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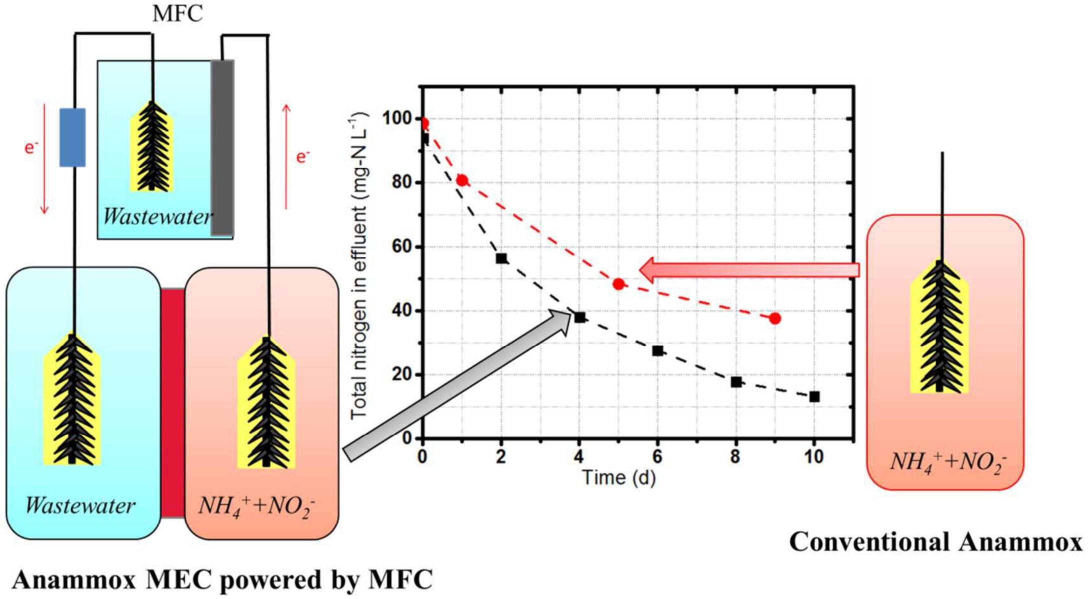
This pioneer study provides a practical solution to the imminent problem of anammox: slow growing anammox bacteria. Unique integration of MECs and MFCs alleviates the dependence on anammox bacteria, and accelerates total nitrogen (TN) removal efficiency 30% higher than conventional anammox. A novel bioelectrochemical anammox kinetic model is developed. This study greatly improves the scientific understanding and engineering application of energy-positive high rate biological nutrient removal processes.

Graphic abstract:



Self-sustained high-rate Anammox: from biological to bioelectrochemical process

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Abstract

Slow growth rate of anammox bacteria is the imminent problem for system efficiency and stability. An innovative solution was explored in this study by accelerating anammox in microbial electrolysis cells (MECs) and alleviating the dependence on anammox bacteria. The batch tests showed that 85 % of total nitrogen (TN) was removed in the MEC system, while only 62 % of TN was removed in conventional anammox. Simulation of the modified Nernst-Monod model revealed that the maximum specific utilization rate (0.30-0.38 mmol g^{-1}VSS h^{-1}) in the anammox MEC was 60 % higher than in conventional anammox (0.18-0.20 mmol g^{-1}VSS h^{-1}). Harvesting the power generated in microbial fuel cells (MFCs) to support MECs substantially saved energy consumption and effectively utilized the low power output of MFCs. Simulation of power management system (PMS) interface demonstrated the charge/discharge cycles for power supply by MFCs and power consumption by MECs. The integrated MEC-MFC system accelerated anammox, avoided external carbon requirement, effectively utilized wastewater energy, and thus achieving self-sustained nitrogen removal.

Keywords: microbial electrolysis cell, anammox, nitrite, Nernst-Monod model, microbial fuel cell, power management system.

