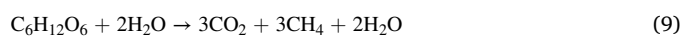
2. Factors that can impact methane-production in AD-MECs

AD-MECs are designed to increase the methane production rate and yield by inserting a set of electrodes as done in MECs into AD reactors and applying external voltage across the electrodes to generate current from the oxidation of organic matter. In MECs, the main gas product is H₂ gas, which is produced from electrons and protons at low pHs (Eq. (1)) or from water dissociation at neutral to higher pHs (Eq. (2)). In AD-MECs, any hydrogen production is rapidly followed by its conversion to methane by hydrogenotrophic methanogens (Eq. (3)), methane can be also produced by using other indirect mediators like acetate (Eqs. (4) and (5)) or formate (Eqs. (6) and (7)), or direct electromethanogenesis (Eq. (8)) at the cathode.



The activities of exoelectrogenic bacteria at the anode, and

electrotrophic or hydrogenotrophic methanogens at the cathode are thought to play an important role in AD-MEC efficiencies [19]. Theoretically, more electrons produced at the anode would yield more hydrogen (or methane) at the cathode if water is split at the anode. However, if the anodic potential is not sufficiently positive to enable water splitting (or chlorine evolution from chloride ion oxidation), the ultimate methane yield will be the same for either direct substrate conversion to methane or through H₂ evolution from the cathode. For example, direct conversion of glucose produces 3 mol of methane (Eq. (9)) and complete glucose oxidation at the anode to produce current (Eqs. (10) and (11)) or glucose fermentation to acetate and H₂ (Eq. (12)), followed by acetate oxidation to current and H₂ (Eq. (13)), will ultimately produce the same amount of methane.



If the only impact of adding electrodes into AD-MECs is hydrogen gas production by current generation, then current density in an AD-MEC could improve methane production rate in accordance with the rate of hydrogen production.

There are possible impacts of adding electrodes into AD-MECs other than current generation (Fig. 1). First, insertion of the electrodes will provide large surface area for microbial adhesion and retention. The addition of inert materials into bioreactors is frequently used to facilitate microbial adhesion to these surfaces and thus increase the solids retention time (SRT) of the microorganisms in the reactors [20–22]. Biofilm formation onto the support materials, such as polyurethane foam, biochar, and sand in various bioreactors has promoted reactor efficiencies in terms of higher final product yield and shorter start-up periods by maintaining higher biomass concentrations [22]. The additional retention of biomass using a biofilm-based technology is particularly beneficial for increasing the retention of slow-growing microorganisms such as methanogens [23]. Support materials are used in many different processes such as fixed bed reactors (FBR), anaerobic fluidized bed reactors (AFBR), and anaerobic packed bed reactors (APBR) [24]. The electrodes in AD-MECs therefore also function as support materials because the electrodes are held in the solution by wires. An important property of the electrode materials, in addition to electrical conductivity, is the bioadhesion properties of the material. Roughness, hydrophilicity/hydrophobicity, porosity, and surface energy can impact the rate and extent of biofilm formation on a surface [25]. The materials typically used for bioelectrodes in AD-MECs, such as graphite felt, graphite brushes, and carbon cloth, all have high porosities, roughness, and are hydrophilic which provide high biocompatibility and thus increases biomass retention.

Another factor relevant to AD-MECs is DIET due to the use of electrically conductive materials compared to non-conductive materials that can be added into conventional biofilm-based anaerobic reactors. DIET is a newly discovered mechanism based on the extracellular electron exchange by physical contact between microorganisms, which is an alternative route for indirect interspecies electron transfer (IIET) based on chemicals such as H₂ and formate [14]. In DIET, electron transfer between different microbial groups is achieved without indirect redox mediators (H₂ and formate). DIET can occur via biological electrical connections using the electroactive proteins (e.g., cytochrome and e-pili) or via electrically conductive materials as non-biological electric conduits [14]. Since several steps including metabolite generation and transport in solution are not required for DIET, it is thought to improve methanogenesis rates. The addition of conductive carbon- and iron-based materials could improve AD performance by enabling electrical

