Using Acetylene as a Low-cost and Effective Methanogenesis Inhibitor in Single Chamber Microbial Electrolysis Cells

By

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Abstract

Microbial electrolysis cells (MECs) for hydrogen production exhibit great advantages over many other biohydrogen production techniques in terms of versatility of substrate and hydrogen yield. However, hydrogen scavenging by methanogens put forward a great challenge to the application of the single chamber MECs when using mixed culture. Various strategies were developed against methanogens but with limited effect or challenges to practical application. This study demonstrated that the presence of 5-10% (v/v) acetylene in headspace can successfully inhibit methanogenesis in MECs using both acetate and glucose as substrates. While 0.1% of acetylene in the headspace was not effective for inhibiting methanogens, 1% acetylene somewhat suppressed methanogens when acetate was used as substrate. Acetylene at 5% and 10% demonstrated superior methanogen inhibition effects over the classic methanogen inhibitor, 2-bromoethanesulfonate (BES) at 5 mM. The acetylene inhibition was specific to methanogens. No evidence of inhibition against exoelectrogens was found, compared to BES and no treatment controls. MEC performance with diluted wastewater was also explored, but more must be done to avoid swings in pH during batch-operation. Our cost analysis suggests that utilizing acetylene is a cost-effective way of inhibiting methanogens in MECs, indicating its great potential for practical application.

Introduction

Hydrogen is a promising energy carrier due to its properties of high energy density and clean combustion by-products. Hydrogen combustion has an advantage over combustion of other energy carriers, like methane, in that it has no carbon dioxide emissions and results in only water vapor and heat. Hydrogen is industrially produced via steam reformation of natural gas or via water electrolysis, however these methods are expensive and energy intensive. Global interests in optimizing hydrogen production to make it more economically and environmental friendly are growing.

Microbial electrolysis cell (MEC) is an emerging technique in hydrogen recovery, whereby current-generating bacteria called exoelectrogens (such as *Geobacter sulfurreducens* and *Shewanella oneidensis*), using an additional supply of energy, are used to evolve hydrogen at the cathode of an electrode assembly [1,2]. MEC for hydrogen production has the advantages of versatility on substrate breakdown and higher hydrogen yield, compared with dark fermentation[3–5]. Though electricity is required for microbial electrolysis, the electricity consumption is only a fraction of that required for water electrolysis [6]. The feasibility of using MECs to harvest hydrogen from inexpensive materials, like wastewater and lignocellulosic hydrolysate increase the potential for practical application [7–12].

MECs could also provide a benefit to wastewater treatment by simultaneously reducing the biological oxygen demand. Wastewater treatment accounts for 3% of electricity consumption in the US, a substantial portion of which is associated with aerating the wastewater to degrade organic substrates. Harvesting a portion of the internal chemical energy of wastewater could result in savings for wastewater treatment. The membrane-less single chamber MEC design further reduces operational and constructional cost and reduces internal resistance, improving economic feasibility and electrochemical performance [13].

However, the single-chamber design lends itself to substrate scavenging by acetotrophic methanogens and hydrogen scavenging by hydrogenotrophic methanogens, reducing the Coulombic recovery and cathodic hydrogen recovery respectively [1,6,14–16]. Methanogens are ubiquitous in mixed, anaerobic cultures, serving as hydrogen sinks in the anaerobic digestion of complex polymers. Various strategies of methanogenesis inhibition have been applied to single chamber MECs with either limited effect or challenges for practical application [17]. Conditions with high heat, low pH or short hydraulic retention time failed to suppress methanogenesis [6,18]. 2-bromoethanesulfonate (BES), low temperature at 4℃, UV radiation, chloroform and actively harvesting hydrogen were reported as effective methanogenesis inhibition strategies, but increased cost or difficulty of operation [4,19–22]. Studies have shown acetylene to be an effective inhibitor of methanogenesis in marine sediments, landfill cover soil, anaerobic digesters and fish intestines [23–26]. However, no studies have investigated the effectiveness of acetylene on inhibiting methanogenesis in MECs. It is also not clear if the addition of acetylene can inhibit or affect the activities of exoelectrogens on the MEC anode.

In this study, we investigated the feasibility of using acetylene to inhibit methanogenesis in single chamber MECs. The focus of the study was to compare the frequency of treatment, in this case 5 mM BES and various concentrations of acetylene, required to inhibit methanogenesis and to discover the lower and upper limitations of acetylene. The inhibitory effectiveness of acetylene was also investigated under different substrate conditions, including acetate, glucose, and wastewater. Results demonstrate that periodically injecting 5% and 10% of acetylene to headspace is more effective than 5 mM BES in inhibiting methane production without affecting current and hydrogen production, demonstrating the potential of this approach for practical applications.

