Selective inhibition of methanogens for the improvement of biohydrogen production in microbial electrolysis cells

Kyu-Jung Chae, Mi-Jin Choi, Kyoung-Yeol Kim, F.F. Ajayi, In-Seop Chang, In S. Kim*

Department of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), 261 Cheomdan-gwagiro, Buk-gu, Gwangju 500-712, South Korea

ABSTRACT
The microbial electrolysis cell (MEC) is a promising technology for producing biohydrogen at greater yield than with conventional technology. However, during a run of an acetate-fed MEC at an applied voltage of 0.5 V, substantial amounts of substrate are consumed in undesirable methanogenesis. Therefore, in order to suppress the methanogens specifically without adversely affecting exoelectrogens, this study examined the effects of sudden changes in pH, temperature and air-exposure, as well as chemical inhibitors, such as 2-bromoethanesulfonate (BES) and lumazine on methanogenesis. An abrupt decrease in temperature and pH from 30 to 20°C and 7 to 4.9, respectively, had no effect on methanogenesis. Exposing the anode biofilm to air was also ineffective in inhibiting specific methanogens because both methanogens and exoelectrogens were damaged by oxygen. However, an injection of BES (286 μM) reduced the methanogenic electron losses substantially from 36.4 ± 4.4 (145.8 ± 17.4 μmol-CH₄) to 2.5 ± 0.3% (10.2 ± 1.2 μmol-CH₄), which in turn improved the overall hydrogen efficiency (acetate to H₂) from 56.1 ± 5.7 to 80.1 ± 6.5% (3.2 mol-H₂/mol-acetate). Once after inhibited, the inhibitory influence was retained even after 10 batch cycles in the absence of further BES addition. In contrast to BES, methanogenesis was unaffected by lumazine, even at much higher concentrations.

The installation of a Nafion membrane resulted in the production of high purity hydrogen at the cathode but hindered proton migration, which caused a serious pH imbalance between the anode and cathode compartments.

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1. Introduction
A microbial electrolysis cell (MEC), which is a modified microbial fuel cell (MFC), has attracted considerable attention over the last three years as a promising technology for the production of biohydrogen on account of its great hydrogen yield compared to conventional hydrogen producing-dark fermentation [1–6]. MEC also uses the same biocatalysts, such as exoelectrogens, which oxidize organic matter and transfer electrons to an anode as with an MFC. Therefore, a MEC operates in the same manner as a conventional MFC, except that the cathode is maintained under oxygen-free conditions and an external potential is supplied to overcome the thermodynamic barrier for reducing the protons to hydrogen at the cathode (i.e., −0.42 − (−0.28) − 0.14 V in case of acetate at pH 7). As acetate is used as the electron donor in MECs, carbon dioxide, protons and electrons are produced from the anodic degradation of acetate by exoelectrogens, while hydrogen gas (a maximum theoretical yield of 4 mol-H₂/mol-acetate) is produced at the cathode (Eqs. (1) and (2)).
Anode: \[ \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 8\text{H}^+ + 8\text{e}^- \] 
\[ E^0 = -0.28 \text{ V(NHE)} \]  
(1)

Cathode: \[ 8\text{H}^+ + 8\text{e}^- \rightarrow 4\text{H}_2 \] 
\[ E^0 = -0.42 \text{ V(NHE)} \]  
(2)

However, acetate is also a favorable substrate for methanogens, which convert it to methane and carbon dioxide. Methane is commonly observed in the anode of both MFCs and MECs, even though its amount varies considerably according to the inoculum, substrate and reactor configuration. Interestingly, no- or negligible methane production (0.9 \(-0.1\% \text{ in headspace}) from acetate was reported from a two-chamber MFC\[7\] and a membrane-less single chamber MEC with intermittent exposure of the reactor to air in order to inhibit methanogenesis \[8\]. Jung and Regan \[9\] reported methanogenic electron loss of only 3.4 \(-1.3\% \text{ in an acetate-enriched MFC inoculated with an anaerobic sludge} \). On the other hand, substantial methane production was reported by Kim et al.\[10\] using a wastewater-fed MFC inoculated with anaerobic sludge, and by Chae et al.\[1\] using an acetate-fed MEC inoculated with anaerobic sludge. Several studies have reported that methanogens still compete with the exoelectrogens for the substrate, which is quite severe with complex substrates, such as glucose and wastewater, on account of their fermentable nature\[1,7,8,10\]. It is expected that there would be a significant decrease in energy loss through \( \text{CH}_4 \) production if methane production can be replaced by exocellular electron transfer by exoelectrogens in a MEC. This suggests that the specific suppression of methanogens essential for enhancing the overall hydrogen recovery in MEC systems.

