Evaluation of limiting factors for current density in microbial electrochemical cells (MXCs) treating domestic wastewater

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This study quantitatively assessed three limiting factors for current density in a microbial electrochemical cell (MXC) treating domestic wastewater: (1) buffer concentration, (2) biodegradability, and (3) particulates. Buffer concentration was not significant for current density in the MXC fed with filtered domestic wastewater (180 mg COD/L). Current density reduced by 67% in the MXC fed with filtered sewage having similar COD concentration to acetate medium, which indicates poor biodegradability of soluble organics in the wastewater. Particulate matters seriously decreased current density down to 76%, probably due to the accumulation of particulates on biofilm anode. Our study quantitatively showed that buffer concentration does not limit current density much, but biodegradability of soluble organics and fermentation rate of particulate matters in domestic wastewater mainly control current density in MXCs.

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1. Introduction

Microbial electrochemical cells (MXCs) that include microbial fuel cells, microbial electrolysis cells, and microbial desalination cells show a promise as sustainable wastewater treatment due to resource recovery (e.g., electric power, H2, CH4, water, H2O2, etc.). However, substantial energy loss in MXCs would trade off the profits of resource recovery, especially for large scale systems, and hence existing studies did not show clear benefits of MXCs, as compared to other anaerobic biotechnologies (e.g., anaerobic membrane bioreactors) [23]. In wastewater treatment perspectives, MXCs still have significant merit of no aeration requirement. Anode-respiring bacteria (ARB) that oxidize organic wastewater and transfer electrons to the anode in MXCs are anaerobes, which mean that MXCs can treat wastewater without significant oxygen supply. Aeration costs account for 30–50% of operating and maintenance costs in municipal wastewater treatment facilities [33]. For instance, MXCs application to sewage treatment would save ~$1.5 billion annually in Canada.

To improve current density is crucial for MXC application to domestic wastewater treatment, since it represents wastewater treatability. Volumetric current density (A/m3 of anode chamber) is equivalent to organic loading rate (kg COD/m3 d), one of the most important design and operating parameters in wastewater treatment facilities. Organic loading rate typically ranges from 0.9 to 1.2 kg COD/m3 d in activated sludge [24,31], while it depends on the concentration of chemical oxygen demand (COD) in given domestic wastewater. MXCs should produce ~150 A/m3 of volumetric current density (equivalent to ~1 kg COD/m3 d) for sewage treatment, while subsequent polishing step seems essential to meet wastewater effluent standards. Lefebvre et al. (2013) [18] reported high current density of 110 A/m3 in an MXC from domestic wastewater, mainly due to high packing density of anodes in a small anode chamber (15 mL of working volume). In comparison, most of literature employing relatively large MXCs has commonly shown small current density from 0.4 to 43 A/m3 for domestic wastewater [19,35,36]. Feng et al. [9] recently reported the maximum current density of 0.43 A/m3 in a large-scale Mxc (1 m3), despite of using carbon brush anode, which implies the challenge of achieving high current density in large MXCs treating sewage.

There are many parameters that are able to influence current density in MXCs, including microbial community on biofilm anode, pH, temperature, oxygen, separator, cathodic catalysts, biodegradability of substrate, alkalinity, biofilm conductivity and so on [7,8,20,21,26,28,30,34]. Microbial community would show functional redundancy consistently once kinetically-efficient ARB are well proliferated on biofilm anode [1,29]. The limitations in cathodic reaction or ohmic resistance can be alleviated by using better materials or optimizing M XC design [6,20]. However, characteristics of wastewater are uncontrollable factors that can
substantially affect the substrate-utilization rate of ARB and current density in MXCs [17,27]. When municipal wastewater is compared to acetate medium, there are three key differences: [1] biodegradability, [2] alkalinity, and [3] presence of particulates. Literature have commonly reported that the biodegradability of the wastewater was very poor, as compared to acetate, which seems to account for low current density in sewage-treating MXCs [1,9,36]. However, it is daring to conclude that poor biodegradability of domestic wastewater mainly decreases current density in the MXCs because the other two important factors of alkalinity and particulates can also limit current density in the MXCs. For instance, it is well known that low alkalinity can acidify a part of biofilm anode, which can seriously decrease current density in MXCs [12,34]. Alkalinity concentration in the domestic wastewater, however, is extremely lower than that in the acetate medium having 50–200 mM phosphate buffer [1,11,25].