Materials and methods

Fermentation trials

Preliminary trials to investigate the effectiveness of various methanogenesis inhibition methods in fermentative conditions were performed. These included heat shocking, 5 mM BES, and acetylene at concentrations of 0.1%, 1.0%, and 5.0%. A control group (no inhibition) was included. Each treatment was performed in triplicate. Serum bottles of total volume of 120 ml contained 50 ml of media consisting of 5 ml anaerobic digestion sludge from a local wastewater treatment plant as the inoculum, 11 mM glucose and 100 mM sodium phosphate buffer with necessary minerals and vitamins as reported previously [27]. Heat shocking the inoculum was performed by submerging a tube of anaerobic digestion sludge in a hot water bath at 70 ˚C for 10 minutes. This was performed only once at the beginning of the study. Media was replaced inside of an oxygen-free glovebox to avoid exposing methanogens to oxygen. Media was also centrifuged at 4,000 rpm for 10 minutes to recover the biomass and solids. The bottles were sealed with rubber stoppers and aluminum crimps before purging with nitrogen for 10 minutes to achieve anaerobic conditions. Gas production was measured with a 30 ml gas displacement syringe and composition was analyzed using gas chromatography every 24 hours.

Construction of single chamber MECs

Single chamber MECs were constructed to investigate the effect of acetylene on methanogens and exoelectrogens. The MECs were made from glass narrow mouth media bottles sealed with butyl septum topped caps (VWR International, LLC). Total reactor volumes were 320 mL with a liquid volume of 100 mL and a gas volume of 220 mL. The cathode was stainless steel mesh (200*200 Mesh, 304 Stainless steel woven wire cloth, McMASTER-CARR) with a projected surface area of 50 cm^2 and the anode was plain carbon cloth (Type-B, fuelcellearth.com) with a

projected surface area of 10 cm^2. Two electrodes were separated by a layer of J-cloth (First Brands Corporation, USA) and wrapped into a tubular shape.

MEC inoculation and operation

The bacteria used in the MECs originated from conventional single-chamber MFCs using local domestic wastewater from the Corvallis Wastewater Treatment plant as the inoculum. When the power output of the MFCs was stabilized at 1.2 W/m^2, the anodes were removed and placed in MECs. The medium solution that was used for MEC operation contained 30 mM sodium acetate as substrate, 100 mM sodium phosphate buffer and necessary minerals and vitamins as reported previously [27]. After purging the MECs with nitrogen for 10 minutes to maintain anaerobic condition, a voltage of 1.0V was applied to the MECs. MEC current output was recorded as described previously [6]. When stable generation of current density was achieved, 5 mL anaerobic digester sludge (Gresham, Oregon, USA) was introduced to allow methanogenesis in MECs. All MECs were operated at 32^oC.

The MECs were divided into four groups: 1% acetylene group, 5%/10% acetylene group, BES control group, and no treatment control/0.1% acetylene group. 1% and 5%/10% acetylene group had an initial acetylene concentration of 1% and 5% respectively. Acetylene was increased to 10% in 5%/10% acetylene group on day 80 to investigate whether high concentrations of acetylene would have a negative effect on exoelectrogens. 5mM BES was present initially in the BES control group and 0.5 mmol BES was dosed into the MECs during each injection when methanogens revived. The no treatment control/0.1% acetylene group had no methanogen inhibition method initially to investigate the viability of methanogens in MECs. On day 81 of the testing period, 0.1% acetylene was injected to investigate the effect of 0.1% acetylene on methanogens.

Sodium acetate was utilized as substrate from day 1 to day 40 as well as day 80 to day 135. Substrate was switched to glucose on day 41 to investigate the effect of substrate type on the effectiveness of inhibition methods. Substrate was supplemented to the desired concentration through injection of 1 mL concentrated substrate solution or replacing the solution with fresh medium. Each condition was tested in duplicate MECs.

Wastewater Characterization and MEC Performance

Brewery wastewater from Sky High Brewing in Corvallis, Oregon was acquired and characterized. Chemical oxygen demand (COD) was measured using a traditional COD test procedure following EPA Method 410.4. Total COD and soluble COD were of interest, wherein soluble COD was distinguished from total COD by centrifuging the wastewater and the supernatant was collected and used for dilution. The wastewater was diluted fivefold, twentyfold, and fiftyfold in duplicates. 2.5 ml of diluted samples were dispensed into test tubes containing 1.5 digestion solution and 3.5 catalyst solution. The digestion solution was comprised of 5.1 g potassium dichromate $K_2Cr_2O_7$, 84 mL concentrated sulfuric acid H_2SO_4, and 16.7 g mercuric sulfate $HgSO_2$ in 500 mL water. The catalyst solution was comprised of 22 g silver sulfate Ag_2SO_4 in a 4.09 kg bottle of concentrated sulfuric acid H_2SO_4. The test tubes were shaken and sealed tightly before being placed in a heat block and digested at 150 ˚C for two hours. After cooling, samples were analyzed using a Shimadzu UV-Visible Spectrophotometer (Model UV-1700) at wavelength 600 nm to measure the absorbance of the samples. The absorbance outputs were then used as inputs in a pre-calibrated curve to estimate the COD.

Media for MECs was prepared by diluting the wastewater tenfold in 200 mM phosphate buffer media along with trace vitamins and minerals. Two MECs that were previously operated using the methods outlined in previous sections and treated with 1.0% and 5.0% acetylene were

selected to evaluate the diluted wastewater performance. Each MEC had 10 mL wastewater and 90 mL of buffered media. MECs were sealed and purged with nitrogen as previously described.