Methanogens employ unique coenzymes, such as methanofuran, methanopterin \((5,6,7,8\text{-tetrahydromethanopterin}; \text{H}_2\text{MPT})\), and coenzyme M \((\text{CoM}; \text{HSCH}_2\text{CH}_2\text{SO}_3^-)\), which play important roles as \( \text{C}1 \) carriers in methanogenesis \[11–13\]. Coenzyme M is a cofactor involved in the terminal step of methane biosynthesis and is present only in methanogens. Methanopterin carries a \( \text{C}1 \) group during most of the reductive steps in the methanogenic pathway \( \text{(reduction of CO}_2 \text{ to CH}_4 \text{), from the formyl (–CHO) level to the methyl (–CH}_3 \text{) level, before transferring it to coenzyme M, which is involved in the final step of methanogenesis. Coenzyme M is converted to methyl coenzyme M (CH}_3\text{–S–CoM), which then reacts with coenzyme B (CoB), finally releasing methane (Eq. (3)) by the catalysis of methyl coenzyme M reductase. Some chemicals such as 2-bromoethanesulfonate (BES) and lumazine have been reported to be act as specific inhibitors for methanogens as a result of their structural similarity to above mentioned coenzymes [14–16].}

\[ \text{CH}_3 - \text{S} - \text{CoM} + \text{HS} - \text{CoB} \rightarrow \text{CH}_4 + \text{CoB} - \text{S} - \text{S} - \text{CoM} \]  
(3)

In this study, it was hypothesized that methanogens are much more susceptible to inhibitory conditions than exoelectrogens based on the fact that methanogens are weak microbes and are sensitive to environmental changes \[17\]. Therefore, the selective suppression of methanogens was carried out by introducing inhibitory conditions, such as temperature, pH and oxygen shock, and methanogen-specific inhibitors, such as BES and lumazine.
was then changed to MEC mode with an applied voltage of 0.5 V over a 10 month period. After a 10 month run, the mode was changed again to MFC mode and run for 2 months to confirm the activity of the exoelectrogens because a gradual decrease in hydrogen yield was observed over the previous 10 month run. After verifying reproducible current generation, they were considered fully acclimated and then were operated in MEC for more than 5 months. All the data was obtained from the final 5 month run. The MECs were run in a fed-batch mode in a temperature controlled room at 30 ± 2 °C.

2.2. Operational shock experiments

A normal MEC was operated as a control at 30 °C, pH 7, and under anaerobic conditions. However, in order to examine the response of methanogens to temperature shock, the temperature was decreased suddenly from 30 to 20 °C by replacing the medium with a cold one (20 °C). The temperature was maintained at 20 °C for 3 batch cycles, and then increased again to 30 °C. Similarly, to determine the relative sensitivity of the methanogens to a lower pH, the anode pH was decreased suddenly by replacing the pH 7.0 medium with pH 4.9 medium. For oxygen shock, the anode biofilm was exposed to air for 50 min followed by 5 min of air-sparging of the anode medium between batch cycles. All experiments were conducted in quintuplicate.

2.3. Methanogen-specific inhibitors

The chemical inhibitors studied were BES (Na salt; BrCH₂CH₂SO₃Na; Sigma, USA) and lumazine (2, 4-Pteridinediol; C₆H₄N₄O₂; Aldrich, USA) at 0, 143, 286 and 572 μM. BES and lumazine are structural analogues of coenzyme M and methanopterin, respectively, which are unique coenzymes for methanogens. BES and lumazine were dissolved in deionized water to make 20 and 5 mM stock solutions, respectively. In order to inhibit the methanogens, the target amount of each stock solution was injected directly into the anodic compartment of the MEC reactors through a butyl rubber septum using a syringe to achieve concentrations of 0, 143, 286 and 572 μM.