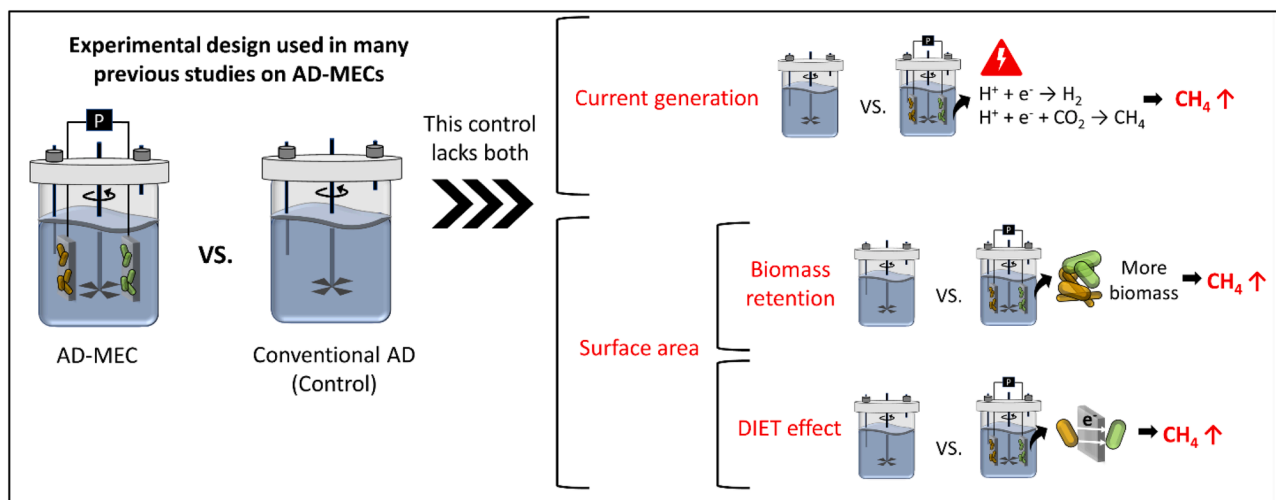


Fig. 1. The experimental design used in many previous studies on AD-MECs and possible impacts of adding electrodes into AD (biomass retention and DIET) when comparing the performance with a conventional AD reactor.

Table 1

Summarized results of previous AD-MEC studies. The table is organized by substrate type and then by substrate concentration.

Substrate	Sub con. (g COD/L)	A_s (m ² /m ³) ^a	E_{ap} (V)	I_v (A/m ³)	Q_{CH_4} (m ³ /m ³ /d)	M^b (%)	Control ^c	Ref.
Defined and synthetic wastewaters								
Acetate	1.6	3.7	0.5	3.1	0.05	74	-E	[29]
Acetate	1.6	3.7	1	72.2	0.08	110	-E	[29]
Acetate	1.6	3.7	1.5	2.9	0.03	38	-E	[29]
Acetate	2.5	35.3	-0.9 ^d	10.0	0.04	384, 752 ^e	-E, +E-C	[27]
Glucose	2.1	3.7	0.5	5.8	0.09	18, 15 ^e	-E, +E-C	[2]
Glucose	2.1	3.7	1.0	19.0	0.11	30, 27 ^e	-E, +E-C	[2]
Glucose	2.1	3.7	1.5	2.4	n.a.	18, 16 ^e	-E, +E-C	[2]
Glucose	2.1	8.6	1.0	51.5	0.05	91	-E	[30] ^f
Glucose	4.3	8.6	1.0	44.4	0.15	143	-E	[30] ^f
Glucose	8.5	8.6	1.0	52.8	0.17	36	-E	[30] ^f
Glucose, starch, beef extract, xylose and cellulose	15.4	3.0	0.8	0.9	0.15	33	-E	[5] ^g
Glucose, starch, beef extract, xylose and cellulose	15.4	3.0	0.8	0.9	0.16	27	-E	[5] ^g
Syn. brewery wastewater	5.8	2.3	0.1	1.4	n.a.	23	-E	[3]
Real wastewaters								
Food waste	14.4	20.0	0.3	9.9	0.04	87	-E	[31]
Food waste	60.3	13.5	0.3	n.a.	0.61	68	-E	[32]
Food waste	63.0	3.0	0.3	n.a.	n.a.	70	-E	[33]
Waste activated sludge	15.7	1.3	0.8	n.a.	0.07	115	-E	[28]
Waste activated sludge	36.7	1.5	0.6	24.9	0.09	30, 15 ^e	-E, +E-C	[34]
Waste activated sludge	114.2	0.7	0.3	4.3	0.21	23, 16 ^e	-E, +E-C	[35]
Swine manure	19.6	7.2	0.1	n.a.	0.12	30, 9 ^e	-E, +E-C	[6]
Swine manure	19.6	7.2	0.3	n.a.	0.12	28, 8 ^e	-E, +E-C	[6]
Swine manure	19.6	7.2	0.5	n.a.	0.11	19, 0 ^e	-E, +E-C	[6]
Swine manure	19.6	7.2	0.7	n.a.	0.12	37, 16 ^e	-E, +E-C	[6]
Swine manure	19.6	7.2	0.9	n.a.	0.11	25, 5 ^e	-E, +E-C	[6]

^a Calculated by the cathode projected surface area to the liquid volume of reactor.

^b Increase in methane production in AD-MECs compared to each control without electrodes.

^c -E, AD without electrode; +E-C, AD with electrode but without current (open circuit operation).

^d Cathode potential was controlled versus Ag/AgCl electrode.

^e Increase in methane production in AD-MECs compared to each open-circuit control reactor with electrodes.

^f Different initial substrate concentration was used.

^g Stainless-steel with different mesh size was used as cathode materials.

conduits between microorganisms for DIET [13]. DIET could be occurring in AD-MECs due to use of electrically conductive materials as electrodes even in the absence of external voltage application and current production.

To reveal the actual significant role of MECs in AD, the factors of current generation, applied voltage, and surface area-to-volume ratio in MECs and AD-MECs were explored for their impact in each system by using data in many different studies. Then we investigated if the importance of each factor in MECs can be translated in AD-MECs.

3. Analysis methods

The data was collected from recent AD-MEC studies (published in 2015–2021) or MEC studies (published in 2009–2021) where cathode projected surface area, applied voltage, volumetric current density, and CH_4 (or H_2) production rate were available. Only AD-MEC studies which had controls without electrodes were included in the analysis in order to calculate an increase in methane production due to the presence of the electrodes (with or without current). The MECs and AD-MECs analyzed for this study can be mainly differentiated by the final product, which is H_2 for MECs and CH_4 for AD-MECs.

