Bioelectrochemically-assisted ammonia recovery from dairy manure

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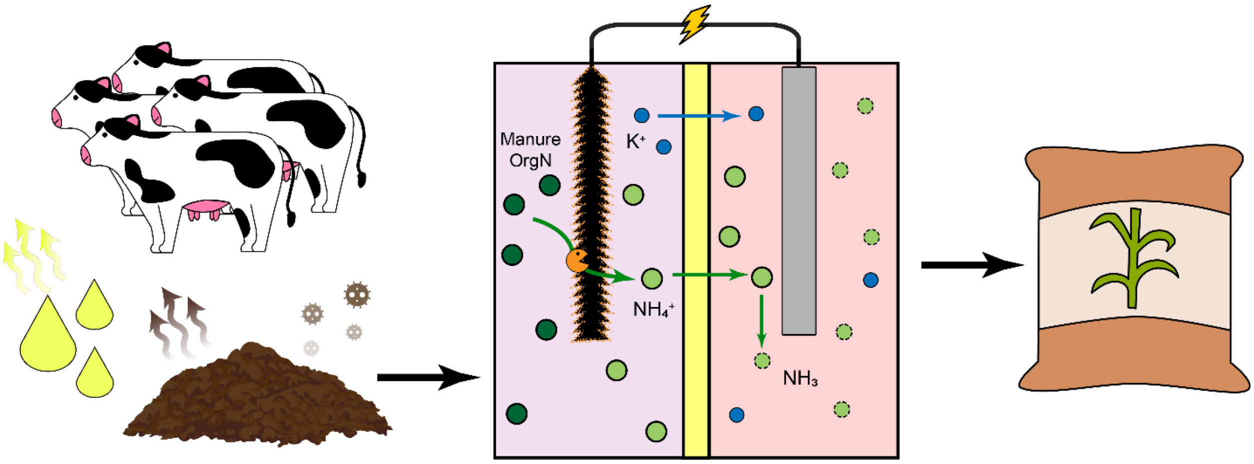
Abstract

The sustainability of direct land application of dairy manure is challenged by significant nutrient losses. Bioelectrochemical systems for ammonia recovery offer a manure management strategy that can recover both ammoniacal and organic nitrogen as a stable ammonia fertilizer. In this research, a microbial fuel cell (MFC) was used to treat two types of dairy manure under a variety of imposed anode compartment conditions. The system achieved a maximum coulombic efficiency of 20 ± 18% and exhibited both COD and total nitrogen removals of approximately 60%. Furthermore, the MFC showed a maximum organic nitrogen removal of 73.8 ± 12.1%, and no differences in organic nitrogen (orgN) removal were detected among different conditions tested. Decreasing concentrations of anolyte ammonia nitrogen coupled with the observed orgN removal from the anolyte indicate that the MFC is effective at recovering orgN in dairy manure as ammoniacal nitrogen in the catholyte. Additionally, ion competition between NH_4^+ and other relevant cations (Na^+, K^+, and Mg^{2+}) for transport across the CEM was investigated, with only K^+ showing minor competitive effects. Based on the results of this research, we propose three key processes and two sub-processes that contribute to the successful operation of the MFC for nitrogen recovery from dairy manure. Bioelectrochemical systems for nitrogen recovery from dairy manure offer a novel, robust technology for producing a valuable ammonia nitrogen fertilizer, a thus far untapped resource in dairy manure streams.

Keywords: microbial fuel cell, resource recovery, manure management

Graphical Abstract:



1. Introduction

In 2022, the United Nations projected global population to grow to approximately 10 billion by 2050 (Population Division, 2022), which is estimated to correspond to a 30% to 62% increase in global food demand (Van Dijk et al., 2021). The ever-increasing food demand will intensify the pressures on agricultural production, leading to an increased reliance on nitrogenous fertilizers. Fixed nitrogen is most often the limiting element required for plant growth, with ammonia (NH_3) being the most common form of nitrogen used in fertilizers (Lassaletta et al., 2014; Sinclair and Rufty, 2012; Tilman et al., 2002). Upwards of 80% of NH_3 produced chemically in the US, a total of around 140 million tons, is used in fertilizer production (Lim et al., 2021; Tullo, 2021; Wang et al., 2021). This NH_3 is predominantly produced via the Haber-Bosch process, a high-temperature and pressure thermochemical nitrogen fixation reaction that combines atmospheric nitrogen and hydrogen gas to produce NH_3 (Lim et al., 2021; Smith et al., 2020; Wang et al., 2021). The Haber-Bosch process constitutes almost 1% of global energy use and is estimated to contribute 1.4% of total carbon dioxide (CO_2) emissions from anthropogenic sources (Capdevila-Cortada, 2019; Lim et al., 2021; Liu et al., 2022). With such staggering numbers attributed to the production of NH_3, it is imperative that more environmentally friendly and sustainable methods of NH_3 production be developed.

Increased demand for animal products has led to similar increases in production of animal wastes, such as manure. Livestock manure is a mixture of animal feces, urine, and other system by-products (e.g., wash waters, waste feed), and often contains large quantities of organic matter, pathogens, and nutrients. The nitrogen content present in on-farm manure makes it an attractive supplement to manufactured nitrogenous fertilizers for application to crop fields, a manure management strategy known as direct land application. Crop soils amended with manure directly are subject to significant nutrient losses through runoff and volatilization (in the case of NH_3-N), with one study citing as much as 12.87 mg L^{-1} of total nitrogen (TN) in runoff in as little as 5 minutes after a rain event (Jokela et al., 2016; Little et al., 2005). Furthermore, up to 80% of manure nitrogen is in the organic form (e.g., proteins and amino acids), which needs to be mineralized to inorganic forms (e.g., NH_4^+) before it can be successfully used by plants (Kessel and J, 2002; Long et al., 2018).