2.4. Calculations and analyses

The Coulombic efficiency (CE; acetate to e⁻ in the anode), cathodic hydrogen efficiency (CHE; e⁻ to H₂ in the cathode), and overall hydrogen efficiency (OHE; acetate to H₂) were calculated, as previously described [1,2]. Gas withdrawals (500 μL) from the reactor headspace were performed using a lockable gas-tight syringe (Hamilton #1002), and then analyzed by gas chromatography (GC-2010, Shimadzu, JP) with a thermal conductivity detector equipped with a capillary column (CP-Pora PLOT Q 27.5 m × 0.53 m, 20 μm) and nitrogen as a carrier gas. The acetate concentration in the solution was calculated, as previously described [1,2]. Gas withdrawals (500 μL) from the reactor headspace were performed using a lockable gas-tight syringe (Hamilton #1002), and then analyzed by gas chromatography (GC-2010, Shimadzu, JP) with a thermal conductivity detector equipped with a capillary column (CP-Pora PLOT Q 27.5 m × 0.53 m, 20 μm) and nitrogen as a carrier gas. The acetate concentration in the solution was measured in duplicate using a high performance liquid chromatography (LC-20AD, Shimadzu, JP) with an Aminex HPX-87H column (Bio-rad, USA).

3. Results and discussion

3.1. Hydrogen production and methanogenic electron loss

Although, in theory, exoelectrogens can completely oxidize organics to carbon dioxide, methane is still produced in large quantities in the anode compartment. This indicates acetate consumption by undesirable methanogens, which are non-electricity-generating bacteria, resulting in a lower CE and overall hydrogen efficiency (OHE). Fig. 2 shows methane production and hydrogen recovery efficiency over consecutive batch cycles with an external voltage of 0.5 V. Whilst methane production in the anode compartment increased from 9 μmol (= a methanogenic electron loss of 2.2%) to 116 μmol (= a methanogenic electron loss of 29%) for 38 repeated feeding cycles (86 days), the OHE decreased from 77.1 to 50.1% over the same period. This clearly shows that the specific suppression of methanogens is essential for enhancing the production of hydrogen in a MEC system. In addition, based on our former MEC study using acetate as a substrate, the anodic loss of electrons was more detrimental than the cathodic loss, particularly at the higher applied voltage range, and the major anodic electron loss was due to methanogenesis [1].

The level of methane production increased gradually with time, as shown in Fig. 2, because acetate is a favorable substrate for methanogens as well as exoelectrogens. Methanogenic acetate degradation is conducted by either aceticlastic methanogenesis or an anaerobic acetate-oxidizing reaction (a syntrophic acetate oxidation). Aceticlastic methanogenesis (CH₃COO⁻ + H₂O → CH₄ + HCO₃⁻; ΔG° = −31.0 kJ/mol) is an exergonic reaction that is carried out by only two genera of methanoarchaea, Methanosarcina and Methanosaeta [13,19]. However, the anaerobic acetate-oxidizing reaction is energetically extremely unfavorable, and occurs only when the products (e.g., H₂/CO₂) of syntrophic acetate oxidation are subsequently consumed by hydrogenotrophic methanogenesis. In order to confirm the activity of hydrogenotrophic
methanogens, the anode chamber was filled with hydrogen gas only (<5%) without acetate injection after flushing with nitrogen. However, there was no detectable hydrogen consumption or corresponding production of methane (data not shown). Therefore, acetoclastic methanogenesis probably explains the mass production of methane from the anode in MECs based on its energetic advantage.

3.2. Response of methanogens to operational shocks: temperature, pH, and oxygen

Methanogens are more sensitive than other bacteria and thus be very stressed under adverse conditions such as reduced temperature, less than optimal pH, or toxicant exposure. Previous studies reported that the operating temperature of MFCs had no significant affect on performance [20,21]. Decreasing the temperature from 32 to 20 °C decreased the power density by 9% but showed almost same CE [21]. Therefore, we postulated that an abrupt decrease in temperature can selectively inhibit the methanogens without damaging exoelectrogens. In order to determine the response of CH4 production to temperature shocks, the temperature of the anode was lowered suddenly from 30 to 20 °C, maintained for 3 batch cycles, and then increased again to 30 °C. Temperature shock led to a decrease in CH4 production compared to the control (Table 1). However, the decrease was unremarkable and the level of CH4 production returned to the value in the control reactor within a short period of time. Once recovered, no lasting damage was observed. Similar findings were also reported from the anaerobic digestion of swine manure [22].

