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The effect of high applied voltages on bioanodes of microbial electrolysis cells in the presence of chlorides

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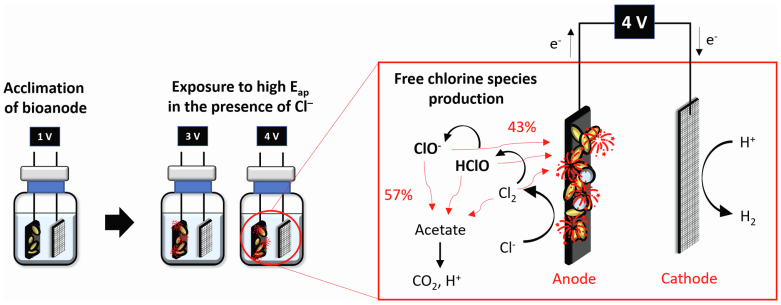
HIGHLIGHTS

- MEC operation at high applied voltages can damage the bioanodes.
- Chloride ions in MEC medium irreversibly inactivated bioanodes when adding 4 V.
- Chlorine species were mainly responsible for irreversible biofilm damage.
- Abiotic tests with same coulombs as biotic tests resulted in higher COD removals.
- Among total coulombs in biotic tests, 43% were consumed for microbial inactivation.

GRAPHICAL ABSTRACT



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ABSTRACT

While most microbial electrolysis cell (MEC) tests and other bioelectrochemical tests use applied voltages (E_{ap}) of 1 V or less, higher voltages are used in some tests that could lead to the generation of free chlorine species (FCS), from chloride ions, and hydroxyl radicals. To examine the impact of high E_{ap} on bioanodes, MECs were acclimated at E_{ap} = 1 V, tested for one cycle at an E_{ap} of 3 or 4 V until the same total coulombs were achieved as E_{ap} = 1 V, and then returned to cycles of E_{ap} = 1 V. All biotic MECs with chloride ions showed severe biofilm damage based on the absence of current production, lack of acetate oxidation, and the absence of hydrogen gas production in subsequent cycles at 1 V. Abiotic tests conducted at E_{ap} = 4 V, with same amount of total coulombs transferred as that which occurred in biotic tests at E_{ap} = 4 V, showed 1.8-fold higher acetate removal than biotic cells at 4 V, suggesting 43% of generated coulombs could have contributed to microbial inactivation. FCS generation, rather than hydroxyl radical production, was concluded to be the major contribution to oxidation of organics due to small changes in acetate oxidation in the presence of a hydroxyl radical scavenger, and the measurement of FCS. These results demonstrated that high applied voltages should be avoided if bioanodes are needed in bioelectrochemical systems when chloride species are present in the solution.

1. Introduction

Microbial electrolysis cells (MECs) are used for electrochemical hydrogen gas production from biological degradation of organic matter assisted by an external power source [1]. Anode-respiring exoelectrogenic microorganisms oxidize organic substances and release the electrons directly to the anode, which are then used at the cathode to produce hydrogen [2]. Many studies have demonstrated the successful operation of lab-scale and pilot-scale MECs for treating real wastewaters [3–5]. Because the oxidation of organic matter is

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thermodynamically favorable, with a potential of approximately −0.3 V (vs a standard hydrogen electrode, SHE), hydrogen gas production can occur at the cathode with only a small applied voltage (E_{ap} = 0.114 V in theory), with voltages typically restricted to a range of 0.2 to < 1 V. The use of E_{ap} < 1 V is usually chosen in order to minimize energy consumption by the MEC, so that a greater amount of energy can be recovered in the hydrogen gas produced compared to that used based on the applied voltage [6].

In several studies on bioelectrochemical systems (BESs), including MECs and anaerobic digesters with MEC-type electrodes added into the reactors, potentials much higher than 2 V to as much as 6 V have been used compared to those typical of MEC studies (≤1 V) [6–11]. For example, 3 V was applied for biohydrogen production using a single-chamber MEC to treat the liquid fraction of pressed municipal solid wastes [6]. In another study 4 V was applied to a BES to achieve nitrogen and organics removal [7]. It was assumed that organic matter would be degraded by exoelectrogens on the anode in addition to heterotrophic bacteria, while hydrogen gas produced from the cathode would be beneficial for autotrophic denitrification. In other studies on anaerobic digestion 2 or 3 V have been applied to electrodes to remove sulfides from the wastewater in order to avoid its evolution in the biogas [8]. In an MEC study where electrodes were operated at 0.8 V another separate pair of electrodes was immersed in the same solution and 3.5 V was applied to evolve oxygen for inhibiting methanogenesis [9]. A range of 0.2–6.0 V was used in MECs to induce efficient phosphate removal at the cathode while an acclimated bioanode was used for substrate oxidation [10]. High voltages were used in these previous studies to increase hydrogen production rates or drive other abiotic chemical reactions at the anode. Unfortunately, the use of high voltages can impact microbial reactions on the anode or in the solution due to the production of unintended products from abiotic reactions. However, the impact of high applied voltages on the chemicals released into the solution or the ability of the biofilm to sustain current generation when these high voltages are applied has not been examined in MECs or other BESs.

High applied voltages (> 1 V) have been used in certain electrochemical treatment systems to generate free chlorine species (FCS) from chloride ions and hydroxyl radicals to abiotically degrade organic matter. For example, electrochemical oxidation of organic matter has been used for removal of organic contaminants in various types of wastewaters [12] as well for the disinfection of microorganisms ranging from bacteria to viruses and algae [13]. Typically, these electrochemical systems are designed to optimize FCS production in order to generate chlorine gas from chloride ions and produce other reactive chemical species effective in oxidation of organic molecules [14]. The oxidation of Cl^- to Cl_2 can be more favorable at lower applied potentials than water splitting with the oxygen evolution reaction (OER) due to the more facile one electron transport for chloride oxidation than OER [15]. Depending on reaction conditions (e.g. the pH, concentration of chloride, and electrode materials), the three main FCS are chlorine gas (Cl_2), hypochlorous acid (HClO), and hypochlorite ion (ClO^-). FCS have a strong bactericidal activity and could damage multiple cellular components [16]. Different anode materials are used to enhance (mixed metal oxides) or reduce (boron-doped diamond, BDD) formation of FCSs [13,17]. In addition, the application of 1.2 V or more to carbon electrodes can lead to carbon oxidation that occur at anode potentials higher than 0.21 V (vs SHE) [18]. The generation of FCS in MECs, particularly for carbon-based electrodes commonly used in MECs, and anode oxidation reactions, however, have not been considered or examined in any previous MEC or BES studies at any applied voltage.