Introduction

Nitrogen in wastewater is normally removed using biological nitrogen removal (BNR) processes consisting of aerobic nitrification and anoxic denitrification. However, conventional BNR is energy-negative and carbon-intensive due to the requirements of high aeration in nitrification, pH adjustment, long sludge retention time (SRT), and exogenous organic carbon in denitrification.[1] In the past decade, anaerobic ammonium oxidation (ANAMMOX) has been developed in which ammonium (NH_4^+) and nitrite (NO_2^-) are directly reacted to form nitrogen gas (N_2) by anoxic anammox bacteria, and thus possessing unique advantages over conventional BNRs including no need for aeration and external carbon sources.[2] Nevertheless, anammox suffers from long growth time (doubling time of 11 days) of anammox bacteria and difficult cultivation, making it barely operated in full-scale wastewater treatment plants.[3]

Bioelectrochemical systems (BES) have drawn global attention for converting the chemical energy stored in wastewater to electrical energy.[4-10] Microbial fuel cells (MFCs) utilize the difference between anode and cathode potentials as the driving force for electricity generation in spontaneous reactions (e.g. oxygen reduction, metal (Cr^{6+}, Cu^{2+}) reduction) and have been studied to reduce the energy cost of denitrification with nitrite and nitrate as the electron acceptors in the cathode chamber.[11-16] On the other hand, microbial electrolysis cells (MECs) that the difference between anode and cathode potentials is deficient can be supported with an external power supply (voltage < 1.0 V) to make non-spontaneous reaction proceed and accelerate spontaneous reactions. For instance, chromium (VI) reduction was accelerated in MECs by changing cathode potential.[17] Nitrogen removal with nitrification on anode and denitrification on cathode was achieved in MECs.[18] Previous study found that ammonium served as the anodic fuel in MFCs and current generation was promoted during the process of electron transport from ammonium to nitrite.[19] Therefore, installing power supply to anammox in MECs could accelerate ammonium oxidation.

The limited electric power output (less than 5 W m^{-2}) of MFCs has posed a problem for using MFCs as the sole power source.[20,21] Thus, an efficient utilization of low MFC power is critical for its real-world application. Past several years have seen MFCs as the power source for low power subsea devices, water quality sensors, and MECs of hydrogen production and metal treatment.[13,22-25] Especially, the low voltage requirement of MECs (<0.5 V) makes the MFC a proper power supply. But the interface between power production in MFCs and power consumption in MECs has not been established, making it difficult to predict the power supply and consumption between MFCs for MECs.

Reliable estimation of kinetic parameters in anammox modeling is critical to better understand anammox mechanisms and enhance its performance. Monod kinetics, pseudo first order model and Haldane-type model were used for anammox simulation.[26-29] A wide range of half-velocity constants (0.003-13.7 mM) and maximum specific utilization rate (0.09-3.74 mmol N g^{-1}VSS h^{-1}) has been obtained.[29] With two reactants (ammonium and nitrite) being involved in anammox reactions, Monod model with multiple and/or dual substrates should be used, which could reflect the dependence of each substrate in reactions. In the meantime, the Nernst-Monod model has been developed for electron donation and acceptance between substrates and biofilm electrodes in MFCs and MECs, and modified for the reduction of nitrate and nitrite in cathode.[30,31] Until now, there has been no model for anammox in MECs or MFCs. Due to the complexity of redox reactions and multiple substrates involved in anammox, a novel Nernst-Monod model coupling with multi-substrates Monod model should be developed to simulate the anammox in BES systems and the dependence of each substrates.

The breakthrough of this study was to accelerate anammox in MECs without solely counting on anammox bacteria and to explore the feasibility of the integrated MFC-MEC in wastewater treatment plants. MECs were powered by MFCs treating wastewater to achieve self-sustained nitrogen removal without extra energy input and efficiently utilize the low power output of MFCs, which none of existing BNR and BES has accomplished. There were four tasks in this study. First, ammonium and nitrite were fed in MECs to examine their degradation rates. The accelerated anammox mechanisms in MECs were verified by conducting three control tests (conventional anammox, ammonium/nitrite alone, and abiotic MEC test). Second, the kinetic model of accelerated anammox in MECs was developed to fundamentally understand the self-sustained anammox and the dependency of ammonium and nitrite in anammox reactions. Third, the variation of important parameters (e.g. pH, redox potential) was examined to determine the occurrence of anammox in MECs, and relate with nitrogen removal rate. Finally, the interface of the integrated MFC-MEC system was modeled to predict the power supply of MFCs to anammox MECs. Energy saving of the integrated system was calculated and compared with traditional BNR and anammox processes to confirm the novel self-sustained anammox MFC-MEC with high nitrogen removal rate and minimal energy consumption.