Similarly, anode pH was reduced suddenly in order to investigate if methanogens can be suppressed specifically at lower pH without damaging the exoelectrogens. According to the pH shock results, CH4 was still produced in large quantities, even with fairly significant changes in pH from 7.0 to 4.9 (Table 1). Similar electron loss of 27.3 ± 3% was measured by methanogenesis at pH 4.9, as compared to 36.4 ± 4.4% for control at pH 7, resulting in an almost comparable overall hydrogen recovery (61.1 ± 11.2% for pH 4.9; 56.1 ± 5.7% for pH 7).

Molecular oxygen not only inhibits the growth of methanogens, but also irreversibly inactivates many methanogenic enzymes. Methanogens are strict anaerobes, but facultative exoelectrogenic bacteria have been reported in MFC systems.

It was hypothesized that exposing an anodic biofilm to oxygen may not be detrimental to electricity generation by exoelectrogens but would probably inhibit methanogenesis. CH4 production was inhibited successfully by air-exposure of the anode biofilm, which is consistent with a previous study [8] (Table 1). However, the substrate saved by inhibiting methanogenesis did not lead to a corresponding increase in CE, possibly because exposure to air inhibits the key biocatalysts in anodic extracellular electron transfer, such as Geobacter spp., which are also strict anaerobes. The reduction in current generation and hydrogen gas produced at the cathode was indirect evidence showing the inhibition of exoelectrogens by the oxygen shock. Consequently, these approaches are unsuitable for the specific control of methanogens in a MEC system.

3.3. Response of methanogens to selective inhibitors: BES and lumazine

Specific methanogenic inhibitors, such as BES and lumazine, were tested. BES is a structural analogue of coenzyme M and is regarded as a methanogen-specific inhibitor [10,14–16]. Lumazine is an analogue of the methanopterins that carry out catalysis of Coenzyme M in methanogenesis. The methane yield from the anode during an operational time of 800–1000 h reached 0.46 mol/mol-acetate but decreased to <0.01 mol/mol-acetate after the BES injection (Fig. 3). Fig. 4 shows methanogenic electron losses and corresponding decrease in hydrogen production of the control (no BES) and methanogens-inhibited MEC (BES 286 μM). Methane production was significantly lower in the presence of BES (10.2 ± 1.2 μmol-CH4 for BES-system; 145.8 ± 17.4 μmol-CH4 for BES-free system), which in turn results in an improvement in overall hydrogen efficiency from 56.1 ± 5.7 to 80.1 ± 6.5% compared to those obtained in the absence of BES. There was approximately 1.4 times more hydrogen gas produced in the presence of BES (3.20 mol-H2/mol-acetate for the BES-injected MEC but only

| Table 1 – Hydrogen production and methanogenic electron loss under the normal and shocked condition of acetate-fed MEC at an applied voltage of 0.5 V (n = 5). |
|-----------------|-----------------|-----------------|-----------------|
| Experiment      | Methanogenic electron loss (%)<sup>a</sup> | CE (%)          | CHE (%)         | OHE (%)         |
| Control         | 36.4 ± 4.4      | 60.0 ± 6.5      | 93.5 ± 4.2      | 56.1 ± 5.7      |
| Temperature shock| 25.1 ± 3.8      | 61.0 ± 3.2      | 92.3 ± 6.8      | 56.3 ± 5.1      |
| pH shock        | 27.3 ± 3.0      | 64.6 ± 4.0      | 94.6 ± 4.2      | 61.1 ± 11.2     |
| Oxygen exposure | 6.3 ± 1.4       | 51.2 ± 5.1      | 93.0 ± 3.7      | 47.6 ± 8.2      |

<sup>a</sup> Electron loss associated with methane was calculated based on the electron equivalence of 8e<sup>−</sup> mol equiv/mol of methane produced. The Coulombic efficiency (CE): acetate to e<sup>−</sup> in the anode; cathodic hydrogen efficiency (CHE): e<sup>−</sup> to H<sub>2</sub> in the cathode; overall hydrogen efficiency (OHE): acetate to H<sub>2</sub>.
2.24 mol-H₂/mol-acetate for the control). Interestingly, once inhibited, BES toxicity was retained even after 10 batch cycles in the absence of further BES injections, indicating its practical applicability.

Fig. 5 shows the CE, CHE and OHE as a function of the initial substrate concentrations under methanogen-suppressed conditions with BES (286 µM). Increasing the acetate concentration from 0 to 7.15 mM maintained the CHE at a relatively constant value of 92.4 ± 6.7%. This indicates that once the electron delivered from the anode to the cathode, they can be easily used to produce hydrogen gas. Meanwhile the CE was greatest (87.1%) at 1.43 mM, and a further increase in feed load resulted in a decrease in CE (36% at 7.15 mM) and a corresponding decrease in OHE (from 80.2% to 2.86 mM to 32% at 7.15 mM). This was attributed to the incomplete degradation of acetate due to the low anodic pH caused by the accumulation of protons as a result of improper proton transfer through the Nafion membrane (further discussed in later section).