To determine the impact of applied voltages larger than 1 V in the presence of chloride ions in the media, higher applied voltages of 3 or 4 V were applied to MECs in the absence or presence of NaCl (50 mM) in the media. Commonly used anodes in MEC studies (i.e., graphite plates) were tested in mini-MECs, having a high surface area of electrodes per volume of reactor, in order to directly assess the potential for FCS on subsequent current generation. Abiotic experiments were also conducted under the same applied voltages, based on normalizing current and time for the same total amount of coulombs transferred, to directly examine substrate removals and the potential inactivation of bioanodes due to FCS generation. The potential impact of hydroxyl radicals was assessed by using a scavenger along with measurement of chlorine in the residual solutions.

2. Materials and methods

2.1. Reactor construction and setup

Single chambered mini-MECs were constructed as previously described [19] using serum bottles (5 mL, with a total of 8 mL capacity; Wheaton, Millville, NJ, USA). The anode was a graphite plate (Grade GM-10; GraphiteStore, Buffalo Grove, IL, USA) with dimensions of 1.5 cm × 1 cm × 0.32 cm to provide a projected surface area of 30 m^2/m^3 and a specific surface area of 92 m^2/m^3 based on the liquid volume. The anode was polished using sandpaper, sonicated in acetone for 20 min to remove debris, soaked in 1 N HCl overnight, and rinsed three times with deionized water before use. The stainless steel (SS) mesh (Type 304, mesh size 60 × 60; McMaster-Carr) was used as the cathode with the same projected area as the graphite plate anode. The anode and cathode were connected with a titanium wire (0.08 cm diameter; McMaster-Carr) to an external stainless steel wire (0.10 cm diameter; Malin Co.). The serum vials were filled with 5 mL of media and sealed with a thick butyl rubber stopper and aluminum crimp caps to maintain anaerobic conditions. The rigid titanium wires holding the electrodes were pierced though the stopper and held the electrode spacing to be ca. 0.5 cm.

2.2. Abiotic reactor operation

The medium for abiotic experiments was a 50 mM phosphate buffer solution (PBS, containing 2.45 g/L $NaH_2PO_4{\cdot}H_2O$, 4.58 g/L Na_2HPO_4, 0.31 g/L NH_4Cl, 0.13 g/L KCl; pH = 7.0, conductivity = 7.4 mS/cm) commonly used in MFC and other bioelectrochemical studies [20] with sodium acetate (1 g/L) as the sole organic source. The medium was further amended with 50 mM (2.92 g/L) of sodium chloride (NaCl) to examine the impact of current production at higher chloride concentrations [12]. Abiotic reactors were operated at applied voltages (E_{ap}) of 1, 3 or 4 V by using a potentiostat (VMP3, BioLogic, Knoxville, TN) along with a control (open circuit) for 1 h in the presence or absence of NaCl. Additional control experiments were conducted with 30 mM of sodium sulfate (Na_2SO_4, 11.7 mS/cm) and 60 mM of sodium perchlorate ($NaClO_4$, 12.5 mS/cm) as alternative electrolytes (no addition of 50 mM of NaCl), or PBS solution without any chloride (containing only $NaH_2PO_4{\cdot}H_2O$ and Na_2HPO_4, but no chloride ions; PBS (–) Cl^-), at a single applied voltage of 4 V. The concentrations of the sulfate and perchlorate electrolytes were chosen to produce a similar solution conductivity to that of 50 mM of NaCl (12.5 mS/cm). To investigate the contribution of hydroxyl radicals to organics removal, 30 mM of methanol was added to the solutions containing NaCl as a scavenger [21]. Media were injected into serum bottles by piercing the stopper using sterile syringe, sparged with ultra-pure nitrogen gas for 1 min, and autoclaved (121 °C, 15 min) before experiments. All abiotic experiments were conducted in duplicate at 30 °C without shaking.

2.3. Biotic reactor operation

The biotic reactors were identical to the abiotic reactors except they were inoculated with 1% (v/v) of anaerobic sludge collected from the anaerobic digester at the Pennsylvania State University wastewater treatment plant. Each cycle was one day with the medium completely replaced after each cycle using a syringe. After the first inoculation cycle, the inoculum was switched to 50% (v/v) of wastewater and

medium for 5 cycles, and then they were fed only with 5 mL of fresh PBS medium. Prior to each cycle the headspace was sparged with ultrapure nitrogen for 1 min to remove other residual gases. Bioelectrochemical studies were conducted after at least 3 successive similar current production profiles were observed.

The biotic tests were divided into three parts: acclimation, treatment, and recovery period. For the acclimation period, NaCl (50 mM, 2.92 g/L) was added to the medium from the initial start-up phase to acclimate microbial community to the higher-salinity solution compared to PBS. However, based on previous studies, salinity would not be expected to inhibit microbial growth for NaCl concentrations < 10 g/L [22,23]. Reactors designated R1–R6 were operated with E_{ap} = 1 V, while reactors R7–R9 were left under open circuit conditions as no-current controls. For the treatment period, the applied potential of reactors R1-R3 were set to 3 V, and R4-R6 were set to 4 V for one cycle. These higher voltages were applied for a period of time until the same total coulombs were transferred as that in the previous cycles at E_{ap} = 1 V. For the recovery period, the reactors were all re-set to an E_{ap} = 1 V and operated for an additional three cycles. Control biotic tests were conducted using PBS medium lacking chloride ions. For microbial growth and enrichment, the original PBS solution (containing KCl and NH_4Cl) was fed during the acclimation period and then the medium was switched to Cl-free PBS media (PBS (–) Cl^-) for the treatment under high voltage (3 or 4 V). For each cycle at varying applied voltage, the initial acetate concentration was maintained at the same level (1 g/L of sodium acetate; 780 mg COD/L). All biotic tests were conducted in triplicate at 30 °C without shaking.