Materials and Method

The anammox MEC setup

The batch-mode anammox MEC consisted of an anode chamber (volume: 140 mL) and a cathode chamber (volume: 140 mL) separated by proton exchange membrane (N117, DuPont Fuel Cells, DE) (Fig. 1 Anammox MEC). During the acclimation period, the anammox MEC was powered by a programmable power supply (model 3645A; Circuit Specialists, Inc.) with the stable voltage output of 0.5 V. The power supply was later replaced by two single-chamber microbial fuel cells (SCMFCs) (each volume: 140 mL) connected in series during the experimental period. The anode of the anammox MEC was connected with the cathode of SCMFCs and the cathode of the anammox MEC was connected with the external resistance (R_{ext} 5 Ω) in the circuit connecting the anode of SCMFCs. The voltages across the R_{ext} were continuously recorded every 120 s using a Keithley 2700 data logging system. Carbon brushes (4cm long by 4cm diameter as projected area, Mill-Rose Carbon Fiber Brush) were used as the anode and cathode of anammox MEC, and the anode of the SCMFCs. Carbon cloth (4×4 cm^2, Fuel Cell Earth LLC, MA) loaded with platinum (Pt) (0.5 mg cm^{-2}) was used as cathode of SCMFCs.

Please add Fig. 1 here

Inoculation and operation of anammox MEC and control experiments

The anode chamber of the anammox MEC was inoculated with the mixture of aerobic and anoxic sludge (volume ratio: 1:1) from the University of Connecticut Wastewater Treatment Plant (UConn-WWTP). The ammonium chloride (NH_4Cl, ~ 50 mg-N L^{-1}), sodium nitrite ($NaNO_2$, ~ 40 mg-N L^{-1}), and the medium solution (0.2 mM Na_2HPO_4, 0.1 mM NaH_2PO_4, 0.2 mM KCl, 0.2 mM $MgSO_4 \cdot 7H_2O$, and 6mM $NaHCO_3$) were fed to stimulate the anammox bacteria growth in the anode. The inoculation lasted five weeks by changing half of the anodic solution (around 70 ml) weekly and refilling with fresh ammonium, nitrite and nutrient solution. When the experiment started, the initial volatile suspended solids (VSS) concentration was about 0.4 g L^{-1} in the anammox MECs. The cathode was fed with the influent wastewater from UConn-WWTP. Both the anode and cathode were sealed with plastic caps to secure anaerobic conditions and prevent the occurrence of nitrification. The SCMFCs were filled with the mixture of the influent wastewater from UConn-WWTP and sodium acetate (15 mM) with the chemical oxygen demand (1500 mg L^{-1}) and operated more than one month to provide stable voltage (0.5 V) for anammox MECs.

Along with the experiment of anammox MECs, three control experiments were operated side by side for comparison. Control 1 (conventional anammox) was conducted in a sealed plexiglass bottle (volume: 140 mL) with a carbon brush for biofilm growth (Fig. 1 Control 1). No power supply or SCMFCs was connected. Control 2 had the similar setup to the anammox MEC, except feeding with either ammonium or nitrite solution (Fig. 1 Control 2) to examine the nitrogen removal under single nitrogen species, and the dependence of ammonium and nitrite for anammox and denitrification. Control 3 (abiotic test) was the similar setup to the anammox MFC, expect no sludge was inoculated in the anode (Fig. 1 Control 3) to compare with Control 1 (biotic anammox tests) and determine the role of bacteria in nitrogen removal in MECs. All the tests of anammox MECs and Controls were carried out at 30°C in duplicate.

Analysis of anammox MEC and control experiments

Ammonium concentration, nitrite, nitrate, chemical oxygen demand (COD), alkalinity were measured with a spectrophotometer (DR 2800, HACH company, CO) and TNTplus^{TM} series (HACH company, CO). The pH in anammox MEC and controls were measured with a portable pH meter (Thermo Fisher Scientific Orion 3-star). The redox potential (ORP) of the anammox MEC and control experiments were measured with ORP meter (Thermo Fisher Scientific Orion 3-star). The open circuit potentials (OCPs) of anodes and cathodes were measured using an Ag/AgCl (+197 mV vs SHE) as the reference.