To identify the factors that impacted hydrogen production rates (Q_{H_2}) in MECs, data were compiled from 90 experimental results published in 37 different MEC studies using data on the ratio of electrode (cathode) projected surface area to the liquid volume of the reactor (S/V ratio), applied voltage (E_{ap}), and volumetric current density based on the liquid volume (I_V) (Table S1). The methane production rates (Q_{CH_4}) and an increase in methane production (M) in AD-MECs were examined as a function of the same factors used for the MEC analysis for 13 different studies ($n = 24$ experimental results) (Table 1). The M refers to an increase in methane production in AD-MECs compared to each control AD without electrodes. The inoculation and operation conditions are slightly different for MECs and AD-MECs. MECs were mostly inoculated with the effluent or anode biofilm from previously operated microbial fuel cells (MFCs) or MECs, while AD-MECs were mostly inoculated with

anaerobic sludge (initial biomass concentration of 5,900–33,700 mg/L of volatile suspended solids). The MECs were operated at room temperature or mesophilic temperature (30–35 °C) (except for one study operated at 4 °C) [26] while AD-MECs were operated under mesophilic condition (35–38 °C) except for two studies (10 °C [27], and 20 °C [28]). For both MEC and AD-MEC studies, the initial pHs were adjusted to 7.0–7.2 and thereafter maintained at near neutral pH using a phosphate buffer solution except for the reactors fed with real wastewaters (e.g., food waste and waste activated sludge). Most of the studies analyzed here were operated under fed-batch modes. Continuously operated reactors had much shorter hydraulic retention time in MECs (3.0–6.7 h) than AD-MECs (20 d). The construction and operating conditions other than those described above are summarized in Table 1 (AD-MECs) and Table S1 (MECs).

4. Possible correlations between biogas production rate and operation factors

4.1. MEC studies

The highest hydrogen production rate (5.4 $\text{m}^3/\text{m}^3/\text{d}$) was obtained at the largest values of S/V (100 m^2/m^3) and volumetric current density (460 A/m^3), and the second highest rate at an applied voltage of 1.1 V. Among the variables examined, the only significant relationship obtained was between I_V and Q_{H_2} ($R^2 = 0.72$, p -value < 0.01; Fig. 2C). There were no significant correlations between the amount of electrode area used in the reactor (S/V ratio) ($R^2 = 0.08$; Fig. 2A), or the voltage added into the circuit (E_{ap}), and Q_{H_2} ($R^2 = 0.14$; Fig. 2B). On average, the hydrogen production rate was 0.8 ± 1.0 $\text{m}^3/\text{m}^3/\text{d}$, the current density was 124 ± 99 A/m^3 , reactor size was 51 ± 76 mL, the S/V ratio was 29 ± 21 m^2/m^3 , and the average applied voltage was 0.7 ± 0.2 V in these studies.

Increasing the applied voltage should increase current (up to some limiting current), and thus the hydrogen production rate, in any single MEC where other conditions remain unchanged [1,36–38]. However,

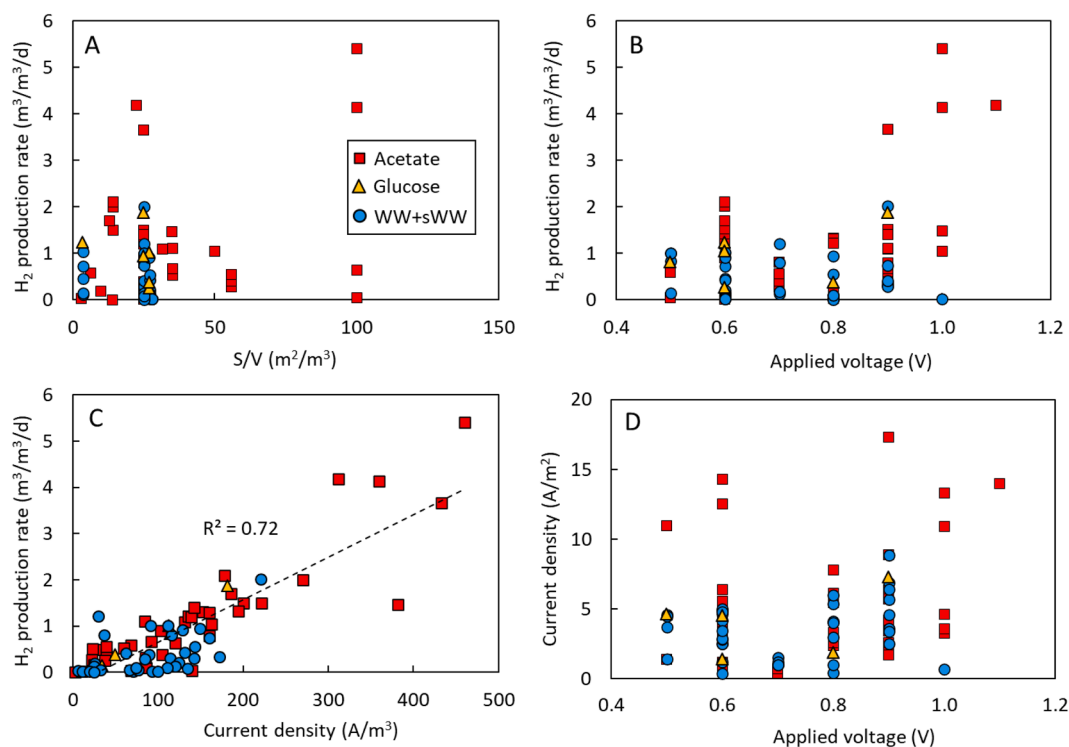


Fig. 2. Comparisons of hydrogen production rate in MECs with acetate (squares), glucose (triangles) and real or synthetic wastewaters (WW + sWW; circles) as a function of (A) surface area-to-reactor volume ratio, (B) applied voltage, and (C) current density. (D) The relationship between applied voltage and current density.