2.4. Chemical analyses

At the end of batch cycle the composition of gas in the headspace was analyzed with a gas chromatograph (GC, SRI Instrument, Torrance, CA, USA) using 250 μL of gas from the headspace that was obtained using an airtight syringe (Hamilton, Reno, NV, USA). Gas volume was measured with syringe method by piercing needle through the rubber stopper and reading the volume of gas in the syringe after the pressure in the headspace dropped to ambient pressure. Chemical oxygen demand (COD) was analyzed before and after the reactor operation using standard methods (TNTplus COD reagent; HACH company). The FCS was measured by N,N-diethyl-p-phenylenediamine (DPD) colorimetric method (DPD free chlorine reagent powder pillows HACH company). The samples from biotic experiments for COD measurement were prepared by using a syringe filter with 0.45 μm of pore size. All measurements were performed in duplicate.

2.5. Linear sweep voltammetry

Linear sweep voltammetry (LSV) was performed with mini-MECs by placing the reference electrode (Ag/AgCl; +200 mV vs. SHE) between anode and cathode. The reference electrode was inserted through a hole cut in the rubber stopper using a cork punch. Three different electrolytes with varying chloride concentration were used: 50 mM PBS without any chloride ion, 50 mM PBS, and 50 mM PBS with 50 mM of NaCl. LSVs were conducted over a range of 0–4 V at a scan rate of 5 mV/s at 30 °C without shaking while current and anodic potential were recorded by potentiostat (VMP3, BioLogic, Knoxville, TN).

2.6. Calculations

The hydrogen production (V_{H2}) was calculated using $V_{H2} = (V_h + V_p)f_{H2}$, where V_h is a headspace volume, V_p is the amount of total gas production, and f_{H2} is a fraction of hydrogen in the total gas. The theoretical hydrogen production based on the integrated current over time (V_{th}) was calculated using $V_{th} = C_t V_m/2F$, where C_t is the total coulombs calculated by integrating the current over time until 90% of coulombs were achieved according to the I_{90} method [24], V_m is the molar gas volume (24.2 L/mol), and F is Faraday's number (96,500C/mol). The cathodic hydrogen recovery (γ_{CAT}) was calculated using $\gamma_{CAT} = V_{H2}/V_{th}$. Coulombic efficiency ($CE$) was calculated as CE = C_t/C_c, where C_c is the total charge consumed based on the acetate removal. To convert the acetate concentration to coulombs, the conversion factors of 8 electrons per acetate and 1.07 g COD per g acetate were used.

The current density was normalized by the liquid working volume (volumetric current density; I_V) or the anode specific surface area (current density per area; I_A). The current density for each experiment was obtained by averaging the top 10 current densities during each cycle according to previous reports [25].

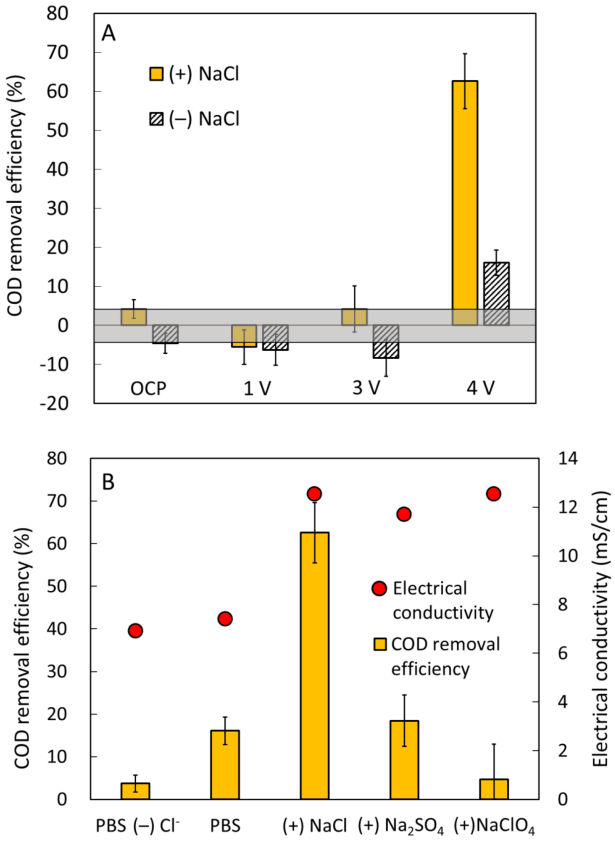

Fig. 1. COD removal efficiencies for acetate (1 g/L) in abiotic tests (A) as a function of the applied voltage in the presence and absence of 50 mM of NaCl, and (B) 50 mM PBS buffer without any chloride ion (PBS (–)Cl^- containing only $NaH_2PO_4{\cdot}H_2O$ and Na_2HPO_4, but no KCl or NH_4Cl), PBS in the absence or presence of NaCl, compared to two different sodium salts in the electrolytes (30 mM of Na_2SO_4 and 60 mM of $NaClO_4$) instead of NaCl. The sensitivity of the COD measurement (4% as COD removal efficiency) was presented as a grey area. Error bars show the errors based on the error propagation method using standard errors of the measured values.

3. Results & discussion

3.1. The effect of chloride ions on COD removal in abiotic tests

An applied voltage of 4 V was required to impact COD removal efficiencies in the presence of 50 mM of NaCl in abiotic tests (Fig. 1A). There was no consistent removal of COD (< 4.5%) at E_{ap} of 1 or 3 V, or under open circuit conditions after 1 h of operation. In contrast, there was 62.6 ± 7.1% of COD removal at E_{ap} = 4 V in the presence of 50 mM of chloride ion. These results demonstrated clear removal of organics due to the high applied voltage when 50 mM of NaCl was added into the electrolyte, presumably due to generation of FCS at the anode. A considerable amount of COD removal (16.1 ± 3.2%) without

50 mM of NaCl at E_{ap} = 4 V could be attributed to FCS generated from the low chloride ion concentrations in the PBS buffer (NH_4Cl, 0.31 g/L, 5.8 mM as Cl^-; and KCl, 0.13 g/L, 1.7 mM as Cl^-) or the production of other oxidants such as hydroxyl radicals. The linear relationship between chloride ion concentration and COD removal efficiency further suggested that chloride ions were responsible for acetate degradation at E_{ap} = 4 V (Fig. S1).