Direct land application operates under the premise that organic nitrogen (orgN) will undergo *in-situ* mineralization to NH_4^+, a microbially-mediated biogeochemical process that can be slow and difficult to control (Barbarika et al., 1985; Bernal and Kirchmann, 1992). Due to this slow bioavailability of nitrogen, manure is often applied to fields in excess of nitrogen demand from plants, which increases the likelihood of nutrient runoff and volatilization. Approximately 60% of NH_3 emissions in the U.S. are attributable to livestock manure, mostly from its storage and after direct land application (Aguirre-Villegas et al., 2023; EPA, 2021). Engineered manure management techniques, such as solid-liquid seperation (SLS) and anaerobic digestion (AD), are currently employed to help mitigate the impact of manure on the environment. While these technologies can recover valuable products, like biogas used for renewable energy in AD and even low density ammonia fertilizer in SLS, no existing technology takes advantage of organic nitrogen mineralization to recover the organic nitrogen fraction of manure in a more usable form (Bareha et al., 2018; Dinuccio et al., 2008; Lee et al., 2013). Bioelectrochemical systems (BES) offer a manure management strategy that recovers both existing ammoniacal nitrogen and converts organic nitrogen to NH_4^+ for recovery. BES employ principles of microbiology and electrochemistry to engineer systems which utilize microbe-mediated degradation reactions to treat waste streams and produce value-added products, such as electricity, hydrogen fuel, or concentrated chemical solutions (Burns and Qin, 2023; Carucci et al., 2022; Gul and Ahmad, 2019; Kelly and He, 2014; Losantos et al., 2021; Lu and Ren, 2016). Microbial fuel cells (MFCs) are one type of BES which rely on electrogenic microorganisms to degrade the organics present in waste streams while spontaneously producing current based on extracellular electron transfer (Almatouq et al., 2020; Logan et al., 2006; Virdis et al., 2008; Wang and Ren, 2013). When an ion exchange membrane separates the electrode chambers of the MFC, ionated forms of nutrients, such as NH_4^+, can be recovered in the catholyte as a cleaner, more concentrated chemical stream. Such recovery of NH_3 not only decreases the likelihood of nutrient toxicity to microbes in the anode compartment, but also concentrates the nutrients in separate solution for recovery, therefore producing a more marketable and dosable fertilizer supplement (Carucci et al., 2022; Cerrillo et al., 2023; Kelly and He, 2014; Losantos et al., 2021; Rodríguez Arredondo et al., 2015; Rodríguez Arredondo et al., 2017; Zhang et al., 2014).

This research investigates an MFC operated for NH_3 recovery from dairy manure. This work specifically investigated the anodic processes related to orgN-to-NH_4^+ mineralization and NH_4^+ removal from the anolyte, and as such, reconcentration and recovery of NH_3 gas from the catholyte was outside the scope of this work but is studied in detail elsewhere (Kuntke et al., 2012; Li et al., 2020; Liu et al., 2016; Rodríguez Arredondo et al., 2015). The objectives of this work were to (1) demonstrate effective manure treatment and orgN removal in an MFC operated with source-separated, recombined feces + urine and dairy manure slurry (containing excess food, debris, urine, feces, bedding wash water, etc.), (2) investigate the impacts of low-level aeration in the anode compartment, supplemental NH_4Cl addition in the anolyte, and the open circuit condition on MFC performance, orgN removal, and competition for NH_4^+ transport from anode to cathode via the CEM, and (3) propose a greater understanding of anodic processes in an MFC operated for NH_3 recovery from the orgN fraction of dairy manure.

2. Materials and Methods

2.1. Experimental operations

2.1.1. MFC experiments

Experiments were conducted using custom-built, stacked-plate reactors, as described in Burns and Qin (2023). Briefly, anode and cathode compartments were separated by a cation exchange membrane (CMI 7000S, Membranes International, Inc., Ringwood, NJ, USA) and electrodes were carbon brush and carbon cloth coated with platinum-carbon catalyst (area = 39 cm^2) for anode and cathode, respectively. Electrodes were connected by a 10Ω external resistor for the duration of the experiments. Figure S1 in the Supporting Information provides a schematic of MFC experimental set up. Experiments were run in batch mode, with electrolyte solution continuously recirculated from external reservoirs at a rate of 76 mL min^{-1} to ensure sufficient mass transfer conditions. The generated current was continuously monitored and recorded using a data acquisition system (Keithley Instruments, Tektronix, Inc., Beaverton, OR, USA).

Electrolyte solutions varied depending on the specific conditions in this study, as reported in Table 1. For each named condition, at least 3 replicate cycles were conducted. Two different dairy manures were used as anolyte substrates in this study. Conditions 1 and 2 were characterized by the use of dairy cow feces and urine mixed in a 1:5 (*m:v*) ratio (UW Madison Dairy Cattle Center, Madison, WI, USA). Such combination of source-separated feces and urine allowed for greater control over the anolyte composition in the early conditions tested in this work. Conditions 3 through 5 used dilute dairy manure slurry ("DMS", Blaine Dairy Cattle Center, Arlington, WI, USA) as the anolyte. DMS is a complex mixture of feces, urine, bedding, food, debris, wash water, etc., that is collected and commonly stored in manure storages at dairy farms. Multiple imposed conditions were investigated to enhance cell performance.

Condition 2 had controlled aeration in the anode compartment (DO $\leq$ 0.1 mg L^{-1}) to investigate if the orgN mineralization process could be enhanced without harming the strictly anaerobic exoelectrogens, as orgN mineralization can happen anaerobically or aerobically. Aeration was accomplished by purging air through gas-permeable silicone tubing (5-inch active length, OD 0.125 inches, ID 0.062 inches, Silastic laboratory tubing #MFLX96115-14, Masterflex, Radnor, PA, USA) suspended through the anode compartment (Fig. S2) for a period of 10 minutes twice daily (Yang et al., 2017). Dissolved oxygen concentration was continuously monitored throughout condition 2 in the anolyte reservoir via probe (FDO 925-P, WTW by Xylem Analytics, Weilheim, Germany). Anolyte in conditions 1 and 2 was sonicated for 5 minutes (Sonicator XL2015, Heat Systems, Inc., New Hartford, NY, USA) immediately prior to use to break up large solids (Shen et al., 2018; Syed et al., 2022). Condition 4 included supplemental NH_4Cl addition at a concentration of 8.38 mM in the anolyte to investigate the impacts of high NH_4^+ concentrations on system performance. Condition 5 was tested at open circuit (OC) to investigate ion transport across the membrane in the absence of electromotive forces.

Anolyte in all conditions was strained immediately prior to use to remove large solids. At the start of each batch cycle, approximately 50% of the anolyte volume was replaced to retain vital biomass in the anode chamber. The catholyte in all conditions was deionized water and was replenished as needed (from evaporation, leakage, etc.) or when the conductivity of the catholyte exceeded 3.2 mS cm^{-1}, as this conductivity indicated the experimentally-determined threshold for catholyte ion capacity. To accurately account for any dilution of initial concentrations, electrolyte solutions were recirculated for approximately 10 minutes at the start of each cycle before initial samples were collected for analysis.

Table 1. Anolyte compositions and conditions for each of the experimental Conditions tested in this research. The catholyte for all conditions was DI water. Anolyte in conditions 1 and 2 was sonicated and strained immediately prior to use in the reactor, and anolyte in conditions 3, 4, and 5 was strained immediately prior to use in the reactor.