Analysis and calculations

The volume of biogas produced was measured using a water displacement method by connecting a gas tight gradual cylinder, which was immersed in 0.005M sulfuric acid solution to preventing CO_2 dissolution. The gas composition in the headspace was measured using gas chromatography (Agilent Technologies 6890N Network GC System). Gas analysis was performed every 24 hours. Voltage over the resistor was recorded using a multimeter with a data acquisition system (2700, Keithley). Voltage was applied by a programmable power supply (3645A, Array Elec. Co. Ltd). Current density (based on anode surface area) and overall hydrogen yield were calculated as described previously [7].

Throughout operation, acetylene was supplemented to restore initial acetylene concentration when methane production occurred. We assumed minimal stratification of gases in the headspace. Doing so, we could calculate the dilution of acetylene as gas was measured and released. The biogas released was assumed to have the same concentration of acetylene (in %) as the headspace. The initial % times the amount of biogas released could be subtracted from the original volume of acetylene injected to determine the new concentration of acetylene in the headspace. Once methane was observed to make a recovery via the gas chromatography analysis, the deficit in acetylene was determined and it was restored to the desired concentration.

Although this study refers to the acetylene concentration injected on a gas volume to volume basis, the inhibitory mechanisms occur through the dissolved acetylene in solution. Henry's law was used to estimate the concentration of acetylene in the liquid phase of the MEC. Based on the solubility of acetylene in water of 0.117 g/0.100 L at standard conditions[28], the

Henry's constant for acetylene was 22.22 L atm/mol. The shifts in liquid concentration as a result of biogas release every 24 hours and occasional acetylene administration were modeled and are summarized in Figure 2.

$$k_H = \frac{P}{C_{aq}} = \frac{P*MW}{S}$$

Results and discussion

Effectiveness of inhibition methods in fermentation bottles

Methane production was observed in all batches of the control bottles (Figure 1a). Methane began to dominate hydrogen production beginning on day 12, the beginning of the fourth batch. The heat shock treatment inhibited methane completely until the fourth batch, beginning on day 12 (Figure 1b). Methane continued to dominate the headspace for the rest of the testing period as hydrogen dropped down to zero beginning on day 19. The 5 mM BES treatment completely inhibited methane as long as it was administered at the beginning of each batch (Figure 1c). However, when 5 mM BES was not incorporated into the media at the start of every batch, methane recovery was observed on day 21 (Figure 1d).

Some methane production was observed at the beginning of the 0.1% acetylene treatment but dropped down to zero at the end of the first batch. Complete methane inhibition was maintained until day 12 when methane recovery occurred. 0.1% acetylene failed to inhibit methanogens for the rest of the testing period even when 0.1% acetylene was administered at the beginning of every batch (Figure 1e and 1f). Both 1.0% (Figure 1g) and 5.0% (Figure 1i) completely inhibited methane production throughout testing when each concentration was administered at the beginning of every batch. However, when acetylene was not administered both treatments only lasted until day 21 when methane production started to occur (Figure 1h and 1j). Based on these results, the MEC

study began by investigating the effects of 1.0% and 5.0% acetylene first. Acetylene at concentrations of 0.1% were later investigated in MECs to validate the literature and preliminary trials.

The pH of the fermentation trials was maintained at neutral until it was observed that hydrogen production was dropping even in bottles that had zero methane production. In response to this observation and fearing that there was too little substrate, a concentrated injection of 11 mM glucose was added in the middle of the fourth batch and fifth. However, this caused the pH to drop down to 6. The possibility of acidic conditions interfering with the analysis prompted a switch to 22 mM glucose and 200 mM phosphate buffer media to provide more substrate and maintain pH at 7. Even with the added substrate and neutral pH, hydrogen production rates still did not improve. It is suspected that hydrogen production was poor because more acidic conditions are optimal for fermentation and this study maintained pH at 7. Neutral conditions were favored in this study because MECs operate optimally at pH 7. Homoacetogens could have also played a role in the drop in hydrogen by scavenging hydrogen for acetate generation. Loss of fermentative biomass could have played a role as well, as centrifuging the media at the highest setting of 4,000 rpm for ten minutes still resulted in a white supernatant indicating the presence of fermentative cultures in the discarded media.

Effectiveness of acetylene on MEC methanogen inhibition

Figure 2 demonstrates the effectiveness of various acetylene concentrations on methanogen inhibition compared with BES under acetate-fed and glucose-fed conditions. The introduction of anaerobic digester sludge allowed methane to compose 20-30% of the headspace. Injection of acetylene (1% in headspace) immediately inhibited methanogenesis, leading to a decrease of methane concentration to less than 1%, which was maintained at this level for 20 days. (Figure 2A). An increase of methane to 3% occurred on day 36 and was suppressed after another acetylene

supplement. After substrate glucose was injected to a final concentration of 11 mM, 1% acetylene did not completely suppress methanogenesis, with a methane percentage of 2-4% throughout the operation. Methanogens revival on day 80 was successfully inhibited by consecutive dosage of acetylene. Methanogenesis was completely suppressed by 5% and 10% acetylene throughout the operation (Figure 2B). Although the addition of 5 mmol BES led to the drop of methane from 25% to 5% in the headspace, methane level started to rise after day 20, despite two consecutive addition of BES on day 15 and day 19 (Figure 2C). Injection of 0.1% acetylene was not effective in inhibiting the methane production in the MECs with significant methane production prior to the injection of acetylene (Figure 2D). Figure 3 shows that the extent of methanogenesis in all the treatments for the first 40 days of testing was significantly lower than the control.