In several tests, there were apparent negative COD removal efficiencies based on averages of COD tests. Part of these negative values can be attributed to carbon from the anode that was released into solution during autoclaving of the reactors and electrolytes, as well as variability in measurements that were near the detection limit for the low COD range used for the sample analysis. Additional control tests were performed to confirm these assumptions by changing experiment conditions (e.g., the presence of acetate, autoclave process, and dilution factor). The amount of COD production was much lower when the autoclave process was eliminated, with details given in the Supporting Information (Fig. S2).

COD removal in the absence of NaCl. The results from a control test without any chloride ion (i.e. PBS without NH_4Cl and KCl) showed a much lower COD removal efficiency (3.7 ± 2.0%) than PBS or PBS with 50 mM of NaCl, indicating the contribution of FCS from chloride ions for acetate oxidation (Fig. 1B). A small amount of COD was still removed in the absence of chloride ions, suggesting that direct oxidation or other produced oxidants, for example hydroxyl radicals, were generated and contributed to acetate degradation.

To further confirm that COD removal efficiency was mainly due to FCS generation, two different sodium salts, Na_2SO_4 (30 mM) and $NaClO_4$ (60 mM), were used instead of the NaCl salt (Fig. 1B). Because all of three electrolytes had similar range of solution conductivity (11.7–12.5 mS/cm), a control (i.e. contain only PBS buffer and acetate, 7.4 mS/cm) was prepared to evaluate the potential impact of solution conductivity on acetate oxidation efficiency. COD removal efficiencies in the presence of 30 mM of Na_2SO_4 (18.4%) and 60 mM of $NaClO_4$ (4.7%) were still lower than that in 50 mM NaCl (62.6%), suggesting that the addition of chloride ions was the major reason for the increased oxidation of acetate and that solution conductivity was not a significant factor. These results are consistent with other reports that there was no appreciable decrease in COD concentrations in the absence of chloride ions [12]. For example in the presence of only F^-, PO_4^{3-}, SO_4^{2-}, and CO_3^{2-} there was little COD removal for arginic acid, compared to over 40% of COD removal using 50 mM NaCl-amended solutions [12]. The slightly higher COD removal with Na_2SO_4 compared to $NaClO_4$ observed here (Fig. 1B) could be due to the formation of sulfate radicals ($SO_4^{\bullet-}$) and persulfate, although these species are not expected to appreciably impact bulk oxidation of organics due to the slow kinetics in the absence of an activator such as heat and UV [26].

The results of these tests showed that the highest COD removal was observed for the electrolyte containing NaCl, followed by Na_2SO_4 and then other salts, which was the same order previously reported at constant current densities (100 mA/cm^2) using platinum electrodes [21]. $NaClO_4$ can be used as an inert supporting electrolyte as it cannot be oxidized to form FCS, which is consistent with its lowest COD removal efficiency here and that reported by others [27]. The pH of the medium was slightly reduced after 1 h of reaction but it remained in a circumneutral pH range (6.6–6.8) due to the use of the PBS buffer (Table S1).

Impact of a hydroxyl radicals on COD removal. An additional set of experiments was conducted to investigate the potential contribution of hydroxyl radicals to acetate oxidation. Hydroxyl radicals can be generated at high potentials from electrochemical water oxidation by a one-electron process ($H_2O \rightarrow \cdot OH + H^+ + e^-$, equilibrium potential (E^o) = 2.73 V) [28]. To investigate the possible contribution of hydroxyl radicals to acetate removal, an excess of methanol (30 mM) was added to scavenge hydroxyl radicals [21], although this increased the initial COD concentration by 194% to 1390 mg/L. Although COD

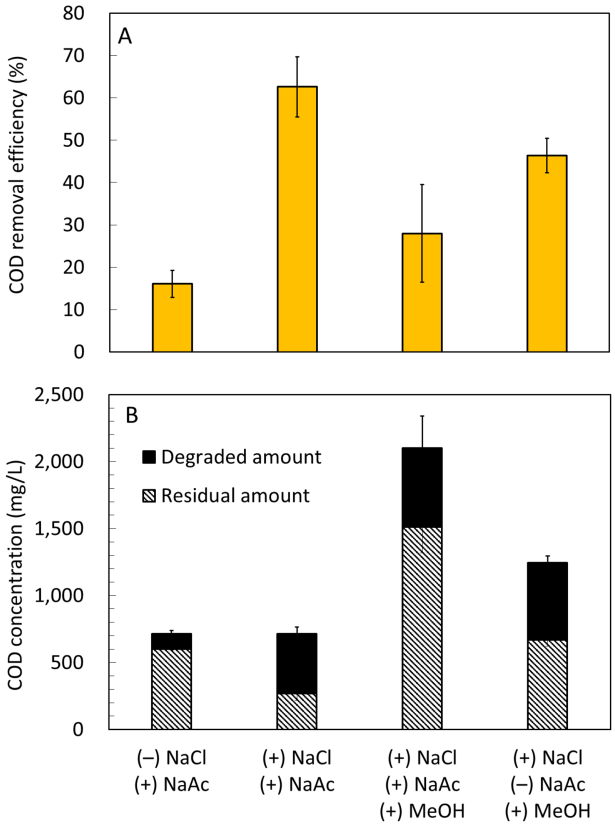

Fig. 2. (A) COD removal efficiency and (B) changes in COD concentrations in abiotic tests in the absence (control) or presence of 50 mM of NaCl in the electrolyte, and in the absence or presence of an hydroxyl radical scavenger (30 mM of methanol).

removal efficiency appeared to substantially decrease with the addition of methanol (Fig. 2A), the absolute mass of COD removed was essentially unchanged in the presence of methanol (Fig. 2B). This observation of a constant amount of COD removal suggests that hydroxyl radicals had a negligible contribution to the acetate oxidation in this study. Methanol as well as acetate could be also degraded by FCS, supported by the 578 mg/L of COD degradation in a control test with only having methanol as organic matter (Fig. 2B). Also, the absolute degraded COD amount was increased from 588 mg/L to 950 mg/L by increasing reaction time from 1 to 3 h in the test having both acetate and methanol. Considering that initial acetate concentration in the mixture was 780 mg COD/L, there was measurable methanol degradation due to FCS generation. It has also been reported that hydroxyl radical formation was negligible in chloride-containing wastewater tests using a boron doped diamond (BDD) in bacterial inactivation experiments [29].