In contrast to BES, lumazine had no effect on CH₄ production. Previously, methanogenesis of pure cultures of ruminal methanogens, including Methanobrevibacter ruminantium, Methanosarcina mazei and Methaomicrobium mobile, was inhibited almost completely by the use of 50- and 100%-lumazine saturated media (≤240 µM) [14]. In contrast to previous observations [14,23], lumazine unaffected methane production in our mixed-culture MECs at a dosage of 0–1144 µM (Fig. 6). This was due to differences between species of dominant methanogens in the previous pure culture and the current specific mixed-culture on the anode: namely, different methanogens showed different levels of resistance to chemical inhibitors. Lumazine is likely to inhibit only H₂ consuming methanogenesis, based on the fact that it is involved in the reductive steps in the methanogenic pathway, from the formyl level to the methyl level, during the reduction of CO₂ to CH₄. Therefore, previously reported methanogens that are sensitive to lumazine are generally hydrogenotrophs [14,23]. However, hydrogenotrophic methanogens were not dominant in our system because hydrogen is insufficiently diffused back from the cathode to the anode. This probably explains why lumazine had no influence on CH₄ production in our MEC.

It should be noted that long-term exposure to BES may not only eliminate the methanogenic archaea but also alter the anodic bacterial community structure including exoelectrogens because it is impossible to specifically remove one group of organism without eventually affecting the metabolically associated members in the community, as previously explained [15]. In our acetate-fed system with relatively short-term exposure to BES for 60 days, the exoelectrogens were unaffected by BES because acetate-utilizing methanogens and exoelectrogens are competing organisms that do not have a syntrophic association because acetate is the anaerobic metabolic end product. Exoelectrogens, such as Geobacter, can favorably utilize simple organic acids, such as acetate. Therefore, they rely on fermentative bacteria to produce their required electron donors from other more complex organic compounds [24]. On the other hand, fermentative bacteria that produce substrates (e.g., acetate) for methanogens would be affected indirectly by BES due to the accumulation of metabolic products caused by the elimination of consumers (methanogens). Consequently, exoelectrogens could be affected by BES as more complex substrates are used, which is of considerable concern.
3.4. Membrane-associated proton transfer problem

The installation of a proton exchange membrane (commonly Nafion) for MEC is recommended for the following reasons: (1) to achieve a high purity of hydrogen gas by avoiding contamination with the biogas (e.g., CO₂, CH₄, etc.) produced at the anode; (2) to avoid hydrogen losses from anodic bacterial consumption; and (3) to prevent poisoning of the precious cathode catalyst, such as Pt, with a biofilm. Crossover of gases through the membrane is unavoidable but it can be minimized as a membrane is installed. However, the membrane impedes proton migration between electrodes, which results in a severe pH imbalance between the anode and cathode chamber [18]. Fig. 7 shows the differences in pH between the anode and cathode chamber over consecutive batch cycles without replacing the medium and hydrogen gas production. With time, the anodic pH decreased continuously from 6.82 to 6.22 at the end of the first batch and then reduced further to 5.82 at the end of the second batch, while an opposite substantial increase occurred at the cathode (6.92 → 7.72 → 8.50). This lower pH in the anode inhibits the activity of exoelectrogens, resulting in a 50% decrease in hydrogen production for the second batch (=1.4 mol-H₂/mol-acetate) compared with the first batch (=2.8 mol-H₂/mol-acetate). Interestingly, high cathodic hydrogen recovery (78–96%) was achieved in a membrane-less single chamber MEC using a graphite fiber brush anode at applied voltages of 0.3–0.8 V [8]. However, operating a MEC without a membrane can reduce the purity of the hydrogen produced, requiring further gas purification for end use.