when current density based on the cathode projected surface area (I_A) was plotted as a function of E_{ap} for these 90 experimental results, there was no significant correlation between these two parameters (Fig. 2D). The lack of a correlation between E_{ap} and I_A was likely due to the different internal resistances of the MECs. Large variations in internal resistance of the MECs preclude possible relationships between E_{ap} and I_A for different MECs because the current that results from an applied voltage will vary as a function of the internal resistance. The internal resistance is consisted of ohmic, charge transfer, and mass transfer resistances [39]. The importance of the internal resistance can be seen by calculating current for the same applied voltages for MECs with different internal resistances (Fig. 3). Current was calculated from internal resistance using Ohm's Law, or $I_A = E_{ap}/R$, where I_A is the current density (A/m^2), and R is the internal resistance ($m\Omega m^2$). For any single internal resistance, the calculated current increases with the applied voltage (Fig. 3). However, current density produced varies substantially at each applied voltage due to differences in internal resistances. For example, at 1.1 V the current density is 3.1 A/m^2 for an MEC with 360 $m\Omega m^2$ of internal resistance, compared to 18.3 A/m^2 for an MEC with 60 $m\Omega m^2$ of internal resistance. Unfortunately, it was not possible to determine the internal resistance of these different MEC studies as polarization data were not routinely reported, and typically only a single applied voltage was used in these studies.

4.2. AD-MEC studies

No significant correlation was obtained between methane production rate and applied voltage or the electrode area per volume ($R^2 < 0.11$) (Fig. 4A and 4B). The highest methane production rates did not occur for the highest current densities (Fig. 4C), suggesting current density was not the main factor in methane production rate, and there was no relationship between the current density and the applied voltage (Fig. 4D). The same analysis conducted with only the defined and synthetic wastewaters listed in Table 1 also did not show any significant correlations between these variables (Fig. S2).

5. Analysis and discussion

The average operational conditions in the AD-MECs differed from those in the MECs except for the use of similar applied voltages (0.7 ± 0.4 V in the AD-MECs compared to 0.7 ± 0.2 V for the MECs). The lack of a relationship between E_{ap} and I_A for the AD-MECs (Fig. 4D) was likely a result of large differences in internal resistances as concluded to be the case also for the MECs [40]. The highest gas production rates were not obtained at the highest S/V ratio, applied voltage, or current density, as was observed for MECs, suggesting that the most optimal conditions for H_2 gas production due to the electrodes were not translated into overall

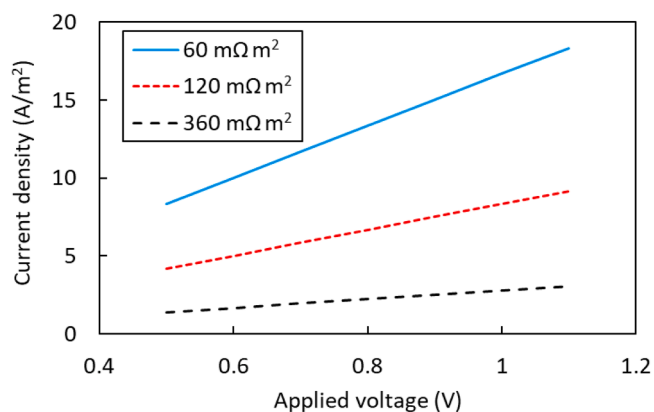


Fig. 3. Theoretical relationship between applied voltage and current density depending on the internal resistance of MEC reactors.

methane production rates in AD-MECs. Substrate concentrations and types of substrates were quite different in AD-MECs and MECs, and therefore the impacts of substrates and overall increases in methane production required further analysis to understand the impact of using electrodes in AD.

5.1. Impact of surface area on increased methane production in AD-MECs

The S/V ratios used in AD-MEC studies of 7 ± 7 m^2/m^3 (range of 0.7 – 35 m^2/m^3) were much lower than those in MEC studies of 30 ± 22 m^2/m^3 (range 3 – 100 m^2/m^3) (Fig. 5). This difference in S/V ratio is partially due to a larger reactor volume used for AD-MECs (2 ± 5 L, range 0.02 – 20 L) than that for MECs (51 ± 76 mL, range 5 – 500 mL) as S/V ratios decline with increases in bioelectrochemical reactor size (Fig. 5) [41]. A lower S/V ratio used in AD-MECs could explain the lower volumetric current density observed and thus a lack of a correlation between I_V and Q_{CH_4} .