Modeling anammox MEC and conventional anammox

In order to predict the variation of ammonium and nitrite concentrations in the anammox MEC and estimate the kinetic parameters, Nernst-Monod Equation (Eq.1 and Eq.2) modified from previous studies was used,[30,31] assuming that ammonium and nitrite (nitrate was negligible in this study) were the electron donors and anode electrode was the electron acceptor. In the meantime, Monod kinetics of double substrates was used to simulate nitrogen removal in conventional anammox without power supply (Control 1) (Eq.3 and Eq.4).

$$\frac{d[\mathrm{NH_4^+}]}{dt} = -\mu_{mNH_4^+MEC}\left(\frac{[\mathrm{NH_4^+}]}{[\mathrm{NH_4^+}]+K_{SNH_4^+MEC}}\right)\left(\frac{[\mathrm{NO_2^-}]}{([\mathrm{NO_2^-}]+K_{SNO_2^-MEC}}\right)\left(\frac{1}{1+\exp\left[-\frac{F}{RT}\eta\right]}\right) \tag{1}$$

$$\frac{d[\mathrm{NO_2^-}]}{dt} = -\mu_{mNO_2^-MEC}\left(\frac{[\mathrm{NO_2^-}]}{([\mathrm{NO_2^-}]+K_{SNO_2^-MEC}}\right)\left(\frac{[\mathrm{NH_4^+}]}{[\mathrm{NH_4^+}]+K_{SNH_4^+MEC}}\right)\left(\frac{1}{1+\exp\left[-\frac{F}{RT}\eta\right]}\right) \tag{2}$$

$$\frac{d[\mathrm{NH_4^+}]}{dt} = -\mu_{mNH_4^+ana}\left(\frac{[\mathrm{NH_4^+}]}{[\mathrm{NH_4^+}]+K_{SNH_4^+ana}}\right)\left(\frac{[\mathrm{NO_2^-}]}{([\mathrm{NO_2^-}]+K_{SNO_2^-ana}}\right) \tag{3}$$

$$\frac{d[\mathrm{NO_2^-}]}{dt} = -\mu_{mNO_2^-ana}\left(\frac{[\mathrm{NO_2^-}]}{([\mathrm{NO_2^-}]+K_{SNO_2^-ana}}\right)\left(\frac{[\mathrm{NH_4^+}]}{[\mathrm{NH_4^+}]+K_{SNH_4^+ana}}\right) \tag{4}$$

Where $[\mathrm{NH_4^+}]$ and $[\mathrm{NO_2^-}]$ are the effluent concentrations of ammonium and nitrite (mg-N $\mathrm{L^{-1}}$); μ_m is the maximum specific utilization rate (mg-N $\mathrm{g^{-1}}$VSS $\mathrm{h^{-1}}$); *Ks* is the half-velocity constant (mg-N $\mathrm{L^{-1}}$); *F* is Faraday's constant (9.64853×10^4 C $\mathrm{mol^{-1}}$); *R* is gas constant (8.314 J $\mathrm{K^{-1}}$ $\mathrm{mol^{-1}}$); *T* is operation temperature (K); η is the local potential (V), it was estimated as 0.2 V in this study.[27]

The results of ammonium and nitrite concentrations in the anammox MEC and Control 1 over the experimental period were fit into the models (Eqs.1-4) to calculate critical kinetic parameters –maximum specific utilization rate and half-velocity constant. Integration of differential equations was conducted using the Matlab software (R2014a).

Modeling power support and consumption of the integrated MFC-MEC

The power support and consumption between anammox MEC and SCMFCs was simulated. Power output from MFCs was evaluated based on total coulombs (*Q*), and the current produced during the operational period (*t*) was determined based on the organics consumption (ΔCOD) in wastewater (Eq.5). Coulombs from SCMFCs (*Q*) were consumed in anammox MEC through the anammox pathways, so that the concentration of nitrogen removed (ΔC_N) could be simulated with the amount of electrons transferred per mole of nitrogen (NH_4^+) (Eq.6). By establishing the interface of power generation in SCMFCs and power consumption in anammox MEC, ΔCOD in SCMFCs and ΔC_N in anammox MEC could be simulated.

$$Q = \int_0^t I dt = \frac{FV_{MFC}\Delta COD}{8} \times CE_{MFC} \qquad (5)$$

$$\Delta C_N = \frac{QM}{nFV_{MEC}} \qquad (6)$$

Where *Q* is the total coulombs harvested from MFCs (C); V_{MFC} and V_{MEC} are the SCMFC volume and MEC cathode volume (L); ΔCOD is the total COD consumed in SCMFCs, (g L^{-1}); CE_{MFC} is the coulombic efficiency of SCMFC (10-27 %),[32] with 20% being used in Eq. 5; *M* is the molecular weight of nitrogen (14 g mol^{-1}); *n* is the electron transfer per mole of nitrogen; *F* is the Faraday's constant (9.64853×10^4 C mol^{-1}) .