Condition	Anolyte Composition/Condition
1	5 g feces + 25 mL urine in DI water
2	5 g feces + 25 mL urine in DI water with minimal aeration (DO ≤ 0.1 mg L^{-1})
3	Dilute dairy manure slurry (DMS)
4	DMS + 8.38 mM NH_4Cl
5	DMS at open circuit (OC) condition

2.2. Analytical methods

2.2.1. Sample analyses

All electrolyte samples were analyzed for pH and conductivity (Fig. S3) via benchtop electrode probe and meter (Orion Versa Star Pro, Thermo Fisher Scientific, Waltham, MA, USA). The chemical oxygen demand (COD), total nitrogen (TN) and combined ammoniacal nitrogen (NH_3/NH_4^+-N) for each electrolyte sample were measured using standard colorimetric methods (COD Digestion Vials High Range, Total Nitrogen Persulfate Digestion Test 'N Tube, and High Range Ammonia Nitrogen AmVer Salicylate Test 'N Tube, Hach, Loveland, CO, USA). Due to the high pH value and aeration of the catholyte solution, much of the catholyte ammoniacal nitrogen is removed via air-stripping. Measurement of this air-stripped NH_3 is outside the scope of this study, which focuses on anode chamber processes. Ammoniacal nitrogen removal from the anolyte solution is used as a proxy for the theoretical NH_3 recovery achieved by the MFC system. Concentrations of ions of interest (i.e., Na^+, Mg^{2+}, K^+) of selected representative samples were determined via ion chromatography (Thermo Dionex 2100 Ion Chromatography System, Thermo Fisher Scientific, Waltham, MA, USA). Nitrate and nitrite concentrations in both electrolyte solutions were tested periodically throughout the experimental duration, such that samples from at least one representative cycle in each condition were tested. The nitrate and nitrite concentrations remained below detectable limits for the duration of this study (data not shown). Organic nitrogen (orgN) concentrations were calculated according to Eq. 1:

$$orgN = TN - NH_3 \qquad \text{(Eq. 1)}$$

where TN is the measured concentration of total nitrogen in the sample and NH_3 is the measured concentration of ammoniacal nitrogen (NH_3/NH_4^+ combined) in the sample. Only aggregate orgN is reported in this paper (i.e., summed concentration of all organic nitrogen compounds) due to limitations in measurement capabilities.

2.2.2. Computational analyses

All measurements reported in section 3 are average values of replicate experiments in each condition. The removal efficiency for COD and TN in the anolyte was calculated by

$$R = \frac{C_i - C_f}{C_i} * 100 \qquad \text{(Eq. 2)}$$

where R is the percent removal, C_i is the initial anolyte concentration of the nutrient of interest, and C_f is the final anolyte concentration of the nutrient of interest. Electrochemical performance was evaluated on the basis of total charge transferred, Q, between the anode and cathode and the nitrogen removal efficiency, R_N, equations 3 and 4, respectively. Q is defined as the area under the current-time curve, calculated as:

$$Q = \int_0^t I * dt \qquad \text{(Eq. 3)}$$

where I is the current (in Amperes) at time t. Columbic efficiency, CE, is the ratio between charge transfer (Q) and electrons generated from COD removal, and provides a metric for how efficiently the MFC converts COD to electrical current. CE is calculated as:

$$CE = \frac{32Q}{4FV_{an}\Delta COD} \qquad \text{(Eq. 4)}$$

where 32 is the molecular weight of oxygen, Q is the total charge transferred (Eq. 3), 4 is the number of electrons exchanged per mole of oxygen consumed, F is Faraday's constant (96,485 Coulombs per mole of electrons), V_{an} is the volume of the anolyte, and ΔCOD is the difference in COD from start to end of the experimental cycle (Logan et al., 2006). R_N is a measure of how current is partitioned to drive nitrogen transport (as NH_4^+) across the CEM, and was calculated for cycles in conditions 1, 2, 3, and 4 (Eq. 5):

$$R_N = \frac{V_{an}F(TN_{i,an} - TN_{f,an})}{Q} \qquad \text{(Eq. 5)}$$

where V_{an} is the total volume of the anolyte (in L), F is Faraday's constant (96,485 Coulombs per mole of electrons), $TN_{i,an}$ and $TN_{f,an}$ are the initial and final concentrations, in M, of total nitrogen in the anolyte, respectively (Burns and Qin, 2023). It is important to note that R_N calculated via

Eq. 5 relies on the assumption that the only TN removal from the anolyte is as ammonium ion transport across the CEM. Further discussion of this is included in section 3.1.2.

Statistical analyses were performed to determine the validity of apparent differences in average values for COD, TN, and orgN removal in different conditions, as well as differences in average Q and R_N between conditions. All statistical analyses were performed in Python using the *statsmodels* package (Seabold and Perktold, 2010). Separate linear models were created with condition as the independent variable and attributes of interest as the dependent variable. Analysis of variances (ANOVA) was run on each linear model, and significant difference was determined as $p < 0.05$. Post-hoc Tukey tests were performed using the models that ANOVA determined had significant difference, to determine which conditions specifically differed. Again, significance was determined as $p < 0.05$.

3. Results and Discussion

3.1. Influence of anolyte conditions on MFC performance

To investigate the influence of anolyte conditions on the system performance, two types of dairy manure and various compartment conditions were tested in the MFC as listed in Table 1. The manure + urine mix used in Conditions 1 and 2 had an initial organics concentration (measured as COD) of 1,400 ± 95 mg L^{-1}, which was more dilute than DMS used in condition 3 through 5 with a COD concentration of 14,000 ± 204 mg L^{-1}. The TN concentration was 450 ± 10 mg L^{-1} and 780 ± 21 mg L^{-1} for the manure + urine mix and DMS, respectively. The dilute nature of the recombined manure + urine mix allowed for slow introduction of complex organics to the MFC system, which had previously only operated with synthetic manure anolyte (Burns and Qin, 2023). The initial NH_3-N concentration was 370 ± 38 mg L^{-1} in the manure + urine mix and 470 ± 25 mg

L^{-1} in the DMS, further reflecting the more dilute nature of the manure + urine mix compared to the DMS.

To explore the orgN mineralization processes in MFC, various anode compartment conditions were imposed on the MFC alongside the different manure types. For example, condition 2 introduced minimal aeration directly to the anode compartment. The hypothesis is that controlled aeration could enhance the orgN mineralization without harming the anaerobic electrogenic microorganisms. OrgN mineralization is a widely observed microbial process that can happen either anaerobically or aerobically. The majority of research on orgN mineralization comes from aerobically-incubated soil experiments, leading us to investigate the impacts of aeration on our system (Barbarika et al., 1985; Girkin and Cooper, 2023; Kandeler, 2007). Condition 4 added supplemental NH_4^+, in the form of NH_4Cl, to the anolyte. We hypothesized that orgN mineralization was the limiting step in NH_3 recovery processes in the MFC, considering that microbes first have to mineralize orgN to NH_4^+ before it can be transported to the catholyte and recovered as $NH_{3(g)}$.