Many previous studies have reported increases in AD performance by combining MECs with AD, with the implication that performance was due to current generation alone. However, the added surface area of electrodes that enabled more biomass to be retained in the reactor could also have been a factor in performance. Thus, we examined the increase in methane production and the amount of surface area added relative to the volume of the reactors. The increase was calculated by comparing methane production rate or yield (if rate is not applicable) between AD-MECs and control AD reactors without electrodes. The increased methane production showed a significant correlation with the S/V ratio ($R^2 = 0.63$, p -value < 0.01) (Fig. 6A). In contrast, there were no significant relationships between the calculated increase and either applied voltage or current density (both with $R^2 < 0.03$) (Fig. 6B and 6C). The finding of a significant correlation between electrode surface area, but not volumetric current density, suggests that current production due to the MECs was not as a crucial factor in improving methane production as the electrode area provided for enhanced retention of biomass on the electrodes [3,28].

The possible impact of electrode materials other than carbon on methane production efficiency was also investigated. There were five studies (a total of six data points) where cathode materials were used that were not plain carbon, that were titanium, stainless steel, copper, or carbon electrodes coated with nickel or platinum. However, there was no trend between the electrode type and the increase in methane production for these other electrodes (Fig. S1).

5.2. Impact of current and H_2 production on methane generation rates

The larger AD-MECs (2 ± 5 L, compared to 51 ± 76 mL for MECs) had volumetric current densities that were an order of magnitude lower on average with $I_V = 20 \pm 24$ A/m^3 (1 – 72 A/m^3) for the AD-MECs compared to $I_V = 124 \pm 99$ A/m^3 (0.02 – 460 A/m^3) for the MECs. The much lower current densities achieved in AD-MECs than those in MECs could have been a factor in the lack of a significant correlation between I_V and Q_{CH_4} in AD-MECs (Fig. 4C). Examination of the data for MECs over a similar current density region (0 – 80 A/m^3) as that reported for AD-MECs also showed no correlation between hydrogen production rate and current density (Fig. S3). Lower current densities in AD-MECs would necessarily produce less hydrogen production, and thus less methane production, than that possible if the reactors had electrode areas per volume similar to those used in MECs.

The maximum possible impact of current production from MECs on methane production in AD-MECs can be calculated from the measured current, assuming complete conversion of current to hydrogen gas. The observed methane production rate, Q_{CH_4} , was compared with the rate that could be theoretically achieved from current production by MECs ($Q_{CH_4, MEC}$). The $Q_{CH_4, MEC}$ was calculated based on the volumetric current density reported from each study according to:

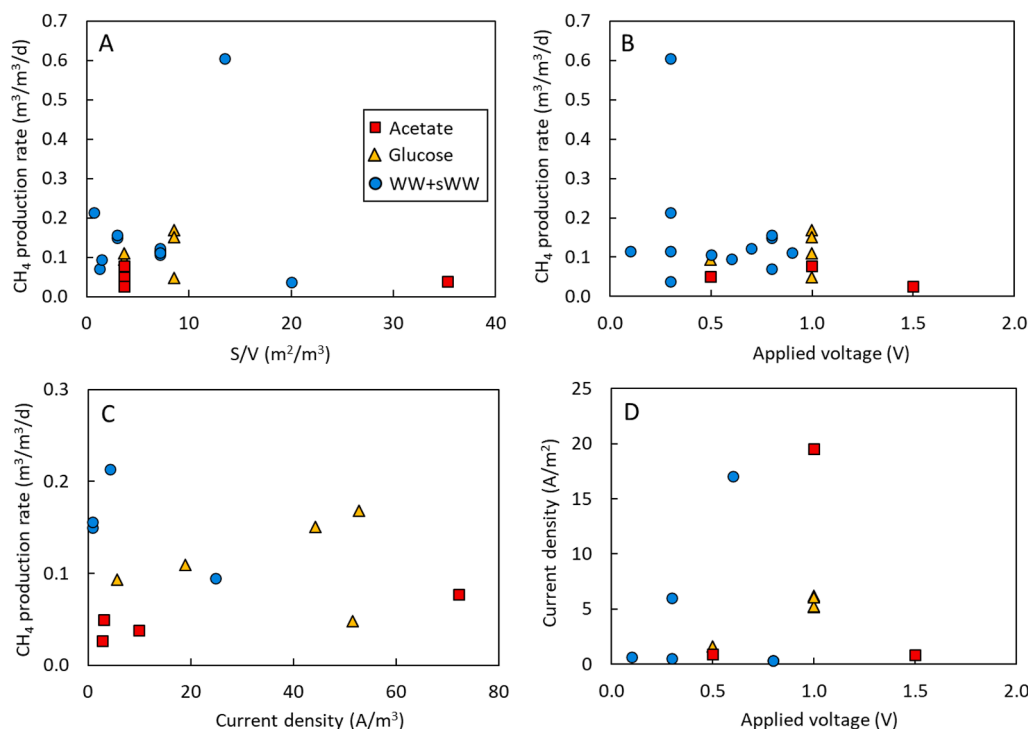


Fig. 4. Comparisons of methane production rate in AD-MECs with acetate (squares), glucose (triangles) and real or synthetic wastewaters (WW + sWW; circles) as a function of (A) surface area-to-reactor volume ratio, (B) applied voltage, and (C) current density. (D) The relationship between applied voltage and current density.