The production of FCS in the presence of 50 mM NaCl was supported by measurements of 23.2 ± 3.2 mg as Cl_2/L at E_{ap} = 4 V, while its concentration was 0.3 ± 0.1 mg as Cl_2/L at E_{ap} = 3 V and below the detection limit at E_{ap} = 1 V after 1 h of operation. These results are in reasonable agreement with other reports of Cl_2 generation at E_{ap} = 4 V (5–6 mg/L) and smaller amounts at E_{ap} = 3 V (< 0.5 mg/L) for 1 h of operation, although chloride concentrations (12–20 mM) and the anode material (a mixed metal oxide) were different than those used here [30].

The presence of chloride ions in the solutions created conditions for a more favorable reactions at the anode, leading to increased current densities with higher applied voltages (Fig. 3). Voltage scans using LSV from 0 to 4 V showed that the presence of chloride ions increased the current density at applied voltages greater than approximately 3 V. The current density was increased more rapidly between E_{ap} of 3 and 4 V with the higher chloride ion concentration in the electrolyte, consistent

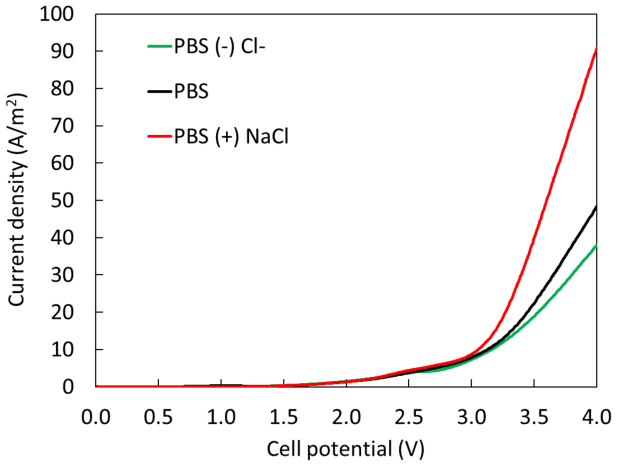

Fig. 3. Linear sweep voltammetry (LSV) curves in three electrolytes, 50 mM PBS without any chloride ion, PBS, and PBS with 50 mM of NaCl. Current density is normalized by the anode surface area.

with findings of larger COD removal efficiencies in these same solutions (Fig. 1B). From the perspective of thermodynamic potentials needed for different reactions, the equilibrium potential (E^o) for the oxygen evolution reaction (1.23 V) is lower than that for the chlorine gas evolution reaction (E^o = 1.36 V), which should make the oxygen evolution more favorable [31]. However, four electrons are required for oxygen evolution, compared to two electrons are required for chloride oxidation, which could make the oxygen evolution slower from a kinetics point of view. Thus, chlorine evolution usually outcompetes oxygen evolution at E^o greater than 1.36 V, which was observed here for the anodes at applied voltages of more than 3 V (Fig. S3). Overall abiotic test results in the present study demonstrated that the presence of chloride ions is the major factor to determine organic removal efficiency under applied voltages higher than 3 V.

3.2. Acclimation of bioanodes in MECs

The six MECs acclimated under the same conditions all produced similar current densities (Fig. 4A), reaching maximum average current densities of I_A = 4.7 ± 0.2 A/m^2 (I_V = 400 A/m^3) after 3–4 h from the start of each cycle. After this peak current, the current density started to decrease, reaching a low constant value of ~ I_V = 80 A/m^3 after ~ 13 h, presumably due to the depletion of the substrate. A similar maximum current density of I_A = 5.7 A/m^2 using different MEC configuration was obtained by others at 1 V [32], with 45% less current (2.6 A/m^2) at a lower applied voltage of 0.7 V in the same mini-MECs [19] (Table 1).

At the end of the cycle, the COD removal efficiency was 89 ± 3%, with a 134 ± 17% coulombic efficiency and 55 ± 14% cathodic hydrogen recovery (Table 1). In the open circuit controls, there was no gas production and only 8.3% of the COD was removed, indicating that the largest percentage of removed COD was utilized for cell synthesis [33]. A coulombic efficiency over 100% is frequently observed in single-chamber MECs due to hydrogen cycling [34]. In the absence of a membrane or separator between the electrodes, hydrogen gas produced by the cathode can be oxidized by hydrogenotrophic methanogens, homoacetogens, or exoelectrogens such as *Geobacter sulfurreducens* on the anode. The fact that significant current was generated after the acetate was likely depleted suggested the existence of anodic hydrogen oxidation (Fig. 4A). Because there was negligible methane production from all MECs (< 0.03% in the headspace composition), methanogenesis was likely not a factor in the loss of cathodic hydrogen gas. The possibility of homoacetogenesis is consistent with lower COD removals and cathodic hydrogen recoveries than previously reported values from similar MEC operating conditions (Table 1). The relatively long cycle time (i.e. one day) after current generation greatly decreased in a batch cycle likely contributed to hydrogen cycling. Long hydraulic retention times (HRTs) in a continuous single chambered MEC was reported to produce more severe hydrogen cycling than shorter HRTs [34]. Taken together, these observations indicated that microbial groups including anode-respiring bacteria and homoacetogens were likely enriched during acclimation and operation.