After the substrate was switched from 30 mM acetate to 11 mM glucose, 30 mM of BES failed to inhibit methanogenesis in the glucose-fed MEC. When the substrate was switched back to acetate, methane production was inhibited, and methane in the headspace dropped from 30% to 5% in 55 days. When glucose was present as the substrate, BES failed to suppress methanogenesis, but 5%/10% acetylene succeeded in methanogen inhibition. 1% acetylene also showed impaired performance against methanogens. These observations could potentially be attributed to a flux of hydrogen and other essential nutrients being formed as by-products of dark fermentation, which could result in the favorable growth of hydrogenotrophic methanogens[29].

Figure 4 illustrates the estimated acetylene concentration change in headspace over time. In the 5%/10% acetylene group, the high acetylene concentration was only maintained for a very short period after each injection due to the significant biogas production. Methanogenesis revival occurred in 1% acetylene treated MEC on day 39 and day 57 while no methane was present in 5% acetylene groups. However, the levels of acetylene in 1% and 5% acetylene MECs were approximately 0.2 to 0.4% when the methanogen revival occurred. This result suggests that higher

percentage of acetylene had an irreversible effect, preventing methanogens from recovering as observed at lower levels of acetylene. Such an inhibitory effect was possibly the result of irreversible binding of acetylene to copper, which is the active site of many metalloenzymes[30]. Furthermore, the irreversible inhibition was also observed in the interaction between acetylene and methane monooxygenase, which requires copper as a critical cofactor[31]. Acetylene has also been shown to inhibit nickel-iron hydrogenases, found in *Methanosarcina* and other methanogens[32]. The [NiFe]-hydrogenase in methanogens plays an important role in the methanogenesis pathway, as it is responsible for cleaving hydrogen and reducing the F_{420} coenzyme. Genomic studies have shown that *Geobacter sulfurreducens* has 4 [NiFe]-hydrogenases encoded in its genome[33]. Of these 4 [NiFe]-hydrogenases, the methyl viologen hydrogenase (*Mvh*) also plays a significant role in energy generation via methanogenesis in methanogens. It is unclear what role *Mvh* plays in the growth of *G. sulfurreducens*, as it has not been shown to have a direct role in hydrogen-dependent growth. It is also unclear if acetylene has the same inhibitory effects on *Mvh* as it does on F_{420}-reducing hydrogenases. However, the risk of acetylene inhibition on exoelectrogen growth is still of concern and an investigation is warranted.

Impact of acetylene on exoelectrogens

To further investigate the impact of acetylene on exoelectrogens, current densities were compared as the most direct measure of exoelectrogenic activities. Figure 1 demonstrated that exoelectrogenic activities were not inhibited by the presence of 1%, 5% and 10% acetylene. Maximum current densities for all testing MECs were maintained at 10-12 A/m^2 prior to the introduction of anaerobic digester sludge. The maximum current densities of 1% acetylene group were at 10-14 A/m^2 with 30 mM acetate and 10-12 A/m^2 with 11 mM glucose, which is similar to the 5%/10% acetylene group (12-16 A/m^2 with 30 mM acetate and 8-9 A/m^2 with 11 mM glucose). However, the BES group showed significant decrease in current density from the maximum

current densities of 6-10 A/m^2 with acetate and 4-8 A/m^2 with glucose to less than 5 A/m^2 after 40 days' operation. Though extremely limited hydrogen was produced, the no treatment control group exhibited similar max current densities, which were 8-10 A/m^2 with acetate and 6 A/m^2 with glucose. However, these current densities were only observed shortly after substrate injection and quickly fell down to current densities of 1 A/m^2 or less. The introduction of 0.1% acetylene resulted in slightly improved and consistent current density, with an average of 4 A/m^2.

We observed higher current densities in 1%, 5% and 10% acetylene treated MECs than MECs treated with BES. BES is a structural analog of coenzyme M, which could compete with coenzyme M to inhibit methanogenesis, and BES had no known inhibitory effect on exoelectrogens[34]. Thus, 1-5% acetylene could not inhibit the activity of exoelectrogens more significantly than BES. The specificity of acetylene inhibition against methanogens could be the result of methanogens failing to maintain or generate the transmembrane proton gradient for ATP synthesis and methanogenesis. Such inhibition occurred specifically in methanogens without gross membrane damage or inhibition on other critical enzymes[35]. Thus, the inhibitory effect might act on certain enzymes or cytochromes of the electron transport chain in methanogens, which could differ from those in exoelectrogens. The method of growth and habitat could also contribute to the specificity of inhibition. Exoelectrogens survived on the electrode as a member of the anodic biofilm, while methanogens might exist in the planktonic cells for higher availability of hydrogen. It has been reported that special physical or chemical structure of exopolysaccharides might improve the biofilm resistance against biocides[36].