Particulates are also present in municipal wastewater and they can directly block the formation of ARB biofilm on the anode, reducing current density in MXCs [14,34]. Alternatively, competitive microorganisms (e.g., methanogens) present in particulates can divert substrate electrons to other electron sinks than coulombs [4,28], which can finally dilute ARB biofilm density on the anode and decrease current density and coulombic efficiency in MXCs. There are, however, no studies that quantitatively evaluate the three limiting parameters separately in MXCs treating domestic wastewater, while those factors co-exist in the wastewater.

The goal of this study is to identify a main limiting factor for current density in MXCs treating domestic wastewater. To exclusively assess biodegradability of domestic wastewater, and the effects of alkalinity and particulates on current density, a dual-chamber MXC was operated with acetate medium, and filtered and raw domestic wastewater as alkalinity concentration was varied.

2. Material and methods

2.1. Microbial electrochemical cell (MXC) configuration

A dual chamber microbial electrochemical cell (MXC) was used for this study. Briefly describing MXC design, two cylindrical plexiglass tubes consisted of anode and cathode chambers, and anion exchange membrane was placed between the two chambers. By integrating carbon fibers with a stainless steel current collector, the anode surface area per membrane was increased at 1600 m²/m² approximately, along with electrode distance less than 1 cm. The literature [2] provides detailed information on MXC configuration; current density was expressed per the surface area of the membrane for simplicity in this study.

2.2. Inoculation, feed, and start-up

Recycle activated sludge (RAS) was collected from the Waterloo Wastewater Treatment Plant (Waterloo, Ontario, Canada) to inoculate the MXC. 15 mL of RAS was added to the anode chamber, the chamber was sparged with ultra-pure nitrogen for 30 min before being fed to the MXC. Medium pH was constant at 7.5 ± 0.15. A reference electrode (Ag/AgCl reference electrode, MF-2052, Bioanalytical System Inc. USA) was placed within ~1 cm distant from the anode to fix the anode potential at ~0.4V vs. Ag/AgCl reference electrode using a potentiostat (BioLogic, VSP, Gamble Technologies, Canada). The cathode chamber was filled with tap water in which hydrogen gas is produced. Under this potential mode, cathode potential responds to current density and over-potentials in the MXC [17,35]. The applied voltage (cathode potential–anode potential) was constant at 0.85 ± 0.5 V during the acclimation phase. Electrode potentials and currents were recorded at every 60 s using EC-Lab for windows v 10.23 software in a personal computer connected with the potentiostat. The MXC was mixed at 150 rpm using a multi-position magnetic stirrer (Model 650, VWR International Inc. Canada), and operated in a temperature-controlled room at 25 ± 1 °C. The MXC was run with 25 mM acetate medium in batch mode for over 3 months until steady-state current density (18 ± 2 A/m² of membrane) was achieved. Then, the MXC was operated in continuous mode. Acetate medium or domestic wastewater (filtered and raw) was fed to the MXC at a flow rate of 37.5 mL/h using a cartridge-type peristaltic pump (Master Flex® L/S digital drive, Model 7523-80, Cole-Parmer, Canada) to maintain hydraulic residence time (HRT) of 8 h in the anode chamber.

2.3. Experimental conditions

MXC performance and effluent quality were evaluated with different feed conditions at a fixed HRT of 8 h. First, buffer concentration effect was assessed with acetate medium (2.7 ± 0.2 mM, 175 ± 10 mg COD/L) amended with 50 mM or 5 mM bicarbonate buffer (Run 1 and 2). Then, wastewater biodegradability against acetate medium was investigated at Run 3. To avoid particulate (i.e., SS) effects on current density, particulates were separated from the wastewater in two filtration steps using glass fiber filters (Fisherbrand glass fiber filter, 1.6 μm, G6, Cat. No. 9-804-55 A) and glass microfiber filters (Whatmann microfiber filter, 1.2 μm, GF/C, Cat. No. 1822-070). The average soluble COD (SCOD) for the domestic wastewater was close to the COD concentration of the acetate medium. Table 1 summarizes the characteristics of the domestic wastewater. At Run 4, buffer effect on current density was re-assessed in the MXC fed with the filtered wastewater having 50 mM bicarbonate buffer. At Run 5, the MXC was operated with the acetate medium having 5 mM bicarbonate buffer to recover current density. After that, SS collected from the wastewater were added to the acetate medium at Run 6. To collect SS, the domestic wastewater was centrifuged at 5000 rpm for 15 min with a centrifuge (Beckman TJ-6 Tabletop Centrifuge, Beckman Coulter Inc. CA, USA). The SS was added to the acetate medium (L) having 230 ± 28 mg SS/L, which is close during experiments the medium was autoclaved and then sparged with the ultra-pure nitrogen for 30 min before being fed to the MXC. Medium pH was constant at 7.5 ± 0.15.