3.1.1. COD and TN removal

The MFC effectively removed COD and TN in all conditions, as shown in Figure 1. In conditions 1 and 2 with the manure + urine mix substrate, the MFC achieved a COD removal of 52 – 59%, corresponding to between 950 ± 200 mg L^{-1} and 1100 ± 130 mg L^{-1} of absolute removal. In comparison, in Conditions 3-5 with DMS substrate, the COD removal was between 25 and 40%, or 3000 ± 2600 mg L^{-1} and 5500 ± 3400 mg L^{-1} absolute removal. Note that, although the absolute COD removal is higher in conditions 3-5 than in conditions 1 and 2, the initial COD concentration in the later conditions was much higher (section 3.1). In order to make a fair comparison, COD removal is normalized to the initial COD concentration with the COD removal percentage (Eq. 2).

As shown in Fig. 1a, the COD removal in condition 2 was 59 ± 6%, much higher than that in condition 3, which was 25 ± 18%. The decrease in COD removal in condition 3 is likely due to the increased complexity of the DMS introduced as the anolyte in this condition, and is indicative of the microbial community's required adaptation time when substrates are changed. Additionally, it is of note that the COD removal did not decrease significantly from condition 1 to condition 2, despite the introduction of low-level aeration in condition 2. This finding suggests that the low-level aeration did not adversely affect the strictly anaerobic microbes responsible for COD removal and current production (also discussed in section 3.1.2). COD removals varied much more in the DMS conditions, particularly in conditions 3 and 5, than in either of the feces + urine conditions (Fig. 1a). We infer that this is due to the higher concentration of the DMS, as well as the increased complexity of this substrate. The consistency in COD removal across both types of manure and most conditions indicates that the MFC is able to achieve stable organics removal, regardless of the manure type and cell conditions.

For TN removal, the MFC achieved removal efficiencies between 61 ± 8.5% in condition 1 and 22 ± 18% in condition 5 (Fig. 1a). TN removal in condition 5 is much lower than other conditions ($p < 0.02$). The low TN removal in condition 5 confirms that, in the absence of current flow and the corresponding electromotive force for NH_4^+ transport across the CEM, there is only limited TN removal from dairy manure in the MFC. The feces + urine conditions (1 and 2) demonstrated higher TN removal when compared to modified DMS anolyte conditions (conditions 4 and 5), and no significant difference when compared to unmodified DMS (condition 3). The consistent TN removal across both types of unmodified manures is again indicative of the stability of this treatment system regardless of manure composition.

3.1.2. Electrochemical performance

In all conditions where current flow was present (conditions 1-4), the total charge transferred between anode and cathode (*Q*) was higher compared to previous research using synthetic manure substrates (Burns and Qin, 2023). This can be attributed to the higher COD concentration real manure compared to that of synthetic substrates, which provides a richer source of electron donors in the anode compartment. We observed a large increase in *Q*-values for DMS conditions compared to feces + urine conditions. For example, Q was 950 ± 127 C in condition 1 while it was 1,880 ± 540 C in condition 3 (Fig. S4). This increase in total charge transferred is in part due to the more concentrated nature of the DMS, specifically in organics concentration, since organics degradation contributes the most to current production (Burns and Qin, 2023; Logan et al., 2006; Ren et al., 2008; Virdis et al., 2008). It is important to note that the cycle time for the different manure conditions varied from approximately 4 days in conditions 1 and 2 to 7 days in conditions 3 and 4, which could also contribute to the increase in *Q* between manure types based on the calculation in Eq. 3 (Fig. S4). As such, Columbic efficiency (*CE*) and maximum current density achieved are also useful in evaluating system performance.

Columbic efficiency is a critical metric to provide a measure of how efficiently the MFC converts organic matter to electrical current. *CE* is calculated as the ratio of charge transferred from anode to cathode to the amount of electrons generated from COD removal (Eq. 4) (Logan et al., 2006). The MFC exhibited a maximum *CE* of 20 ± 18% in condition 3 and a minimum *CE* of 6.9 ± 1.2% in condition 4. Despite this broad range, no statistical differences between *CE* across conditions were observed (one-way ANOVA, F = 1.74, $p = 0.217$, overall large effect $\omega^2 = 0.128$). The maximum current density, which is the maximum current normalized by the active area of cathode electrode, is also quite stable in all conditions. In the feces + urine conditions, the MFC achieved maximum current densities ranging from 1.72 ± 0.30 A m^{-2} in condition 1 to 1.32 ± 0.02

A m^{-2} in condition 2. The drop in maximum current density in condition 2 compared to condition 1 could be indicative of a decrease in electroactive species in the aerated condition. In the DMS conditions, the maximum current density ranged from 1.53 ± 0.14 A m^{-2} in condition 3 to 1.66 ± 0.42 A m^{-2} in condition 4. The consistency in both CE and maximum current density across different manures and experimental conditions suggests that MFC operation achieves a level of stability regardless of substrate variation and operational parameters.

Another system performance metric used to evaluate the MFC performance is the nitrogen removal efficiency (R_N), calculated as the ratio of the amount of nitrogen removed from anolyte to the current production (Eq. 5). A key assumption in the evaluation and comparison of R_N is that all nitrogen removal from the anode compartment is as NH_4^+ ion transport across the CEM, driven by the electron transport from anode to cathode in the external circuit. This assumption was proven to be true in systems where orgN is not in ultra-low concentrations (Burns and Qin, 2023). An R_N equal to 1 indicates the scenario where one mole of electrons drives the transport of one mole of NH_4^+ ions (Burns and Qin, 2023; De Paepe et al., 2020; Rodríguez Arredondo et al., 2017). In condition 1, R_N is 1.17 ± 0.25 mol N mol^{-1} e^-, indicating that there is slightly more N transfer than charge transfer. This could result from the diffusion of NH_4^+ across the CEM. An $R_N > 1$ could also indicate that the assumptions are incorrect, for example, if some of the TN removal can be attributed to uptake by the microorganisms retained on the anode brush. As such, further analysis into the microbial community and elemental composition of the biofilm on the anode brush is warranted. R_N in condition 2 is 1.02 ± 0.24 mol N mol^{-1} e^-, which did not significantly differ from condition 1 ($p = 0.751$). This similarity indicates that minimal aeration did not increase orgN mineralization to NH_4^+ and subsequent removal from the anolyte. This finding is further confirmed based on orgN removal and combined NH_3/NH_4^+ concentrations reported in Fig. 3 and further discussed in section 3.2.1. R_N in conditions 3 and 4 decrease to 0.55 ± 0.16 mol N mol^{-1} e^- and 0.68 ± 0.19 mol N mol^{-1} e^-, respectively. The decrease in R_N from conditions 1 and 2 to conditions 3 and 4 could be attributed to the more complex DMS substrate in conditions 3 and 4 with the existence of competing ions which can also be transported across the CEM. Furthermore, R_N in conditions 3 and 4 are not significantly different from each other ($p = 0.875$), providing further evidence for confirmation that supplemental NH_4^+ addition in condition 4 is not beneficial to the system.