MEC Performance on Diluted Wastewater as Feedstock

The results of the wastewater characterization indicated that the COD of the original brewery wastewater was approximately 70,000 mg/L. Figure 5 shows the performance of one MEC operated with dilute wastewater from day 130 to day 150. Methane production in both MECs

was low, only reaching 5% in the headspace. However, current density dropped from 15 A/m^2 to 5 A/m^2 in the MEC previously treated with 1.0% acetylene and from 10 A/m^2 to 1 A/m^2 in the MEC previously treated with 5.0% acetylene. In addition, pH dropped from 7 to 4 in both MECs. The low methane levels and the current density could have resulted from the drop in pH, as methanogens and MECs have previously been reported to show sensitivity to pH[37]. It is also possible that there were not enough usable substrates in the diluted wastewater to maintain current densities as there was when 30 mM acetate was the feedstock. The MECs were switched back to 30 mM acetate to see if the current density prior to wastewater operation could be recovered. Only the MEC that had been previously treated with 5.0% acetylene recovered while the one previously treated with 1.0% acetylene did not.

Maintaining a neutral pH remains an obstacle in batch-mode MECs operating with wastewater as a feedstock. A large drop in pH from 7 to 4 was observed even after the wastewater was diluted tenfold and a strong buffer concentration was applied. An MEC operated in continuous-mode could benefit from the use of a pH controller but batch-mode laboratory evaluations were limited to diluting the feedstock or using buffers. Future work will involve finding the appropriate buffer and possibly other pre-treatments to successfully operate MECs using wastewater.

Possible sources of error in methodology

In any experiment, there are sources of error from instrumentation and the design of the study. The performance of each MEC is largely dependent on having a good connection between the power supply, the MEC, and the multimeter. If the connection is poor at any point, performance issues will manifest in the current density and biogas production of the MEC. The connection was double-checked by using another multimeter. The wrapped electrode configuration may have its own issues as well. The wrapping of the stainless-steel mesh cathode could result in enhanced

hydrogen scavenging at the cathode. Hydrogen bubbles forming on the cathode could become trapped, a phenomenon that could be aggravated by wrapping of the mesh, as well as wrapping of cloth over it. This could give methanogens and homoacetogens greater access to hydrogen. Tight wrapping of the carbon cloth anode could also cause issues in biofilm growth on the inner side of the cloth, resulting in underestimated current density values.

Outlook of utilizing acetylene as methanogen inhibitor for industrial application

Various strategies had been proposed to inhibit methanogenesis in MEC systems. However, they showed either unsatisfactory results on methane inhibition, or challenges to industrial application (Table 2). Given the low concentration needed and the low cost ($0.036 per liter), acetylene has potential to be more economical than other effective methods. Assuming a reactor was designed with 10% headspace and 90% liquid phase per unit of reactor volume, the cost could be as low as 0.18 USD/m^3-reactor per injection for using 5% acetylene, which is significantly more economical than using BES. Furthermore, based on previous results, the injection interval could be as long as 30 days, which would also negate the necessity of ceaseless operation, to minimize operational difficulties. Thus, utilizing acetylene to inhibit methanogenesis exhibited the advantages of being economical and easy to operate over other strategies.

The effect of acetylene on methanogens was demonstrated, but investigations using higher acetylene concentrations and injection intervals are still necessary to optimize the method. A long-term test of using acetylene as methanogen inhibition and prevention method could review the long-term effect of acetylene in the system and whether methanogens would adapt to such condition. Furthermore, using large-scale reactors with real wastewater or real lignocellulosic hydrolysate as well as operating under conditions with various temperatures, hydraulic retention times, substrate concentrations, acetylene concentrations and acetylene injection intervals could benefit the optimization and future industrial application of this novel method.