A reference electrode (Ag/AgCl reference electrode, MF-2052, Bioanalytical System Inc. USA) was placed within ~1 cm distant from the anode to fix the anode potential at ~0.4V vs. Ag/AgCl reference electrode using a potentiostat (BioLogic, VSP, Gamble Technologies, Canada). The cathode chamber was filled with tap water in which hydrogen gas is produced. Under this potential mode, cathode potential responds to current density and over-potentials in the MXC [17,35]. The applied voltage (cathode potential–anode potential) was constant at 0.85 ± 0.5 V during the acclimation phase. Electrode potentials and currents were recorded at every 60 s using EC-Lab for windows v 10.23 software in a personal computer connected with the potentiostat. The MXC was mixed at 150 rpm using a multi-position magnetic stirrer (Model 650, VWR International Inc. Canada), and operated in a temperature-controlled room at 25 ± 1 °C. The MXC was run with 25 mM acetate medium in batch mode for over 3 months until steady-state current density (18 ± 2 A/m² of membrane) was achieved. Then, the MXC was operated in continuous mode. Acetate medium or domestic wastewater (filtered and raw) was fed to the MXC at a flow rate of 37.5 mL/h using a cartridge-type peristaltic pump (Master Flex® L/S digital drive, Model 7523-80, Cole-Parmer, Canada) to maintain hydraulic residence time (HRT) of 8 h in the anode chamber.

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Table 1

The characteristics of domestic wastewater.

| Parameter                  | Demand (mg/L) |
|----------------------------|---------------|
| Total chemical oxygen demand (TCOD) | 660 ± 10 mg/L |
| Soluble chemical oxygen demand (SCOD) | 185 ± 20 mg/L |
| Total suspended solids (TSS) | 260 ± 15 mg/L |
| Volatile suspended solids (VSS) | 225 ± 10 mg/L |
| Volatile fatty acids (VFAs) | ≤5 mg COD/L |
| pH                        | 8 ± 0.05    |
| Alkalinity                 | 200 ± 50 mg/L as CaCO₃ |
to SS concentration in the wastewater (see Table 1). At Run 7, the domestic wastewater was directly used as substrate for the MXC. A feed tank was continuously mixed with a magnetic stirrer (Model VS-C4, VWR International Inc., Canada) at 200 rpm to avoid sedimentation of SS for Run 6 and 7. Data was collected after current density reached at steady state in each condition. Table 2 summarizes different feed conditions.

| Run | Set     | Substrate                          | Buffer concentration             | The maximum current density (A/m²) |
|-----|---------|------------------------------------|----------------------------------|-----------------------------------|
| 1   | AB50    | Acetate medium                     | 50 mM bicarbonate                | 1.2 ± 0.25                        |
| 2   | AB5     | Acetate medium                     | 5 mM bicarbonate                 | 0.9 ± 0.1                         |
| 3   | FW      | Filtered domestic wastewater       | –                                | 0.3 ± 0.25                        |
| 4   | FWB50   | Filtered domestic wastewater       | 50 mM bicarbonate                | 0.3 ± 0.15                        |
| 5   | AB5     | Acetate medium                     | 5 mM bicarbonate                 | 1.7 ± 0.15                        |
| 6   | AB5-SS  | Acetate medium with suspended solids| 5 mM bicarbonate                 | 0.4 ± 0.07                        |
| 7   | RW      | Raw domestic wastewater            | –                                | 0.5 ± 0.10                        |

3. Results and discussion

3.1. Determination of pseudo, apparent \( K_s \) in the MXC

Fig. 1 shows current density at various acetate concentrations, which follows a typical Monod pattern. The maximum current density \( j_{\text{max}} \) was 6.43 A/m² of membrane, and the best-fit of \( K_s \) was estimated at 17.3 mg COD/L. The simulated curve with the estimated \( K_s \) measured \( j_{\text{max}} \) and measured acetate concentration well fitted into experimental data (Fig. 1). The pseudo, apparent \( K_s \) does not represent the half-maximum substrate concentration of ARB for acetate because current density was expressed per the projected area of membrane, instead of anode surface area; the literature provides more detailed information on this aspect [17,35]. However, this pseudo, apparent \( K_s \) is able to provide useful information on the relationship between substrate concentration and current density in the MXC. For instance, the simulation with Eq. (1) predicts 3.9 A/m² for effluent SCOD of 26 mg/L (only 9% error). Hence, this pseudo, apparent \( K_s \) can be used for a design parameter of MXCs.