3.3. MEC operation with high applied voltages

After acclimation at E_{ap} = 1 V, three of the MECs were operated for one cycle at 3 V (R1–R3) and three others were operated at 4 V (R4–R6). Based on matching the total coulombs of each treatment (59 C) by controlling the cycle time, the 3 V MECs produced I_V = 1500 ± 200 mA/m^3 and were operated for 2.3 h, while the MECs operated at 4 V produced 8000 ± 900 mA/m^3 for 0.4 h (Fig. 4B and 4C). During this cycle, 12.1% of acetate was degraded using E_{ap} = 4 V, while no measurable COD decrease was observed for at E_{ap} = 3 V. Both results suggest that application of these higher voltages did not provide proper conditions for microbial activity by the bioanode.

Following a single cycle at 3 or 4 V, the applied voltage was restored to E_{ap} to 1 V. However, no current was produced and there was no measurable COD removal over the next cycle (Fig. 4D and 5). This lack of current generation indicated that the microbial community on the anode was inactivated after being exposed to E_{ap} of 3 or 4 V, likely due to the FCS generation observed in abiotic tests. Although FCS generation at E_{ap} = 3 V for 1 h was only 0.3 ± 0.1 mg as Cl_2/L, the addition of 0.1 mg as Cl_2/L was found to produce a 0.5 log inactivation of *Pseudomonas aeruginosa* in only 5 min [35]. The exact mechanism how FCS inactivate microorganisms is not fully understood, but it is generally accepted that the primary reaction involves oxidation of the cell membrane which causes leakage of essential macromolecules from the cells [36]. In some cases microorganisms can repair the cellular damage after treatment if they are not fully inactivated [37]. However, no microbial activity was observed over three successive cycles by replenishing MECs with fresh media. Considering that current was generated from the first cycle and reached an appreciable current density within three cycles when MECs were enriched during the acclimation period, no current generation at all (< 0.001 mA) during three cycles indicated severe deterioration of the bioanode. In contrast, in control tests without any chloride ions in PBS, there was an obvious recovery of the anode biofilm after 3 or 4 V was added. When the reactors were returned to 1 V there was appreciable current production in the first cycle and a continuous increase in the amount of current in the successive three cycles (Fig. S4). After three cycles at E_{ap} = 1 V, 89 ± 3% (after 3 V) and 73 ± 3% (after 4 V) of COD was removed suggesting that the electroactive microbial community survived despite of the exposure at high voltage in the absence of chloride ions. The lower maximum current density in this recovery phase than acclimation period might be due to the extensive oxygen exposure or partial biofilm detachment by rapid gas production during the high voltage treatments. There was no direct evidence of oxidation of the graphite anode due to the application of a high voltage on the time scales used here based on no observable changes in medium color or turbidity. However, when 4 V was applied for a longer period of 3 h (403 C transferred), there was a clear change in the color of the solution (Fig. S5).

3.4. Comparison between biotic and abiotic tests

To compare acetate consumption in biotic with abiotic results, E_{ap}'s of 3 or 4 V were applied under abiotic conditions until the total coulombs transferred was approximately 59 C. The cycle time and current density were similar between two conditions at each E_{ap} = 3 or 4 V (Fig. 5A and C). However, COD removal efficiencies in abiotic tests were higher than biotic tests in both external voltage conditions

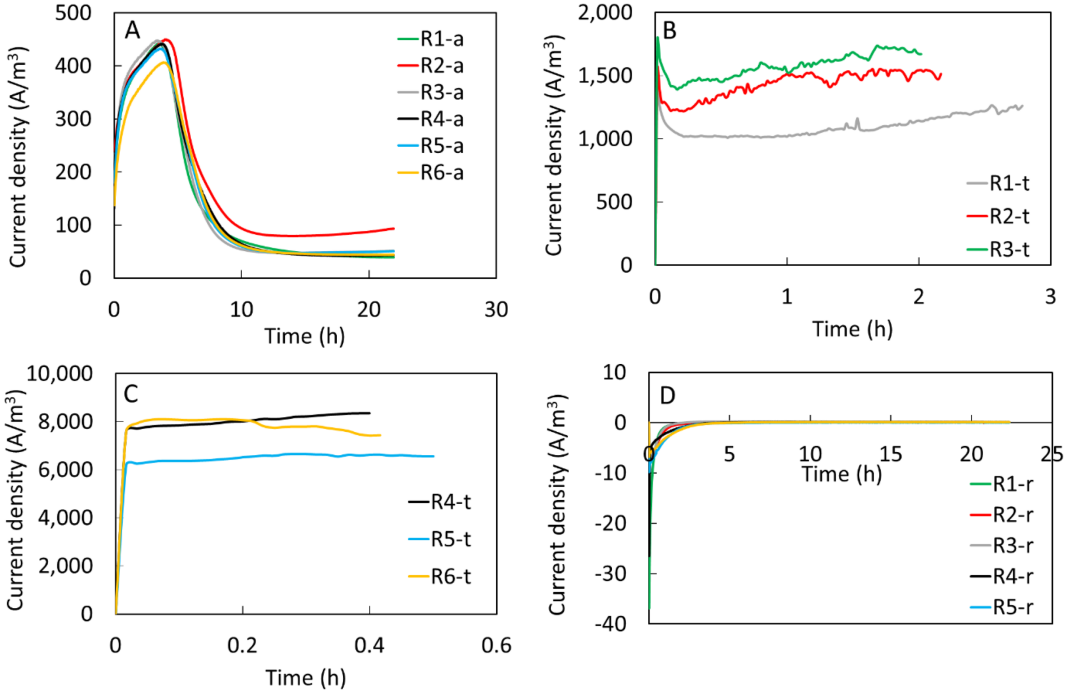

Fig. 4. Volumetric current density at (A) acclimation period at 1 V, (B) treatment period at 3 V, (C) treatment phase at 4 V and (D) recovery period at 1 V of biotic tests.