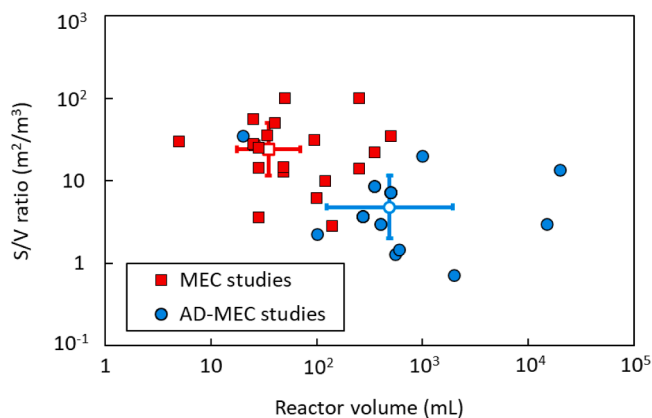


Fig. 5. The surface area-to-volume ratio used in previous MEC and AD-MEC studies as a function of reactor volume. Blank markers represent the average values of each group.

$$Q_{CH_4,MEC} = \frac{86.4I_V}{F c_g n_{CH_4}} \quad (12)$$

where 86.4 is for unit conversions, F is Faraday's constant ($F = 96,500C/mol e^-$), c_g is the concentration of a gas at a temperature T calculated using the ideal gas law (mol/L), and n_{CH_4} is the number of electrons consumed for methane production (8 electrons per CH₄). Based on the average volumetric current density of 20 A/m³, the theoretical gas production would be $Q_{H_2} = 0.24 m^3/m^3/d$ for hydrogen and $Q_{CH_4,MEC} = 0.06 m^3/m^3/d$ for methane, resulting in only 48% of the average $Q_{CH_4} = 0.13 \pm 0.12 m^3/m^3/d$. Due to this low amount of current and gas production from the MECs, it would not be possible to identify a specific impact of the MECs on the methane generation rate.

To see if there was a trend in methane generation rates based on methane that could be produced from current using MECs, the methane production rate was calculated from current using Eq. (12) for the 13

different AD-MEC studies. However, the resulting gas rates showed no significant trend relative to methane production rate (Fig. 7A), suggesting that hydrogen production based on the current produced did not directly impact the overall methane production in AD-MECs. In addition, there was no significant correlation found between the ratio of the methane production rate due to current generation to total methane production rate, as a function of the total methane production rate (Fig. 7B). Except for two points which showed a very high percentage increase in methane produced because of current generation (266% and 307%), the remaining data indicated that current generation contributed $43 \pm 34\%$ (2–89%) to total methane production. The two very high ratios in methane production could have been a result of other reactions than organics oxidation and hydrogen production, such as hydrogen cycling or cathode corrosion that have been observed to increase current densities in MECs [42,43]. Thus, it was clear that adding electrodes contributed to improved methane generation rates in each study, but there was no relationship between methane production and that increased due to hydrogen production based on the reported current densities.

5.3. Impact of substrates used in AD-MECs on current generation

The current densities in AD-MECs were lower than those in MECs despite the use of higher substrate concentrations in AD-MECs. The substrate concentrations averaged 19 ± 26 g COD/L in AD-MECs compared to 2 ± 3 g COD/L in MECs, although the ranges of substrate concentrations in both studies was quite large (2–114 with a median of 20 g COD/L for AD-MECs; 1–15 g/L with a median of 1 g COD/L for MECs) (Fig. 8). If substrate concentration was a crucial factor for current generation by MECs, higher (not lower) current densities should have been observed in AD-MECs than MECs. The type of substrate used in AD-MECs could have been a more important factor than substrate concentrations. In the MEC studies, 52% of experiments used acetate as the substrate which is an optimal fuel for current generation by exoelectrogenic microorganisms in MECs, and 88% (79 out of 90 data points) of experiments were conducted with defined or synthetic wastewaters