Results and discussion

Variation of ammonium and nitrite concentrations in MECs

Please add Fig. 2 here

Ammonium (NH_4^+) and nitrite (NO_2^-) concentrations gradually decreased in the MEC with the power supply of 0.5 V (Fig. 2). The initial concentrations of ammonium and nitrite were set at 50 mg L^{-1} and 40 mg L^{-1}, respectively, to simulate domestic wastewater and the effluent after short-cut nitrification (ammonium to nitrite).[16] It took 10 days to remove 90 % of ammonium (average concentration below 5 mg L^{-1} in the effluent) and remove 85 % of nitrite (average concentration below 6 mg L^{-1} in the effluent). Nitrite decreased fast in the first 4 days and then slowed down. Nitrate (NO_3^-) in the MEC was low (less than 5 mg L^{-1}) and originated from the acclimated sludge residue taken from the anoxic tank of UConn WWTP. The continuous nitrogen removal at the anaerobic condition (DO < 0.1 mg L^{-1}, with both anode and cathode being sealed) indicated the biotic process of this experiment especially the function of anammox bacteria, since ammonium and nitrite could hardly be volatilized in the sealed system, nitrification could not occur under anaerobic condition, and the anammox reaction could only take place with bacteria. The average nitrogen removal rate was 8.2 mg-N L^{-1} d^{-1}, which was in the range of the reported values in BES (3-51 mg-N L^{-1} d^{-1}), but was much lower than BNR (50-400 mg-N L^{-1} d^{-1}).[14,33-35] The main reason was the difference in substrate transfer in these systems. Conventional BNR was mainly operated in the continuous flow mode, but the lab-scale BES (e.g. MEC, MFC) was normally operated in the batch mode, which limited substrate diffusion from wastewater to biomass.[36]

Control tests to verify the occurrence of anammox and denitrification in MECs

Please add Fig. 3 here

Three controls were conducted in order to further elucidate the removal pathways of ammonium and nitrite in MECs. All the control tests were operated for 10 days (the same duration as the MEC tests). Control test 1 (ammonia+nitrite, without power supply) simulated the anammox process (Fig. 1 Control 1), in which the average ammonium, nitrite and nitrate removal rates were 2.80 mg-N L^{-1} d^{-1}, 3.24 mg-N L^{-1} d^{-1}, 0.44 mg-N L^{-1} d^{-1} respectively (Fig. 3). In contrast, the rates for the anammox MEC were 4.38 mg-N L^{-1} d^{-1}, 3.38 mg-N L^{-1} d^{-1}, and 0.33 mg-N L^{-1} d^{-1}, respectively (Fig. 3). The reason for the lower ammonium removal rate in Control 1 than MEC was that power supply in the MEC expedited the ammonium removal, while ammonium removal in Control 1 was solely dependent on anaerobic ammonium oxidation by nitrite. This indicated that ammonium could work as the anodic fuel in MECs and transferred electrons to the anode other than nitrite. In this study, the high potential provided by the power supply intrigued more ammonium oxidation in MECs than in Control 1, implying that MECs had other electron acceptor for ammonium oxidation compared with traditional anammox process. This finding was in accordance with previous study that ammonium oxidation possessed the negative Gibbs free energy to generate electricity.[19] In addition, ferric ion was found to work as electron acceptor by anammox bacteria without nitrite[37] and nitrate-dependent ferrous iron was oxidized by anammox bacteria,[38] indicating that anammox bacteria could utilize different types of electron donors and acceptors, and anammox involves numerous complicated processes. In contrast with ammonium removal difference in MEC and Conrol 1, nitrite removal rates were similar between Control 1 and MEC (Fig. 3), implying that nitrite removal were not related with power supply.