(Fig. 5B). The COD removals were 22.0% (abiotic test) and 12.1% (biotic test) with E_{ap} = 4 V, indicating that 1.8 times more acetate was degraded when there was no biofilm on the anode. At high E_{ap} in the presence of chloride ions, organic matter could be oxidized directly by the anode or indirectly by electrogenerated FCS [38]. From the abiotic test results in Section 3. 1 (Fig. 1), a majority of acetate degradation seemed to occur indirectly via reactions with FCS. However, unlike the abiotic condition, FCS generated from biotic tests could contribute to microbial disinfection as well as acetate oxidation. Since the same total coulombs were produced from biotic and abiotic tests at E_{ap} = 4 V, the difference of COD removal between these two tests can be interpreted as the coulombs (equivalent as COD values) used for biofilm inactivation. As a result of calculation, from the ~ 59 C transferred between the electrodes a total of 9.5 C (abiotic) and 5.4 C (biotic) were utilized for acetate degradation. The difference between two, 4.1 C, was thus concluded to have contributed to microbial inactivation likely through generation of the FCS and the oxidation of soluble products released from microbial lysis.

The observed mechanisms at E_{ap} of 1 or 4 V in this study were different in the presence and absence of chloride ions (Fig. 6). Because an E_{ap} = 1 V could not produce FCS in the present study, stable and healthy microbial activity was possible at the bioanode with 89% acetate removal (Fig. 6A). Hydrogen cycling was also observed with a functioning bioanode. In contrast, there was no observable microbial activity after 4 V was applied to the cell due to microbial inactivation by FCS (Fig. 6B). As a result of conversion of CODs to coulombs from

Table 1
Performance of MECs in this study compared to previous reports using MECs at different applied voltage.

E_{ap} (V)	Anode	Cathode	Substrate	COD removal (%)	CE (%)[a]	I_V (A/m^3)[b]	I_A (A/m^2)[c]	γ_{CAT} (%)	Reference
0	Graphite plate	SS mesh	Acetate	8.3 ± 2.1	–[d]	–	–	–	This study
1.0	Graphite plate	SS mesh	Acetate	89 ± 3	134 ± 17	400 ± 20	4.7 ± 0.2	55 ± 14	This study
3.0	Graphite plate	SS mesh	Acetate	−2.7 ± 4.7	–	1500 ± 200	16.5 ± 2.5	–	This study
4.0	Graphite plate	SS mesh	Acetate	12 ± 2	–	7700 ± 900	83.2 ± 9.7	–	This study
0.7	Graphite plate	SS mesh	Acetate	–	90	240	2.6	–	[19]
0.7	Carbon cloth	CC/Pt[e]	Acetate	90	89	160	–	108	[44]
0.8	Graphite felt	CC/Pt[e]	Acetate	–	–	360	–	90	[45]
1.0	Carbon cloth	CC/Pt[e]	Acetate	–	69	–	5.7	63	[32]
1.0	Graphite Brush	CC/Pt[e]	Acetate	–	–	1830	–	93	[25]
2.0	Carbon felt	Carbon felt	Acetate	76	–	3.6	0.7	–	[11]
3.0	Carbon cloth or SS mesh	Carbon cloth or SS mesh	Dairy manure	–	–	–	–	–	[8]
3.0	Graphite felt	Ti/RuO_2 mesh	LPW[f]	74	152	116	–	10.3	[6]
4.0	Graphite	Graphite	Glucose	–	33[g]	16	–	–	[7]
6.0	Carbon fiber tissue electrodes	Pt-coated carbon	Glucose	–	–	–	–	–	[10]

[a] Coulombic efficiency.
[b] Volumetric current density based on the working volume.
[c] Current density based on the anode specific surface area.
[d] Not available.
[e] Carbon cloth with Pt catalyst.
[f] The liquid fraction of pressed municipal solid waste.
[g] Calculated based on the autotrophic denitrification process.

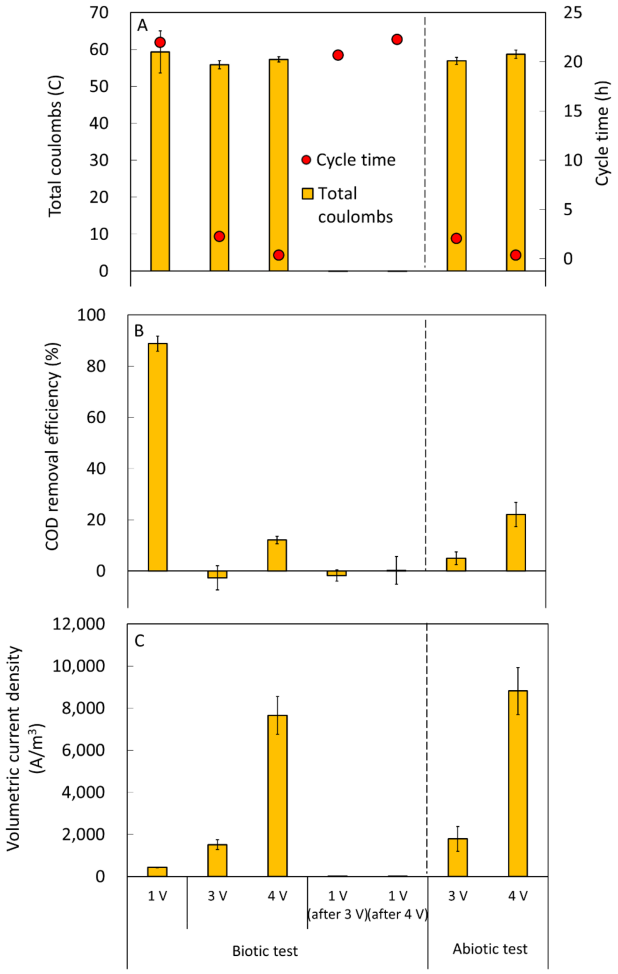

Fig. 5. The electrochemical performance from biotic and abiotic tests. (A) total coulombs, (B) COD removal efficiency and (C) volumetric current density.

biotic and abiotic tests, we estimate that 43% of generated coulombs were used for inactivation of the biofilm. Because the pH of the medium after reaction at E_{ap} = 4 V was in the range of 6.8–6.9 (Table S1), most FCS would have been present as HClO and ClO^-, rather than Cl_2 [39]. FCS mainly exists as HClO in acidic or neutral solution thus HClO, which exhibits stronger oxidation capacity than ClO^-, should be more abundant in the solution [40].