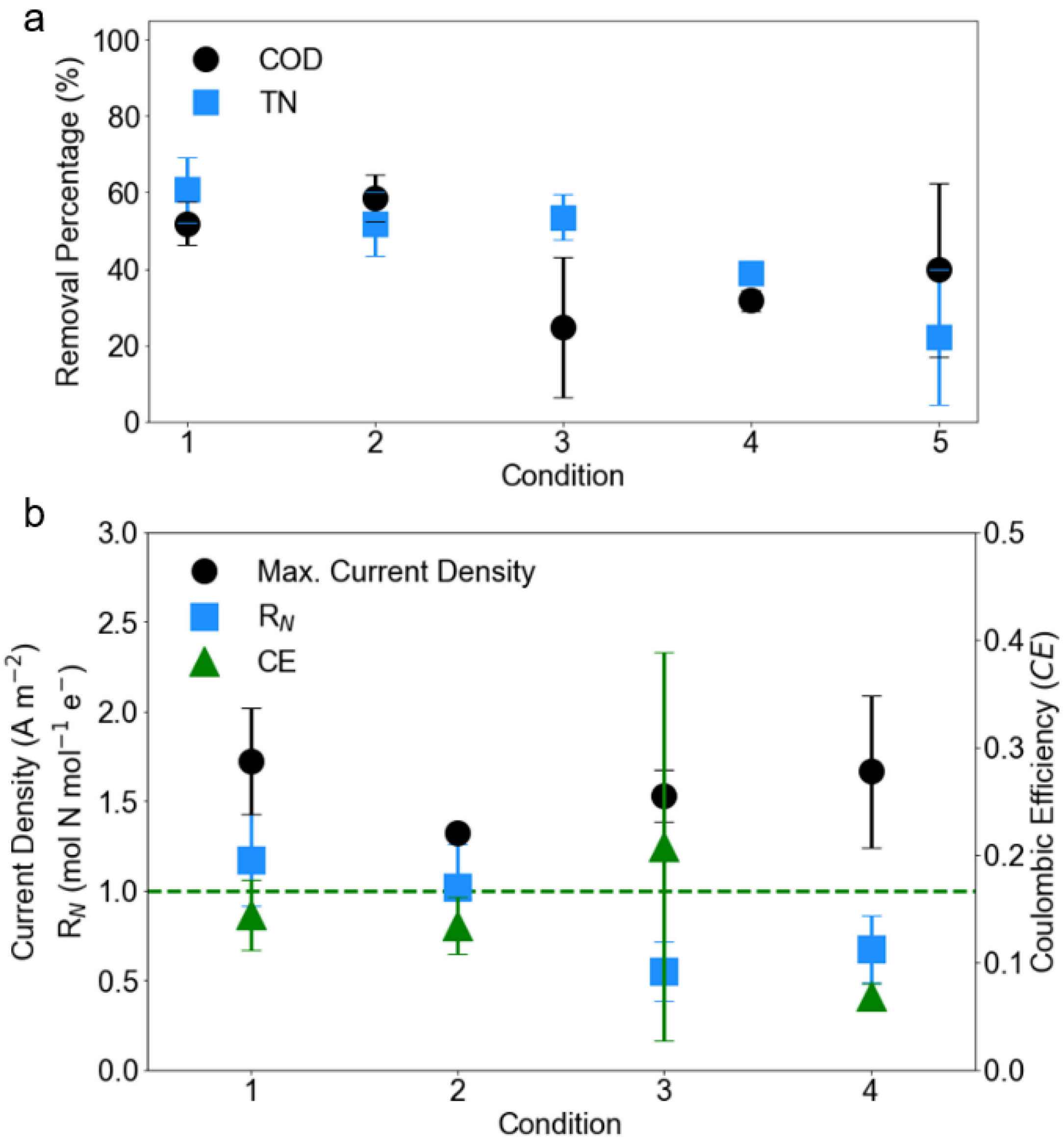


Fig. 1. *COD and TN removal (a) and average maximum current density, nitrogen removal efficiency (R_N), and Coulombic Efficiency (CE) (b) for the MFC under different anolyte conditions as described in Table 1. Data is presented as averages of all measurements/cycles in the given condition, and error bars represent standard deviations of all measurements/cycles in the given condition. No values shown in (b) are calculated for condition 5 as there is no charge transfer from anode to cathode in the OC condition. A dotted line at R_N = 1 shows the balanced nitrogen*

removal efficiency, where exactly one mole of nitrogen is assumed to be transported across the CEM per mole of negative charge, as current, transported from anode to cathode via the external circuit.

3.2. Nitrogen removal and recovery

3.2.1. MFC OrgN Removal

Under all manure types and conditions, the MFC achieved good orgN removal, ranging from a minimum of 39.7 ± 24.8% in condition 4 to a maximum of 73.8 ± 12.1% in condition 1 (Fig. 2a). Statistical analyses proved that there were no significant differences in orgN removal between any of the conditions (one-way ANOVA, $F = 2.75$, $p = 0.073$, overall large effect $\omega^2 = 0.281$). Based on this, we conclude that the MFC is effective at removing orgN from various types of dairy manure and in various conditions. In condition 2, minimal aeration was hypothesized to increase orgN mineralization as the mineralization process can be slightly more efficient in the aerobic condition (Barbarika et al., 1985; Bernal and Kirchmann, 1992; Kessel and J, 2002; Parker and Sommers, 1983). However, there was no observed change in the initial or final anolyte NH_3/NH_4^+ concentrations in condition 2 compared to those in condition 1, and orgN removal was not significantly changed between these conditions (Fig. 2). These findings, coupled with the unchanged R_N between conditions 1 and 2 (see section 3.1.2), support the conclusion that minimal anode compartment aeration at $DO < 1$ mg L^{-1} does not improve orgN mineralization. Furthermore, orgN removal was much more variable in both conditions 2 and 4, the two modified conditions (aeration and supplemental NH_4Cl addition, respectively) for each type of manure. Despite the overall stability of the MFC, this increased variability indicates the sensitive nature of the microbial community in the MFC. Based on these results, we conclude that orgN mineralization in the MFC is largely governed by specific microbes that have yet to be identified, and further microbial community analysis and enrichment is required to better enhance orgN mineralization in the MFC.

Interestingly, there is still significant orgN removal in condition 5, at 63.4 ± 11.1%, despite this being the OC condition. This challenges the initial assumptions regarding nitrogen removal as NH_4^+ transport solely due to current production discussed in section 3.1. However, condition 5 exhibited no change from initial to final measurements for anolyte NH_3/NH_4^+, which was 450 ± 21 mg L^{-1} initially and 460 ± 120 mg L^{-1} finally (Fig. 2b). There was also no NH_3/NH_4^+ measured in the catholyte in condition 5 (Fig. S5). Therefore, there is no evidence that orgN removal from the anolyte is specifically attributed to mineralization to NH_4^+ and transport to the catholyte. OrgN in condition 5 could be removed due accumulation (as either orgN or mineralized forms) into the biomass retained on the anode. As such, further analysis of the microbial matter and elemental composition of the biofilm on the MFC anode is required to fully understand this phenomenon.