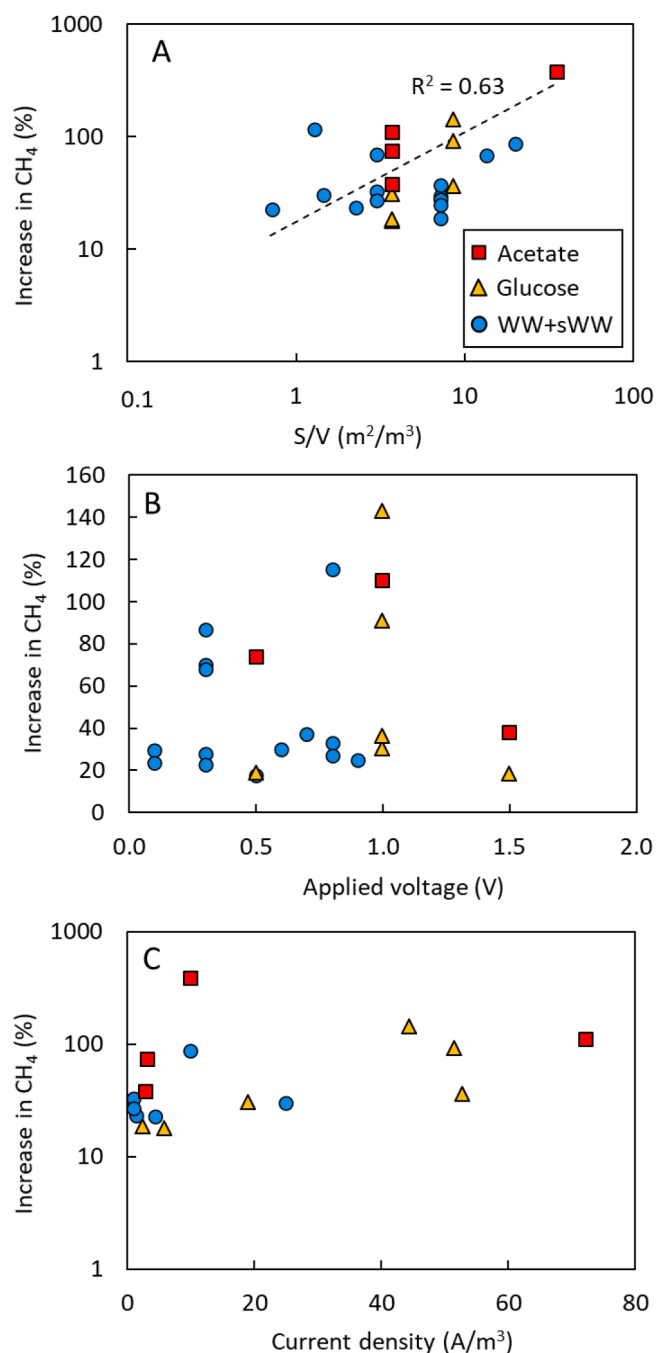


Fig. 6. Comparisons of increases in methane production using AD-MECs compared to controls (no electrodes) for acetate, glucose and real or synthetic wastewaters (WW + sWW) as a function of (A) surface area-to-reactor volume ratio, (B) applied voltage, and (C) current density.

(Table S1). However, in the AD-MEC studies, very few studies (17%) used acetate and about half (54%, 13 out of 24 studies) used defined or synthetic wastewaters (Table 1). The use of complex wastewater will result in slower degradation rates of organic matter and thus likely produce lower concentrations of fermentation products such as acetate. The microbial conversion of organic matter into volatile fatty acids (VFAs) other than acetate, such as propionate and butyrate, would result in lower current densities compared to MECs fed with acetate [44]. Thus, lower current densities in AD-MECs could have resulted from the low concentrations of substrates more optimal for electricity generation in MECs such as acetate.

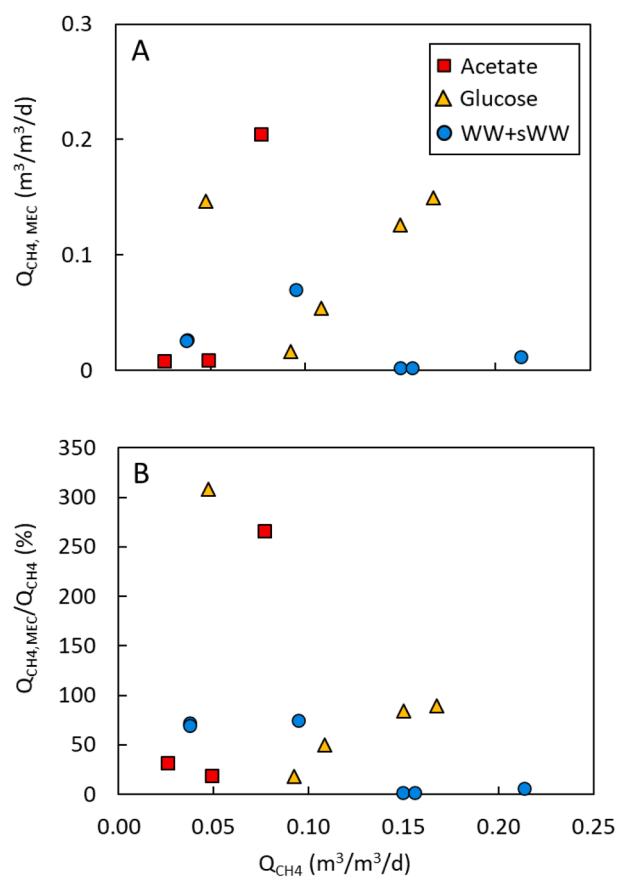


Fig. 7. (A) The calculated methane production rate based on current density and (B) the contribution methane production from current generation on overall methane production as a function of the observed methane production rate.

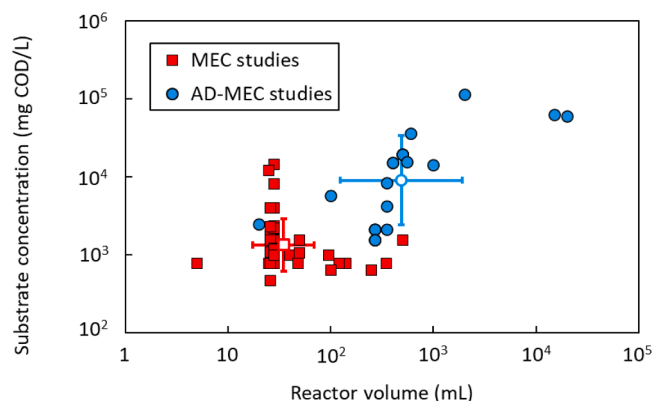


Fig. 8. Substrate concentration used in previous MEC and AD-MEC studies as a function of reactor volume. Blank markers represent the average values of each group.