3.5. Implications for high applied voltage application in bioelectrochemical systems

The results of this study have shown that microbial activity on bioanodes will be inhibited if high external voltages are applied in the presence of chloride ions. Particular caution is required when the applied voltages are 3 V or higher. With E_{ap} = 4 V in this study, a considerable amount of organic matter (12.1%) was degraded and more hydrogen gas (2.4-fold) was generated compared to E_{ap} = 1 V, but the bioanode was completely inactivated due to the high applied voltage. If electrodes like those used here are operated in reactors with high suspended biomass concentrations in the bulk solution (e.g. anaerobic digestion combined with MEC), hydrogen gas will be generated at the cathode but the FCS produced at the anodes will likely damage anodic bacteria and possibly suspended biomass.

The relative impact of the inhibitory effects of FCS produced from high voltages will be affected by specific reactor configurations and operating conditions, such as the amount of electrode area relative to the volume of the reactor and the specific substrates and substrate concentrations. For example, previous experiments using high voltages have been conducted with much smaller projected surface areas (electrode areas per volume), with A_P = 4 m^2/m^3 [8] and A_P = 12 m^2/m^3 [6], compared to A_P = 30 m^2/m^3 (projected area facing the cathode) and A_s = 92 m^2/m^3 (total projected area of all sides) used here. In addition, complex organic matter (dairy manure and the liquid fraction of pressed municipal solid waste) was used in these previous studies which must be degraded to simple substrates for microbial current generation. The conversion of particulate organic matter to soluble substrates is considered as a relatively slow step in anaerobic digestion [41]. The FCS generated using these complex substrates could therefore aid in breakdown of particulate organic matter and complex organics to smaller molecules. Here we only used acetate which can be directly used by bacteria for current generation. The differences in anode surface area and substrates might be reasons why negative impacts on microbial activities were not reported in those studies, while severe deterioration of current generation and COD removal was observed in the present study. Further studies are needed to investigate the impact of the FCS in MECs with different types of substrates.

The production of hydrogen gas at any E_{ap} can be beneficial for MECs or MECs combined with other BESs as long as the amount of FCS is not sufficient to inhibit overall microbial activities. However, chloride ions are ubiquitous in many wastewaters, especially with high salinity toilet wastewaters [30] and food wastes [42]. Treating these wastewaters by electrode-assisted bioreactors with high external voltages would inevitably result in FCS production. Furthermore, there are additional considerations when using high voltages other than FCS generation, such as additional operating costs and potential

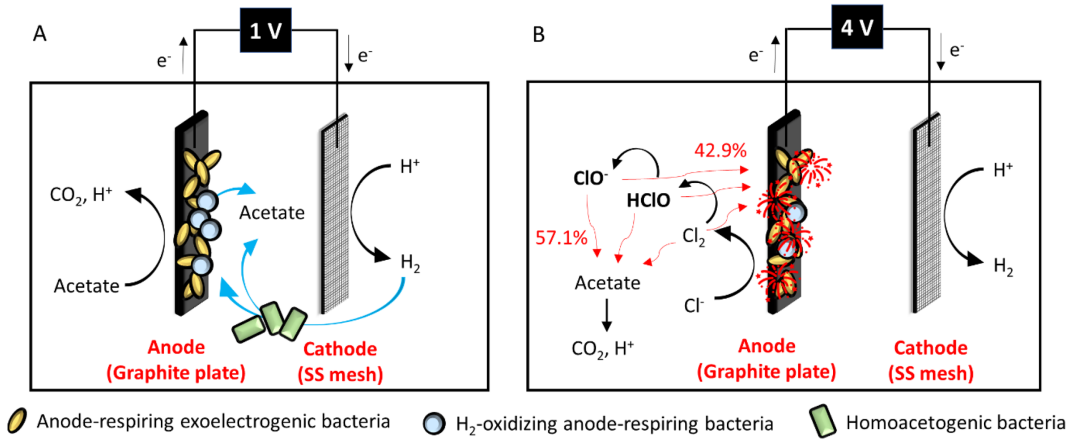

Fig. 6. Proposed bioelectrochemical mechanism of MEC at (A) 1 V and (B) 4 V in the presence of chloride ion.

degradation of the anodes. A previous review concluded that minimizing total costs of MECs would require minimizing applied power and reducing internal resistances [43]. Using a higher E_{ap} to increase current production would increase power input and thus reduce the overall energy efficiency, although it could produce more H_2 gas, potentially increasing operating costs. Also, the use of high applied voltages with anode materials commonly used in MECs, such as graphite blocks or brushes, carbon cloth, or carbon felt, could result in oxidation of the material as shown here using graphite blocks after only 3 h of a high applied voltage (Fig. S5). Therefore, when MECs are operated at high voltages for efficient wastewater degradation the impact of FCS as well as other issues related to the operating costs and the integrity of the materials on the overall process needs to be carefully considered.

4. Conclusions

The significant inhibitory effect of a high E_{ap} (4 V) on bioanodes of MECs was demonstrated by the addition of the 50 mM of chloride ions to PBS. When MECs were acclimated at E_{ap} = 1 V and exposed to E_{ap}'s of 3 or 4 V until same total coulombs were transferred, significant and irreversible deterioration of microbial activity was observed, showing a loss of current generation and decrease in COD removal efficiency. Abiotic experiments with the same coulombs as biotic treatments resulted in 1.8-fold higher COD removal than biotic experiments, suggesting 43% of generated coulombs were consumed for microbial inactivation. FCS production was concluded to be mainly responsible for the observed COD removal in abiotic experiments, while the effects of solution conductivity or hydroxyl radical production provided minor contributions based on measurements in electrolytes other than NaCl and the use of a radical scavenger (methanol). The overall observations suggest that E_{ap}'s higher than those typically used in MECs can cause the inhibitory effects on the bioanode microbial community under chloride-existing environment due to the inactivation effects of FCS.

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.2020.126742.

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