Figures and captions

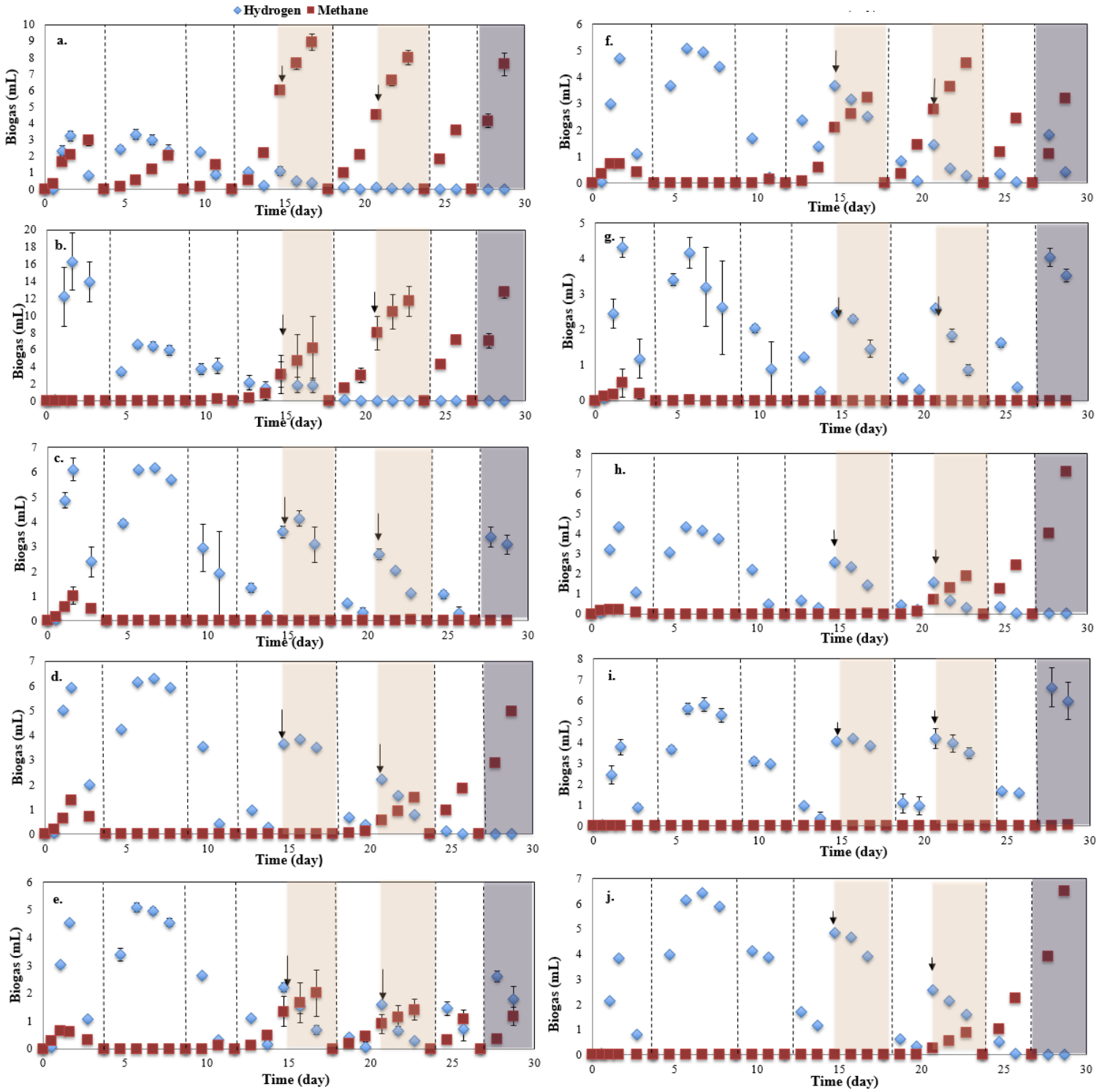

Figure 1. Hydrogen and methane production in control (a), heat shock (b), 5 mM BES (c), 5 mM BES w/o re-administration (d), 0.1% acetylene (e), 0.1% acetylene w/o re-administration (f), 1.0% acetylene (g), 1.0% acetylene w/o re-administration (h), 5.0% acetylene (i), and 5.0% acetylene w/o re-administration (j). Dashed lines indicate new batch. Thin arrows indicate where substrate was injected mid-batch. Lightly shaded areas indicate where pH = 6. Darker shaded area indicates 4 g/L glucose in 200 mM buffer (pH = 7). pH = 7 everywhere else.

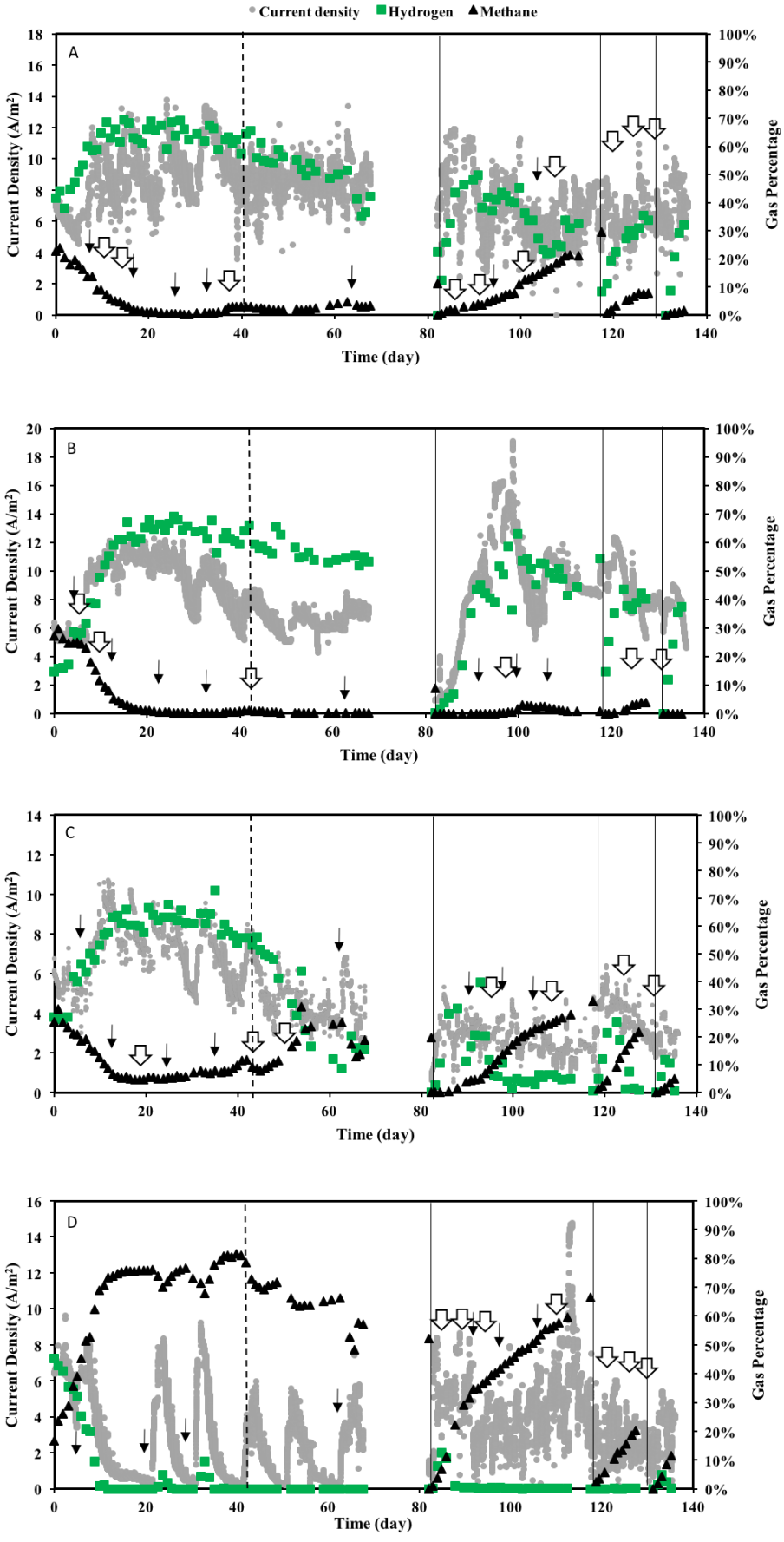
Current density
Hydrogen
Methane
A
B
C
D
Current Density (A/m²)
Gas Percentage
Time (day)