3.2.2. MFC NH_3 recovery

With this application of BES technology, additional recovery of NH_3 through mineralization and recovery of the orgN fraction of dairy manures is enabled. Mineralization is required to convert the nitrogenous fraction of manure organics into charged ions, such as NH_4^+, which can then be transported together with the existing NH_4^+ ions out of the anolyte via the CEM. The MFC achieved NH_3/NH_4^+ removal for all manure types in all conditions with current flow, as shown in Figure 2b. The initial anolyte NH_3/NH_4^+ concentrations in conditions 1 through 3 were all similar, with an average concentration of 350 ± 34 NH_3-N mg L^{-1}. This similarity was observed despite notable differences in NH_3/NH_4^+ concentrations in the different raw manure substrates used in conditions 1 and 2 (manure + urine mix) compared to that used in condition 3 (DMS). This consistency could be due to the dilution effect which results from the 50% replacement of anolyte solution volume at the start of each batch cycle (see section 2.1.1). Comparatively, conditions 4 and 5 saw an increase in initial anolyte NH_3/NH_4^+ concentrations (590 ± 8.6 NH_3-N mg L^{-1} in condition 4 and 450 ± 21 NH_3-N mg L^{-1} in condition 5).

Conditions 1 through 4 exhibited a decrease in anolyte NH_3/NH_4^+ concentration by the end of the experimental cycles. For example, the NH_3-N concentrations in the anolyte effluent were 160 ± 27 mg L^{-1} and 140 ± 45 mg L^{-1} in condition 1 and condition 3, respectively. For all conditions with an observed decrease, the magnitude of the decrease is approximately the same, despite the varied R_N discussed in section 3.1.2 (Figs. 1a and 2b). This finding indicates that the NH_3 removal from the anolyte is relatively stable, which implies that the fluctuations in R_N are likely due to variation in current production (Eq. 5). It is important to note that the final anolyte NH_3/NH_4^+ concentration measured ($NH_{3,f}$ in Fig. 2b) is a combination of multiple ammonia fractions: any remaining initial NH_3/NH_4^+ that was not transported to the catholyte and any NH_3/NH_4^+ produced via microbial mineralization of orgN. However, the decrease in NH_3/NH_4^+ concentration in the anolyte coupled with the calculated orgN removal indicates that the MFC can successfully recover NH_3/NH_4^+ in the catholyte from the orgN fraction of dairy manure. In condition 5, the initial and final anolyte NH_3/NH_4^+ concentrations were 450 ± 21 NH_3-N mg L^{-1} and 460 ± 130 NH_3-N mg L^{-1}, respectively. The stable NH_3-N concentrations under the OC condition indicate that there was little to no NH_3/NH_4^+ removed from the anode compartment (Fig. 2b), which is attributed to the lack of current flow between the electrodes at the OC condition (see section 3.2.1). Furthermore, the significant fluctuation in final anolyte NH_3/NH_4^+ concentration in condition 5 compared to the small variation in other conditions indicates that current flow is required to achieve stable NH_3/NH_4^+ removal.

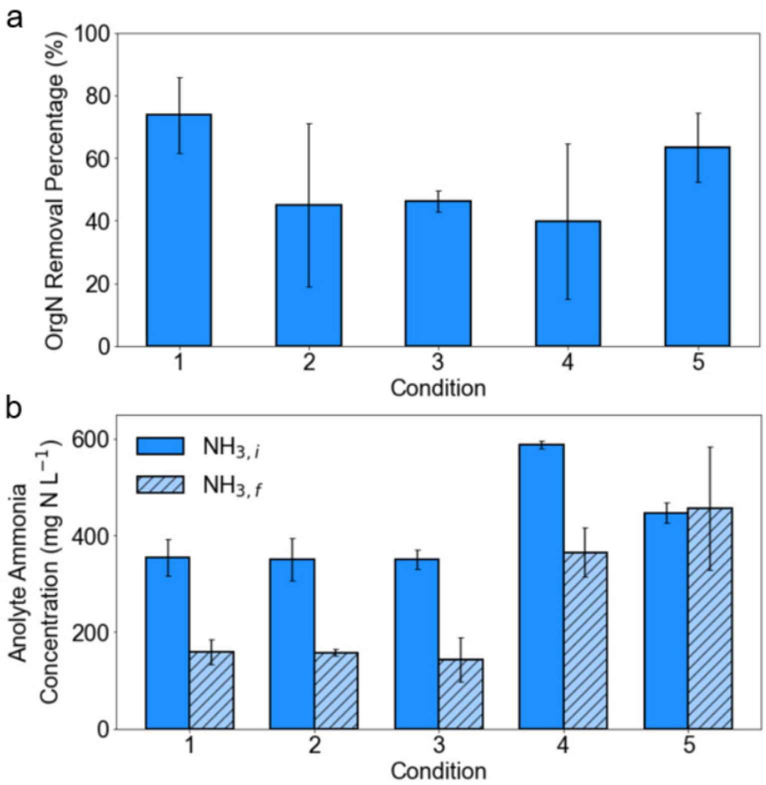


Fig. 2. *Organic nitrogen removal from the anolyte (a) and initial (i) and final (f) anolyte ammonia concentrations (b) in the MFC under different anode compartment conditions.*

3.3. Ion competition

Previous studies investigating cation transport in BES have proven that electromigration, as opposed to concentration-diffusion, is the main driver of cation transport in BES (Cerrillo et al., 2016; Haddadi et al., 2013). Furthermore, some studies have shown that NH_4^+ is the most readily-transported cation to maintain charge neutrality in such systems (Cerrillo et al., 2016). However, the CEM is not exclusive to transporting NH_4^+, and other cations, such as Na^+, K^+, and Mg^{2+}, that exist in dairy manure may compete with NH_4^+ for transport across the membrane. Here, we investigated the initial and final concentrations of these relevant cations (Na^+, K^+, and Mg^{2+}) in the anolyte and catholyte solutions from one representative cycle in each condition. Figure 4 shows the initial and final anolyte and catholyte concentrations of Na^+, K^+, and Mg^{2+}, as well as the concentrations of these ions in the initial manure substrates. The initial ion concentrations measured in catholyte samples, despite the use of deionized water as the catholyte solution, is due to the minimal replacement of catholyte volume as described in section 2.1.1. Different dairy manure compositions contain largely different concentrations of cations that can compete with NH_4^+ for transport. In the feces + urine mix, K^+ was the most concentrated ion measured at around 360 mg L^{-1}, followed by Na^+ at 190 mg L^{-1} and Mg^{2+} at 20 mg L^{-1}. In DMS, K^+ was overwhelmingly dominant at a concentration of 1,200 mg L^{-1}, followed by Na^+ and Mg^{2+} at 430 mg L^{-1} and 390 mg L^{-1}, respectively.