Control 2 was the test of single nitrogen species (ammonium or nitrite) with power supply in order to elucidate whether ammonium and nitrite should be co-present in wastewater for the enhanced anammox-like process in MECs (Fig. 1 Control 2). The ammonium removal rate without nitrite input was 1.12 mg-N L^{-1} d^{-1} (Fig. 3), which was much lower than those of MEC and Control 1, while the nitrite removal rate without ammonia input was 3.28 mg-N L^{-1} d^{-1} (Fig. 3), which was similar with those of MEC test and Control 1. Furthermore, the slopes of linear regression of ammonium, nitrite and nitrate removal rates were analyzed for the MEC, Control 1, and Control 2, showing an obvious change of ammonium removal rate compared with those of nitrite and nitrate (Fig. 3). The slope of ammonium data from MEC to Control 2 was -1.64, meaning MEC was effective at ammonium removal, while the slope of nitrite was only -0.025, meaning there was literally no change of nitrite removal among these three systems. These implied that co-presence of nitrite facilitated ammonium oxidation and nitrite was the main oxidant of ammonium. In contrast, nitrite removal was not affected by ammonium absence, indicating that nitrite could be reduced by anammox bacteria as well as by denitrifying bacteria. Denitrification process was dominant when without ammonium. Control 2 results implied that ammonium oxidation and nitrite reduction were carried out by anammox bacteria and/or anaerobic ammonium oxidation bacteria and denitrification bacteria. This synergic correlation accelerated the electron transfer from ammonium to nitrite and ultimately to anode.

Control 3 (ammonium+nitrite, with power supply, abiotic test) was conducted to verify the role of bacteria in anammox MECs (Fig. 1 Control 3). Both ammonium and nitrite removal rates (0.25 mg-N L^{-1} d^{-1} and 0.41 mg-N L^{-1} d^{-1}) were tremendously lower than those of MEC, Control 1 and Control 2 (Fig. 3), indicating that the removal of ammonium and nitrite was the biological process and bacteria carried out ammonium oxidation and nitrite reduction. All the MEC and

Control tests had low nitrate concentration (<5 mg L^{-1}, mainly coming from raw wastewater) and low nitrate removal rate (less than 0.49 mg-N L^{-1} d^{-1}) (Fig. 3), indicating that nitrification hardly occurred in anoxic conditions. Compared with complete nitrification (ammonium to nitrite and then to nitrate), short-cut nitrification (ammonium to nitrite) saved substantial reaction time, provided the essential electron acceptors (nitrite) in the following anammox, and saved carbon sources for partial denitrification (nitrite reduction).

The simultaneous removal of ammonium and nitrite in the anoxic condition in 10 days (Fig. 2) ascertained the occurrence of anammox in the MEC system. Nitrification was completely inhibited under anoxic condition (DO < 0.1 mg L^{-1}, with both anode and cathode being sealed), which was verified by the stable decrease of nitrite (no accumulation) over the 10-day period. This was a clear evidence that ammonium oxidation was carried out by anammox bacteria with nitrite as the electron acceptor. Several studies of anammox MFCs treating wastewater had the similar configurations (two chamber system), inoculation protocols (wastewater and nutrients ingredients), operational duration (5-14 days), and the same trend of decrease in ammonium and nitrite as this study [39-42], among which some validated the anammox reactions through engineering tests[39,42], and some confirmed the existence of anammox bacteria by microbial community analysis (e.g. anammox 16S rRNA gene as a molecular marker and functional biomarkers (e.g. nitrite reductase gene) involved in the anammox metabolisms)[40,41]. Furthermore, simultaneous removal of ammonium and nitrite in anaerobic and anoxic units had been observed in traditional anammox systems (not BES) treating various types of wastewater (e.g. municipal wastewater, swine wastewater, landfill leachate, salinity wastewater) in bioreactors (e.g. sequencing batch reactor, rotating biological contactor, upflow anaerobic sludge blanket (UASB), moving bed biofilm reactor)[43-51], among which some confirmed the occurrence of anammox reactions through biochemical measurement and engineering operation[43-48] and some detected anammox bacteria using microbial analysis (e.g. cloning with anammox gene primers and gene sequencing, fluorescence in situ hybridization (FISH) with specific anammox bacterial probes) [36,49-51]. By side-side comparing the experimental results in this work and previous anammox studies, the existence of anammox bacteria in the MFCs was ascertained.