Figure 2. Current generation and gas composition in (A) 1% acetylene group, (B) 5%/10% acetylene group, (C) BES group and (D) Control/0.1% acetylene group. Dashed lines indicate substrate was switched from 30 mM acetate to 11 mM glucose. Solid lines indicate solution was replaced with fresh medium containing 30 mM acetate. Thin arrows indicate where substrate was supplemented. Thick arrows indicate acetylene was supplemented to maintain initial concentration.

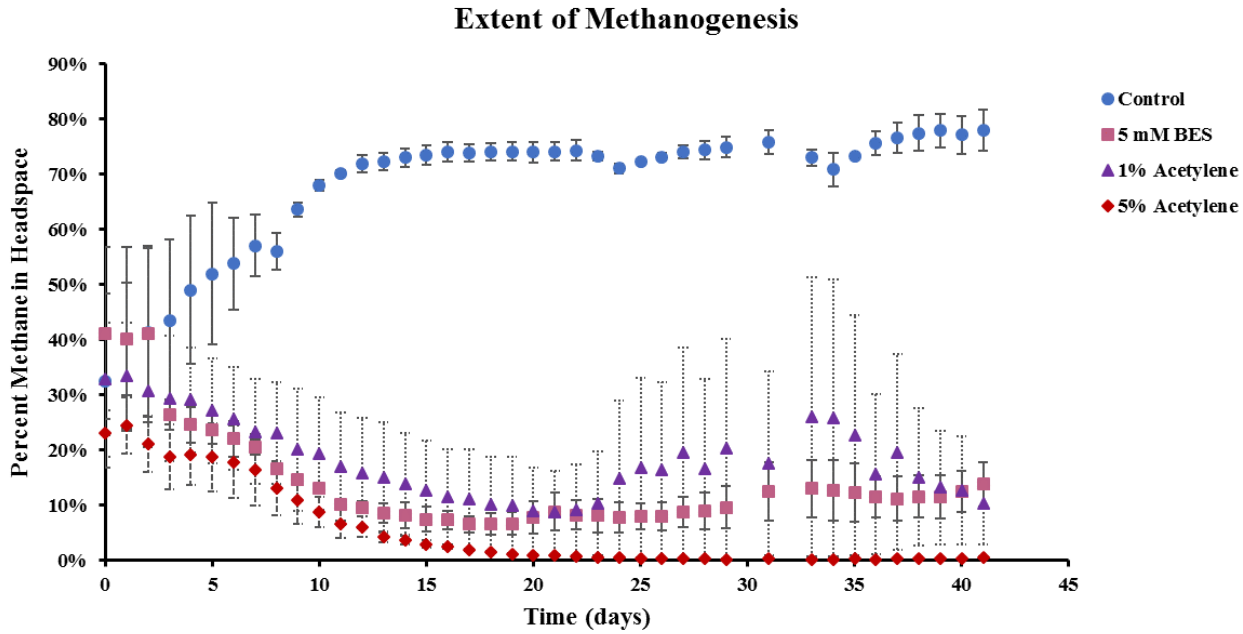

Figure 3. The extent of methanogenesis between all the treatments.