In all conditions except the OC condition, there was good NH_3/NH_4^+ removal from the anolyte, as discussed in section 3.2.2 (Fig. 2b). Here, we compare the previously discussed NH_3/NH_4^+ removal to transport of the other cations examined (Na^+, K^+, and Mg^{2+}). Similar to NH_3/NH_4^+ transport, the transport of Na^+, K^+, and Mg^{2+} in the OC condition (condition 5) was negligible. For example, the initial and final K^+ concentrations are 550 mg L^{-1} and 530 mg L^{-1}, respectively. Furthermore, all cations measured exhibited near-zero concentrations in the final catholyte samples in condition 5 (Figs. 3 and S3), and ammonium transport was found to be negligible (Fig. 2b, discussed in section 3.2.2), indicating that current flow is required in the system to drive the transport of cations from anode to cathode compartments.

Na^+ and Mg^{2+} had minimal transport to the catholyte across all manure types and conditions, indicating that there is little competition for transport between these cations and NH_4^+ (Figs. 2 and 3a&c). For both Na^+ and Mg^{2+}, the concentration variations in the catholyte were negligible (< 30 mg L^{-1}) compared to the 190 mg L^{-1} and 220 mg L^{-1} increases in catholyte NH_3/NH_4^+ concentrations (calculated as the decrease in anolyte NH_3/NH_4^+ concentration) in conditions 2 and 4, respectively. K^+ was the only cation of those measured which indicated potential competition with NH_4^+ across the CEM. For example, competition between K^+ and NH_4^+ was most evident in condition 3, where the catholyte K^+ concentration increased by 107 mg L^{-1}. This is 51.0% of the 210 mg L^{-1} increase in catholyte NH_3/NH_4^+ concentration in condition 3, indicating some minor competition for transport. The competition between K^+ and NH_4^+ was less in conditions 1, 2, and 4, where the catholyte K^+ concentration increased by less than 70 mg L^{-1}, corresponding to, at most, 36.8% of the increase in catholyte NH_3/NH_4^+ concentration. Competition between K^+ and NH_4^+ could be attributed to the relatively high concentration of K^+, which was 2.8 to 3.2 times more concentrated than Mg^{2+} in conditions 3 and 4. Similarly, K^+ was 2.0 to 2.7 times more concentrated than Na^+ in conditions 3 and 4 (both around 210 mg L^{-1}), and 1.7 times more concentrated than Na^+ in condition 2 (230 mg L^{-1}). Higher concentrations can enhance concentration diffusion to a non-negligible effect in the MFC, explaining the higher final catholyte concentrations of K^+ in the MFC in these conditions. However, there is no transport of K^+ observed in condition 5, the OC condition, due to the lack of current generation. Despite the observed competition, NH_3/NH_4^+ is better removed than K^+ in all conditions and under both manure types, indicating that ion competition for transport across the CEM with the cations tested is not a major concern in this work.

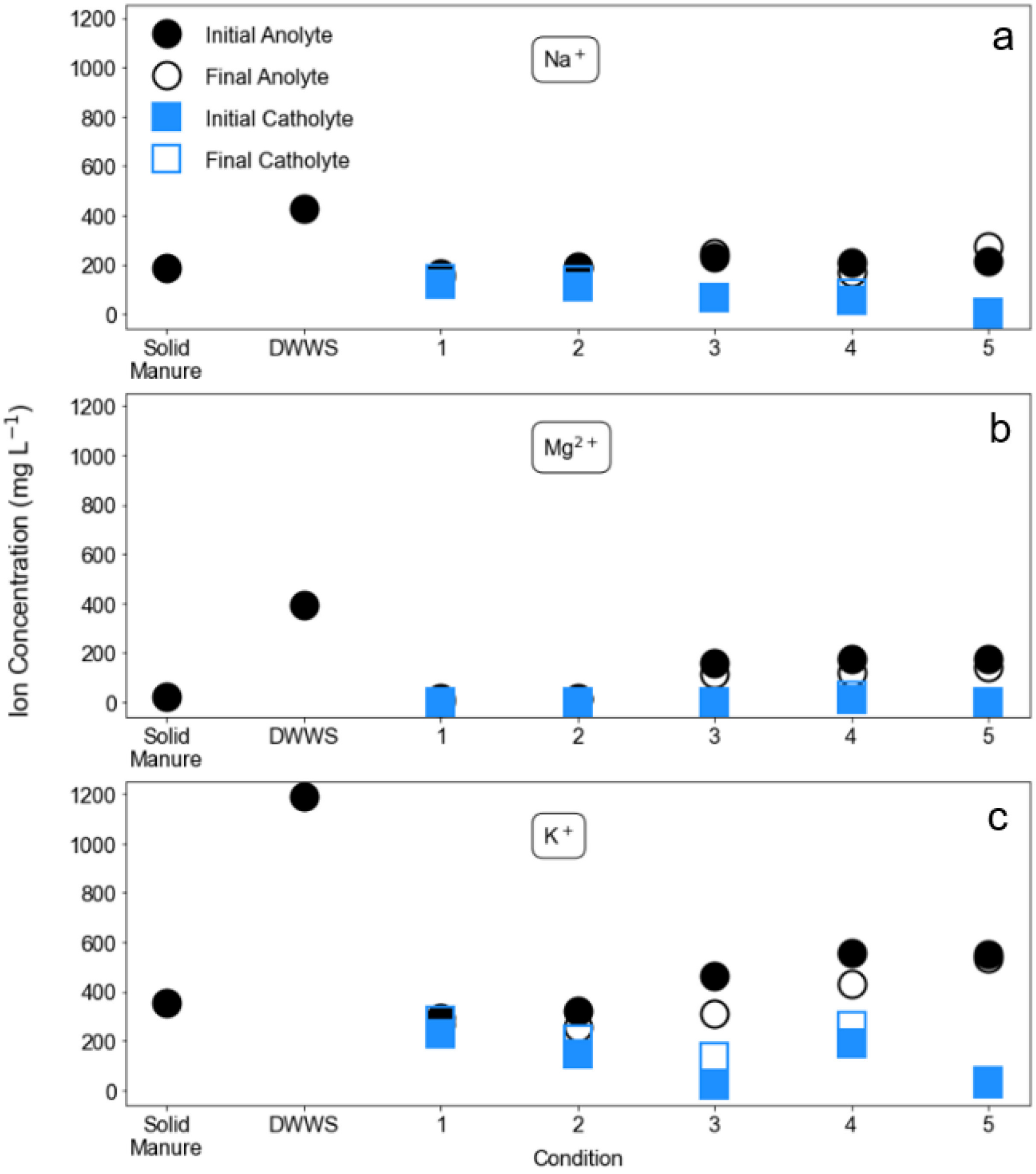


Fig. 3. *Initial and final anolyte and catholyte concentrations for selected cations from one representative cycle in each condition and in the dairy manure substrates:* Na^+*(a),* Mg^{2+}*(b), and* K^+*(c). Anolyte concentrations are circles, catholyte concentrations are squares, initial concentrations are filled markers, and final concentrations are unfilled markers. Initial anolyte and catholyte samples were collected after 10 minutes of recirculation to capture any dilution effects from partial volume preplacement in each electrolyte at the start of each cycle.*