Kinetic modeling of the accelerated anammox in MECs

Please add Fig. 4 here

Please add Table 1 here

The experimental data of ammonium and nitrite concentrations in anammox MEC and Control 1 (conventional anammox) were fitted in Models (Eq. 1-4) to determine critical kinetic parameters. Specifically, the modified Nernst-Monod equation was used for anammox MEC (Fig. 4a), even though not all of the electrons were accepted by the electrode but consumed by other processes (e.g. anammox and denitrification). Total nitrogen concentration change was measured over time (Fig. 4b). Multiplicative Monod modeling with dual substrates was used for Control 1 (Fig. 4c). The fittings of the model simulated data and the experimental results were examined using statistical analysis. The coefficient of determination (R^2) of the experimental results and the model simulated data was all higher than 97%, with ammonium in MECs 99.12%, nitrite in MECs 98.68%, ammonium in Control 1 97.24%, and nitrite in Control 1 99.48%, respectively. In addition, the models were examined by the difference (the subtraction of predicted data from the observed or actual data) between the ammonium and nitrite experimental data in the MEC and Control 1 (MEC experiment duplicated 4 times as shown in Fig 4a, Control 1 duplicated twice as shown in Fig 4c) to validate the model fitness (the inserted figures in Fig. 4). The distribution of the difference over the independent variable (time) indicated that they were randomly distributed and tended to cluster towards the middle of the plots (y=0) in both MEC and Control 1. In addition, the maximum difference was single digits (MEC maximum difference: 6.0 and -7.3, and Control 1 maximum difference: 5.2 and -6.0) without high bias, demonstrating that the models well fitted to the experimental data.

The calculated maximum specific utilization rate of ammonium in the MEC was much higher (0.38 mmol-N g^{-1}VSS h^{-1}) than that of Control 1 (0.18 mmol-N g^{-1}VSS h^{-1}) (Table 1). The maximum specific utilization rate of nitrite in the MEC was also higher (0.3 mmol-N g^{-1}VSS h^{-1}) than that of Control 1 (0.2 mmol-N g^{-1}VSS h^{-1}), implying MEC accelerated anammox reactions. A wide range (0.09-3.74 mmol-N g^{-1}VSS h^{-1}) of the maximum specific utilization rate had been reported for conventional anammox, and associated with anammox bacteria enrichment and operation modes.[29] In this study, anoxic bacteria (e.g. denitrifying bacteria, electrogenic bacteria) in the MEC co-present with anammox bacteria, which might cause the maximum specific utilization rate (0.3-0.38 mmol-N g^{-1}VSS h^{-1}) not higher than the reported values. Besides, high specific utilization rate was reported in the continuous flow mode with high N-loading (concentration: 230 mg-N L^{-1}),[28] while the MEC was conducted in the batch mode in this study with total nitrogen concentration of 90 mg-N L^{-1}. Nevertheless, the ammonium utilization rate in the MEC (anammox with power supply) was twice as in Control 1 (conventional anammox) in this study, indicating the substantial acceleration of anammox in MECs.

As for the half-velocity constant (K_S), a wide range (0.003-13.7 mM) had been obtained due to various model simulations.[26-29] K_S for ammonium (3.47 mM) and nitrite (3.40 mM) were almost same in Control 1 (conventional anammox) (Table1), which corresponded with the equal consumption of ammonium and nitrite in anammox reaction ($NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$). In contrast, nitrite constant (2.33 mM) was lower than ammonium constant (4.11 mM) in anammox MEC (Table1), indicating that ammonium was more easily utilized than nitrite, and thus the power supply of MECs promote the ammonium utilization than nitrite. These results corresponded well with the results of the removal rates of ammonium and nitrite in anammox MEC and Control 1 (Fig. 3), implying that power supply of MEC assisted the ammonium uptake more than nitrite.

Variation of ORP and pH throughout nitrogen removal processes

Please add Fig. 5 here

ORP (redox potential) and pH are the critical indicators for BNR processes.[1,52] ORP values increased over time in the anammox MEC, Control 1 and Control 2 (fed with nitrite), but decreased greatly in Control 2 (fed with ammonium) and remained stable in Control 3 (Fig. 5b). For Control 2 with ammonium input, there was no oxidizer in the system, so that ORP dropped as anoxic bacteria consumed the residue dissolved oxygen and fermentation took place, which also explained its low ammonium removal rate (Fig. 3). For Control 3 (abiotic test) without anoxic bacteria, ORP did not change, indicating that reactions barely happen without anoxic bacteria, and further proved the function of bacteria in the anammox MECs. For MEC test, Control 1 and Control 2 (fed with nitrite), ammonium was oxidized with nitrite as the electron acceptor and ORPs increased over time.

pH values increased in the MEC, Control 1, and Control 2 (fed with nitrite) (Fig. 5b), indicating the occurrence of denitrification, since pH did not change in anammox ($NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$). In contrast, pH dropped from 7.61 to 6.87 in Control 2 (fed with ammonium), which verified the occurrence of fermentation and corresponded to the ORP drop (Fig. 5a). pH remained constant in Control 3 (abiotic test without bacteria), indicating the absence of biochemical reactions that corresponded to low nitrogen removal rate (Fig. 3).