3.5. Cathodic methane production

Cathodically produced methane was observed consistently, even though its origin is still unclear and amount was quite small (maximum <3.5% of the hydrogen produced) (Fig. 8A). Theoretically, hydrogen is only expected as a product gas in the cathode headspace of a two-chambered MEC. However, methane was observed consistently in previous studies and considered to be the result of diffusion from the anode to cathode [1,2]. Fig. 8A shows a typical methane production in the anode and cathode chamber under anodic methanogens-inhibited conditions after a BES injection. The methane concentration in the cathode was substantially higher than that in the anode, indicating it does not fully originate from back diffusion from anode based on a concentration gradient. Instead, there is another way to produce methane on the cathode. It is possible that bacterial contamination of the cathode as a result of the prolonged run enables the bacterial conversion of hydrogen to methane through hydrogenotrophic methanogenesis. Moreover, aceticlastic methanogenesis is also expected due to possible acetate diffusion from the anode through the Nafion membrane. Therefore, PCR amplification was carried out to detect the methanogens

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Fig. 5 – Coulombic efficiency, cathodic hydrogen efficiency, overall hydrogen efficiency and maximum current as a function of the initial acetate concentration from the methanogen-suppressed MEC (BES = 286 μM; 0.5 V).

Fig. 6 – Methane production as a function of methanogen-inhibitor concentrations (at 2.86 mM acetate; 0.5 V).

Fig. 7 – pH differences between the anode and cathode chamber and hydrogen gas production over a consecutive batch cycle without replacing the NMB and PBS medium.
Cathode activity at 120 °C when the cathode compartment was autoclaved to eliminate aceticlastic methanogens. The presence or absence of each microbial group was detected at consistently low levels in the cathode headspace. However, cathodic methanogenesis has not yet well understood, thus further investigation is still need on this matter.

4. Conclusions

Methanogenesis is one of the most critical causes of electron losses in MEC. Hence, the specific suppression for methanogens is essential for improving the overall hydrogen recovery in a MEC system. Control of the operational factors, such as pH, temperature, and oxygen, was ineffective in inhibiting the methanogens specifically. Oxygen shock effectively inhibits aceticlastic methanogenesis but simultaneously suppresses extracellular electron transfer. BES is a potent and selective inhibitor of methanogens because it inhibits the reductive demethylation of methyl coenzyme M (M. (B) Abiotic methane production with an autoclaved cathode compartment (120 °C for 20 min) occurred during anodic methanogen-suppressed operation.

Fig. 8 – (A) Methane production in the anode and cathode during methanogen-suppressed operation with the BES injection into anode (at 2.86 mM acetate). The arrows indicate chemical injections into the cathode: solid for BES of 286 μM, dashed for lumazine of 286 μM. (B) Abiotic methane production with an autoclaved cathode compartment (at 120 °C for 20 min) occurred during anodic methanogen-suppressed operation.

using a family-specific PCR primer, Msc380F (5′-GAAAC CGYGA TAAGG GGA-3′) and Msc828R (5′-TAGGG ARCAT CTTTT AGC-3′) [25] for Methanosarcinaceae, Mst702F (5′-TAATC CTYGA RGGAC CACCA-3′) and Mst862R (5′-CCTAC GCCAC CRACM AC-3′) [25] for Methanosetaeae and order-specific primer MBT857F (5′-CGW AGG GAA GCT GTT AAG T-3′) and MBT1196R (5′-TGC CTG GTT CCA TTC TT-3′) [25] for Methanobacterales which are hydrogen-utilizing methanogens. The presence or absence of each microbial group was identified based on PCR amplification of each target gene. Both aceticlastic (Methanosarcinaceae and Methanosaetaceae) and hydrogenotrophic (Methanobacterales) methanogens were detected when PCR carried out with the specific primers for methanogens (data not shown). Acetate was not detected in the cathode and a BES injection into the cathode chamber did not reduce the level of methane production (Fig. 8A). This demonstrates that methane production in the cathode is mainly a hydrogenotrophic methanogen-associated process with a negligible contribution of aceticlastic methanogens.

Interestingly, methane was still produced at the cathode when the cathode compartment was autoclaved to eliminate the biogenic activity at 120 °C for 20 min as an abiotic control test, even though its quantity was relatively small compared to that of unaucloved cathode (Fig. 8B). Hence, another pathway for cathodic methane production is also expected. The direct conversion of an electrical current to methane (e.g., $\text{CO}_2 + 8\text{H}^+ + 8e^- \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$) or catalytic conversion of hydrogen to methane (e.g., $4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$) with the assistance of an external power source and a Pt catalyst is likely. If this is the case, then $\text{CO}_2$ originates from the anode and it is detected at consistently low levels in the cathode headspace.

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