3.2. The effects of buffer concentration and substrate biodegradability on current density

Table 2 shows an average of the maximum current density observed in the MXC at different feed conditions. The maximum current density was small at 1.2 ± 0.25 A/m² for Run 1 (bicarbonate buffer 50 mM), due to substrate limitation (acetate 2.7 ± 0.2 mM and 175 ± 10 mg COD/L); in comparison, the maximum current density was 18 ± 2 A/m² at 25 mM acetate during acclimation. SCOD concentration in domestic wastewater was 185 ± 20 mg/L close to 2.7 mM acetate. This substrate limitation for current density explicates why MXCs cannot generate high current density from domestic wastewater, although the wastewater would be

**Table 2**

Feed conditions and current density in the MXC.

**Fig. 1.** Observed and simulated current density as a function of acetate concentration. Current density to acetate was simulated with the best-fit \( K_s \), and Eq. (1).
completely available for ARB, like acetate. Literature commonly reported low current density in a range of 0.18–2.4 A/m² in MXC fed with domestic wastewater [1.5,32]. At Run 2, the decrease of bicarbonate buffer from 50 to 5 mM reduced current density down to 0.9 ± 0.1 A/m² (25% reduction, based on 1.2 ± 0.25 A/m² at Run 1), which indicates partial acidification of anode biofilm due to proton accumulation, as expected [12,34]. However, this current reduction is relatively small as compared to literature. Torres et al. [34] reported more than 60% current reduction from ~6 A/m² to 2 A/m² when phosphate buffer decreased from 50 mM to 12.5 mM. This result implies that alkalinity effect on current density would be small for the MXC treating domestic wastewater, since low substrate concentration or other limiting factor already limits ARB catabolism and current density.

At Run 3 (filtered domestic wastewater) current density substantially decreased down to 0.30 ± 0.1 A/m², as compared to 0.9 ± 0.1 A/m² at Run 2 (67% reduction). The alkalinity in the domestic wastewater was 250 ± 50 mg/L as CaCO₃ which is equivalent to the buffer concentration of 5 ± 1 mM as HCO₃⁻ in acetate medium for Run 2. Therefore, the considerable reduction of current density at Run 3 clearly indicates that organic compounds in the wastewater are not readily available for ARB. Low current density was kept at Run 4 where filtered domestic wastewater was supplemented with high bicarbonate buffer 50 mM. This consistent, low current density confirms that the biodegradability of domestic wastewater for ARB is one of the key factors responsible for low current density in MXC, not the buffer concentration. Acclimation of ARB with acetate medium for over 3 months would shift complex microbial community structures mainly to acetate-utilizing ARB, as shown in the literature [16,15]. Furthermore, the microbial community structure analysis for the MXC acclimated under similar operating conditions in our previous study also supports that the biofilm anode would primarily consist of acetate-utilizing ARB with small numbers of non-ARB (e.g., fermenters, methanogens, homoacetogens) [13]. When complex forms of organic compounds in domestic wastewater are exposed to the ARB, their substrate-utilization rate can be significantly limited. Trivial acetate present in domestic wastewater (not detected in our study) or generated from fermentation of complex organics via small numbers of non-ARB (due to filtration) would be used by ARB for current generation in MXC. Therefore, the community structure on biofilm anode that well balances hydrolyzing fermenters, H₂ scavengers, and ARB is preferred for domestic wastewater treatment, since the fermenters and H₂ scavengers break down complex organics in the wastewater into simple acids available for ARB; then current density can be improved in sewage-treating M XC. Our study evidently proves that alkalinity effect on current density is not important in MXC treating domestic wastewater. Instead, the biodegradability of the wastewater is significant for current density in the M XCs.