6. Implications

The overall results of this study indicated that providing high surface area electrodes into the AD system, by adding in electrodes that produced high S/V ratios, was an effective strategy to increase methane production in AD-MECs by 65% compared to conventional ADs. This increase was correlated with surface area of the electrodes but not with the current produced. Despite of the importance of surface area, methane production from AD-MECs has been frequently compared only

with a control reactor without electrode materials (-E) in many of the previous studies (Table 1), which hinders distinguishing the impact of current generation from that of electrode surface area. Our results suggest that a control reactor with the same S/V ratio and same electrode materials should be always included in the experimental design to identify the contribution of surface area separate from current generation. There have been a few studies that used two controls, an AD reactor without electrodes (-E) and an AD reactor with electrodes but operated with an open-circuit mode so in the absence of current (+E-C) [2,6,27,34,35]. In all of these studies with both types of controls, except for one [27], the open-circuit AD-MEC reactors yielded smaller increases in methane production than conventional AD reactors compared to the AD-MEC reactors (Table 1). Higher methane production from open-circuit AD-MECs than conventional AD reactors suggests that an insertion of electrodes itself is beneficial despite of the absence of current production.

Simply increasing the amount of surface area for biofilm growth could be a better strategy to improve AD efficiency than current generation based on inserting MECs, especially when wastewaters with high organic concentration are treated. The strategy of adding surface area to improve methane production rates is well known based on the use of conventional anaerobic biofilm-based reactors, which have inert carriers that induce an attached growth to the inert materials and thus help to increase the retention of biosolids [45,46]. The area of packing added into these biofilm-based reactors have been higher than those used in the AD-MECs. For example, synthetic protein and carbohydrate waste was treated in up-flow anaerobic packed bed reactor using polyvinyl chloride (PVC) rings which had S/V ratios of 132–187 m²/m³, compared to an average of 7 m²/m³ here [47]. Although these packed bed reactors had a lower input organic concentration (10 g COD/L) than that in the AD-MECs studies examined (19 g COD/L), the methane production rate were much higher with Q_{CH_4} of 2.0–3.4 m³/m³/d, likely due to these much higher S/V ratios than those used in AD-MEC studies. In another study using an anaerobic fixed film reactor with PVC rings (S/V ratio of 224 m²/m³) with 25 g/L of COD, the methane production rate ranged from 0.14–1.57 m³/m³/d [48] compared to 0.03–0.61 m³/m³/d in AD-MEC studies. These packed bed studies show that much higher methane generation rates are possible using inert packing materials than using electrodes with current generation in AD-MECs (an average of 0.13 m³/m³/d).

Improving performance and evaluation of AD-MECs. Based on the analysis here, several steps could be taken to improve the performance and evaluation of AD-MECs. First, it is suggested that electrodes inserted into AD have high biocompatibility for biofilm formation, as well as large surface area, to maximize the potential benefits of additional biomass as well as current generation and hydrogen production from MEC electrodes. The amount of surface area (S/V ratio) and materials used for the electrodes in MECs have been recognized as a way to improve H₂ production efficiency, for example by using high surface area brush electrodes, as more electrode area will provide more current per reactor volume [49–51]. A similar approach of high surface area electrodes is therefore necessary in AD-MECs to maximize benefits from current generation, but for AD-MECs the S/V ratio is also important for biomass retention of methanogens. The biocompatibility of the electrode is therefore a crucial factor for enhancing biomass retention in AD-MECs. These materials should be beneficial for increasing microbial adhesion, not be made of materials that could be toxic to microorganisms, and also be resistant to corrosion [52]. Our conclusion in this regard relative to all types of AD additives is consistent with another report that emphasized the importance of the biocompatibility of additives for methane production efficiency as a consequence of biomass retention [53]. Additional studies that further focus on the effect of electrode properties in terms of biocompatibility could help to improve the contribution of electrodes in AD-MECs beyond that obtained due effects only related to current generation.

Second, it is important to analyze the contributions of biofilm and

suspended biomass, due to surface area and current, separately from that of overall methane production by the suspended bulk sludge. If the impact of biomass retention by electrodes is crucial, then methane production from the biofilm should be comparable to (or higher than) the theoretical methane production calculated based on the current generation. Although it can be difficult to completely separate the methane production from biofilm and suspension during operation of the reactors, the efficiency of AD-MECs before and after removal of electrodes could be examined using the same reactor (assuming steady state conditions). The difference between two could be attributed to the electrodes, and therefore this could enable methane production to be separated into categories of biomass- and current-derived contributions by calculating theoretical methane production from the amount of H₂ gas that would be produced through current generation.

7. Conclusions

Highly variable hydrogen production rates in MECs and methane production rates in AD-MECs resulted from difference in substrate concentrations, different substrate types, internal resistances of the electrode systems, and S/V ratio. Overall, the volumetric current densities produced in AD-MECs were much lower than those demonstrated in MECs. While current density was significantly correlated with hydrogen production rate in MECs with all these differences in conditions, there was no significant relationship between methane production rate and current densities in AD-MECs. Methane production improved by 65% in AD-MECs compared to conventional ADs but this increase was only significantly correlated with surface area of the electrodes and not with the volumetric current density. These findings indicate that the current generation in the ADs due to the MEC electrodes was not critical for improving methane production compared to the more important impact of the electrode surface area on methane production through improved biomass retention. The overall results suggest that AD-MECs should have a high surface area of electrodes to maximize their methane-producing efficiency rather than applying high potential.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2021.131281>.

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