Table 1. P-values for treatments of interest (ANOVA Single-factor)

	5 mM BES	1% acetylene	5% acetylene
Control	1.16E-35	1.06E-35	1.25E-41
5 mM BES		0.02	4.63E-05

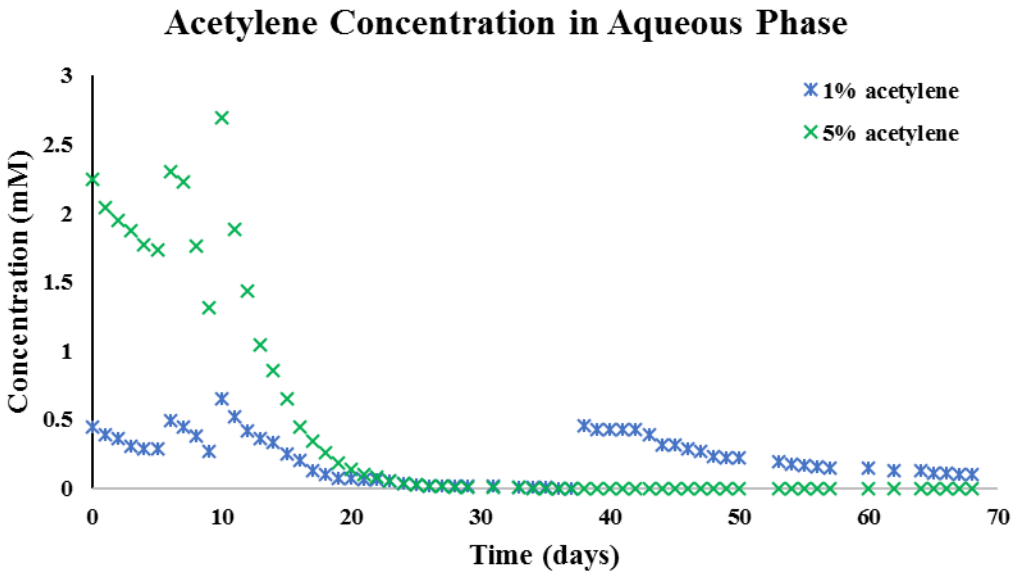

Figure 4. Estimated acetylene concentration change in headspace through the first acetate phase (days 0-40) and glucose phase (days 41-68) with initial injection of 1% and 5% acetylene.

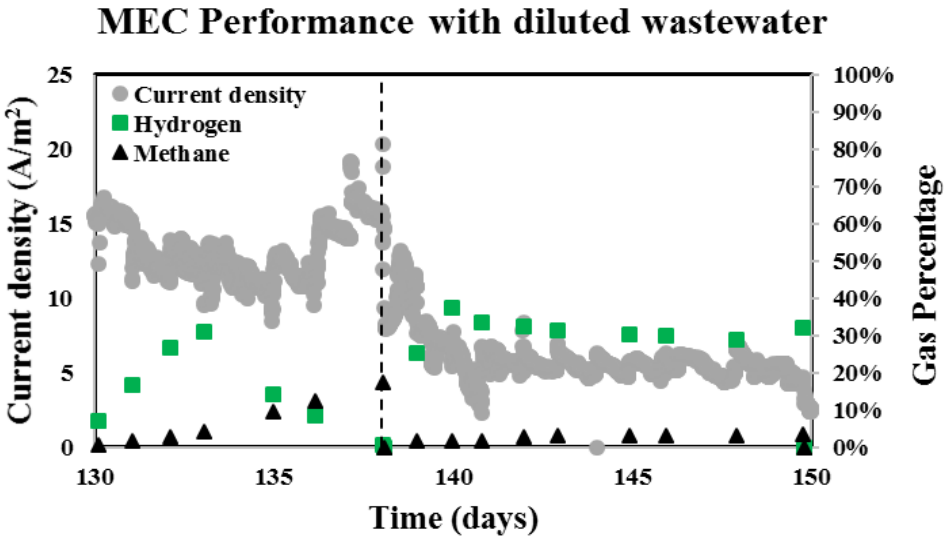

Figure 5. MEC (previously treated with 1.0% acetylene) performance change with diluted wastewater. Dashed line indicates where media changed from 30 mM acetate to diluted wastewater (COD 7,000 mg/L). pH was observed to drop from 7 to 4 over this period.

Table 2. Comparison of methanogen inhibition methods in MECs

Inhibition Method	*MEC Configuration*	*Concentration*	*Result/Challenges*	*Reference*
Acetylene	Single chamber	1% (v/v)	Methanogens recovered after 80 days	This study
		5% (v/v)	Effective inhibition	
Heat shock	Dual chamber	N/A	Methanogens recovered shortly	Chae et al 2010
pH shock	Dual chamber	N/A	Methanogens recovered shortly	Chae et al 2010
BES	Single chamber	5 mM	High cost and methanogens recovered in 5-20 days	This study
BES	Single chamber	20 mM	High cost	Catal et al 2015
UV radiation	Single chamber	N/A	High cost	Hou et al 2014
Chloroform	Single chamber	5% (v/v)	High cost	Zhang et al 2016
BES	Dual chamber	286 µM	High cost	Chae et al 2010
Low temperature (4°C)	Single chamber	N/A	High cost and increased difficulty of operation	Lu et al 2010
Hydrogen harvest	Single chamber	N/A	Increased difficulty of operation	Lu et al 2016

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