3.3. Particulate effects on current density

At Run 5 (acetate and 5 mM bicarbonate buffer), the current density was recovered from 0.30 ± 0.1 A/m² to 1.7 ± 0.2 A/m². However, the current density sharply dropped to 0.4 ± 0.15 A/m² again at Run 6 (76% reduction against 1.7 ± 0.2 A/m² at Run 5) in which SS collected from the domestic wastewater was added to acetate medium; SS concentration was 230 ± 28 mg/L in the anode chamber, which is close to the average SS concentration in the domestic wastewater (260 ± 15 mg/L). This substantial reduction of current density at Run 6 shows that particulate matters seriously prevent ARB from generating current in anode biofilm. Particulate substances can attenuate current generation via several routes. Particulates readily accumulate on biofilm anode [1,29], and the accumulated particulates can alleviate substrate diffusion from bulk liquid to biofilm anode, accentuating substrate limitation. In addition, particulates can mitigate the opportunity of ARB to proliferate on the surface of the anode and expel existing ARB from the biofilm due to space competition. The growth of non-ARB (e.g., fermenters or methanogens) present in SS can compete with ARB for substrate, and as a result current density can be decreased [4,28]. Particulates can also limit extracellular electron transfer, since their inert fractions accumulated on biofilm anode can deteriorate the conductivity of anode biofilm matrix or bother the diffusion rate of shuttling compounds between ARB and the anode [30]. It is interesting to observe the slight increase of current density from 0.4 ± 0.15 A/m² at Run 6 (acetate with particulates) to 0.5 ± 0.15 A/m² at Run 7 (raw domestic wastewater). This current density at Run 7 is even higher than 0.30 A/m² observed at Run 3 and 4 (filtered wastewater with and without 50 mM bicarbonate buffer). The small increase of current density at Run 7 is not meaningful in terms of energy recovery, but seems to provide a clue on how to improve current density in M XCXs treating domestic wastewater. Particulates added to the anode chamber at Run 6 mainly worked as physical/chemical barriers to ARB metabolism in anode biofilm or extracellular electron transfer, as discussed above. It is expected that air exposure during SS collection (30–45 min) would suppress the activity of anaerobic microorganisms present in SS, so the syntrophic interactions between ARB and non-ARB (fermenters and methanogens) would not be promoted well. In comparison, particulates introduction to the anode together with the domestic wastewater (Run 7) can stimulate the syntrophic interactions (fermenters, H₂ consumers, and ARB) [7,13], which can efficiently provide substrate available for ARB (e.g., acetate and H₂). The current density of 0.5 A/m² at Run 7, which is 0.2 A/m² higher than that at Run 3 and 4, supports the importance of the syntrophy, since the number of non-ARB would be trivial in the anode for Run 3 and 4 (filtered wastewater). Hence, stimulation of the syntrophic interactions seems very critical for improving current density in MXC treating domestic wastewater.

A simple way of driving the syntrophy is to extend HRT for the anode. Fermenters proliferated in suspension would better offer acetate and H₂ to ARB at longer HRT. Recent literature presents current increase in MXC fed with mixture of propionate and acetate at longer HRT due to improved propionate fermentation to acetate and H₂ [7,13]. However, the increase of planktonic fermenters driven by long HRT will deteriorate effluent water quality (e.g., TCOD and SS). HRT increase also means the large footprint of M XC system (more investment costs). Thus, M XCs need advanced reactor configurations that allow long solids retention time (SRT) for fermenters with short HRT. Membrane separation, packed-bed, sludge blanket, or fluidized bed integrated with the anode enables M XCs to keep SRT long, but HRT short. Such reactor designs can strengthen the syntrophic interactions between ARB and fermenters, and improve current density and effluent quality.

3.4. COD removal

Fig. 2A shows SCOD concentrations in feed and effluent, and its removal efficiency. Effluent SCOD concentrations were quite constant at ~55 mg/L for the M XC run with acetate medium, except for Run 6 (acetate medium mixed with suspended solids). As expected, SCOD removals observed for both raw and filtered domestic wastewater were much lower than the acetate medium (25–30% in the wastewater vs. ~70% in acetate medium). Poor biodegradability of the wastewater would decrease COD removal, as observed in the evolutions of current density. SS addition to the
acetate medium apparently reduced SCOD removal efficiency from 70% to 41 ± 6% at Run 6. Fig. 2B shows effluent SCOD concentrations as a function of current density; organic loading rates were constant at ~0.5 kg SCOD/d m³ of anode chamber during experiments. No relationship between effluent SCOD concentration and current density was observed, which is totally different from the Monod relationship between effluent SCOD concentration and current density lower than 0.5 A/m² in Run 3, 4, 6, and 7, which evidently supports the significance of particulates and biodegradability of domestic wastewater for generating high current density.

4. Conclusions

Buffer concentration did rarely affect current density in the MXC fed with filtered sewage ~180 mg COD/L. The biodegradability of domestic wastewater for ARB was one of the key factors to reduce current density in the MXC, not buffer concentration. Particulate matters critically prevented ARB from generating current in anode biofilm, showing 76% reduction of current density. Direct utilization of raw sewage improved current density up to 20%, indicating the significance of fermenters and their syntrophy with ARB.

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