3.4. Complete processes in MFC for NH_3 recovery from orgN in dairy manure

Successful MFC operation is ultimately achieved through the balancing of three key processes (KPs): (1) organics degradation leading to the production of extracellular electrons, (2) transport of these electrons as current from anode to cathode, and (3) cation transport from anode to cathode compartments via the CEM (Fig. 4). These processes must happen both sequentially and simultaneously. In the batch reactor, the initial stage after feeding marks the easiest period for the system to engage in all three KPs simultaneously. Simple organics present in the manure are readily degraded by microorganisms and the produced extracellular electrons are sent as current to the cathode. Meanwhile, readily available cations present in the anolyte are transported across the CEM. However, once any one of the reactants in the KP reactions is depleted, challenges are introduced. For example, once all the easily-degradable organics are depleted, the microorganisms must degrade more complex organics, which takes longer and often yields less extracellular electron production as more electrons are directed toward the complex degredation reactions. As a result, current flow is decreased (KP 2) which decreases the electromotive force driving KP 3 and thus decreases cation transport and subsequent recovery in the catholyte (Fig. S4). This restriction can also be flipped; once readily available cations are depleted, KP 3 ceases, upsetting the electromotive force balance required to ensure KP 2 continuation. Here we name two sub-processes (SPs) which are unique to the MFC for NH_3 recovery from orgN in dairy manure that drive cell operation to continue.

In the MFC for NH_3 recovery, orgN mineralization to NH_4^+ (SP1) and transport of mineralized NH_4^+ across the CEM from anolyte to catholyte (SP2) contribute to the sustained operation of the MFC (Fig. 4). SP 1 can contribute a small amount of current towards sustaining KP 2, however, the majority of the benefit from SP1 comes from the products of the mineralization reaction, which are simpler organic intermediates and NH_4^+ (Bernal and Kirchmann, 1992; Girkin and Cooper, 2023; Kandeler, 2007; Kessel and J, 2002). The simple organic intermediates, such as organic acids and simple sugars, are then easily degraded by current-producing organisms and contribute to sustaining KP 1. The NH_4^+ produced directly contributes to SP 2 and KP 3. We separate SP 2 and KP 3 here, as KP 3 represents the cation transport that occurs in every MFC with this setup, while SP 2 represents the added advantage of this specific operation scheme to recover the orgN fraction of dairy manure as NH_4^+ in the catholyte. The NH_4^+ from the orgN mineralization in SP 1 is transported to the cathode in SP 2, thus SP 2 represents the process that recovers additional resources outside typical recovery in MFCs not operated for orgN mineralization.

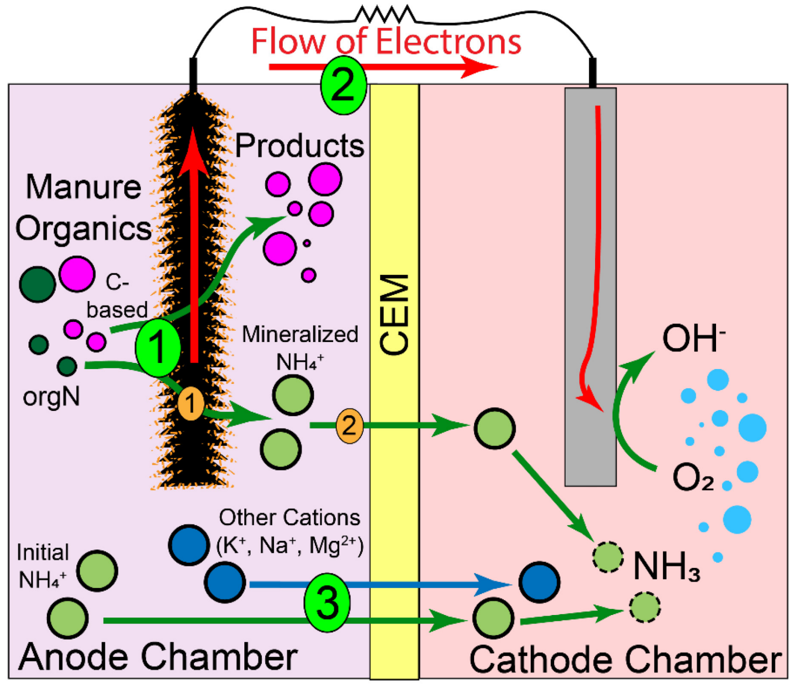


Fig. 4. *Schematic of MFC used for ammonia recovery from organic nitrogen in dairy manure with highlighted key processes (1) organics degradation and extracellular electron production, (2) current flow from anode to cathode, and (3) cation transport to cathode via electromigration and*

bulk diffusion (in green), as well as sub-processes (SPs) (1) orgN mineralization to intermediate organics and NH_4^+ *and (2) transport of mineralized* NH_4^+ *from anode to cathode via the CEM (in orange).*

4. Conclusions

MFCs for NH_3/NH_4^+ recovery from the orgN fraction of dairy manure are a viable alternative to traditional manure treatment. In this work, an MFC was operated using two types of dairy manure under five different conditions: feces + urine mix, dairy manure slurry, low concentration anode compartment aeration, supplemental NH_4Cl addition, and open circuit. The MFC proved robust, achieving good COD and TN removal, with the highest recorded rates being 59 ± 6% and 61 ± 8.5%, respectively. Furthermore, the MFC achieved orgN removal in all conditions, and saw decreases in anolyte ammoniacal nitrogen concentration in every condition where there was current flow. The highest nitrogen removal efficiency (R_N) of 1.17 ± 0.25 mol N mol^{-1} e^- was obtained under condition 1; however, this number decreased to 0.55 ± 0.16 mol N mol^{-1} e^- in the presence of more complex DMS substrate and heightened competition between other cations. Concentrations of other cations, like Na^+, Mg^{2+}, and K^+, were evaluated for comparison with NH_4^+ concentrations in initial and final electrolyte solutions, with only K^+ exhibiting minor competition with NH_4^+ for CEM transport. Based on this work, we propose a more complete understanding of operations within the MFC, including the three key processes and two sub-processes unique to MFC operated for NH_3 recovery from orgN in dairy manure. This work contributes to the growing body of research around manure treatment and offers a novel manure treatment method for both treating the C-based organic load of manure and recovering a valuable resource that is thus far untapped by traditional manure treatment systems.

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