The interface between SCMFCs and anammox MEC

The power required by anammox MEC was provided from SCMFCs treating wastewater. The variation of COD concentration (ΔCOD) in SCMFCs was correlated with the targeted nitrogen concentration (ΔC_N) in anammox MEC (Eq.5 and Eq. 6). For anammox MEC, the amount of electrons transferred per mole of nitrogen to the anode (n in Eq.6) was determined based on the direct donation species of nitrogen. With ammonium as the electron donor for the anode (Fig. 4), the value of n was 3 in Eq.6 since 3 moles of electrons were transferred per mole of N in NH_4^+ to form nitrogen gas. The simulation of Eq.5 and Eq. 6 revealed the linear relationship between the COD change in SCMFCs and nitrogen concentration change in MEC (Fig. 6a).

Please add Fig.6 here

COD consumption in MFCs was selected in the range of 0-4500 mg L^{-1} based on diverse types of wastewater (municipal wastewater COD: 100-500 mg L^{-1}, food wastewater COD: ~8000 mg L^{-1}).[53,54] Nitrogen consumption range of 0-1000 mg L^{-1} in MECs was selected as ammonium concentration could reach ~1000 mg L^{-1} in certain types of wastewater (e.g. reject water).[55] The positive linear correlation of nitrogen and COD concentrations in the integrated MFC-MEC was different from that of conventional BNR where nitrogen removal deteriorated at high COD concentration due to the dominance of heterotrophic bacteria over autotrophic nitrifying bacteria.[1] In the meantime, sufficient carbon source is needed for denitrification in conventional

BNR.[1] Thereby, BNR has a strict requirement for COD concentration. The integrated MFC-MEC substantially overcame this requirement, since COD was consumed in MFCs to provide electric power for MECs, and nitrogen removal in MECs do not solely rely on slow growing anammox bacteria, which greatly reduced the dependence on COD concentrations. With more COD being consumed in MFCs, higher electric power was supplied to MECs for accelerated anammox and autotrophic denitrification.

A reliable interface between MFC and MEC should be built using power management system (PMS) in order to provide the stable voltage of MFCs to MECs.[22,56] In a PMS, the current flew through the MFC to charge a capacitor to a given charge potential value (V_c: 0.5 V in this study), and then the system started discharging the stored energy to MEC until the capacitor reached a discharge potential value (V_d: 0 V in this study). Through these charging/discharging cycles, the PMS could provide more stable voltage and power from MFCs to MEC than the direct connection of MFCs to MEC. The charge and discharge curve of the PMS capacitor was simulated (Fig. 6b). With the initial COD concentration of 2000 mg L^{-1} in the batch-mode SCMFCs, stable charging/discharging cycles lasted for 4 days with a frequency of 0.167 mHz (estimated based on previous research[56]). As the COD was consumed in the batch-mode SCMFC over time, the voltage provided from MFC started to drop after 4 days when COD was below 500 mg L^{-1}. The frequency slowed down to 0.030 mHz at the end of 5th day. With the PMS to connect MFCs and MECs, the drop of power output will be alleviated by adjusting the charge/ discharge frequency.

Energy savings of high-rate anammox in the integrated MFC-MEC system

The operational costs of the integrated MFC-MEC system and conventional BNR were compared in terms of energy and carbon source. The electric power consumption in conventional BNRs was around 0.505 kWh m^{-3} with aeration in complete nitrification as the main electricity cost.[57] In contrast, anammox in the integrated MFC-MEC system only needs short-cut nitrification for nitrite production, so that one third of electric power (~0.189 kWh m^{-3}) was consumed compared with traditional BNR (Table 2). Furthermore, nitrite could be produced without energy consumption. For example, nitrite can be produced in MFCs with oxygen as the electron acceptor in the cathode ($2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$).[15] Nitrite accumulated in autotrophic denitrification MFCs without aeration ($NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$, $2NO_2^- + 8H^+ + 6e^- \rightarrow N_2 + 4H_2O$) can be used for anammox.[31]

Another saving of anammox compared with conventional denitrification is the carbon source, since anammox microorganisms were autotrophic.[2] Nitrogen removal in MECs did not require organic carbon, while in the conventional BNR, carbon source was required for heterotrophic denitrification, which was about 3.5-5.0 of COD NO_3-N^{-1} (Table 2). Moreover, anammox was expedited in MECs powered by two series-connected single-chamber MFCs (SCMFCs) in this study (Fig. 1). One MFC produced the maximum power density of 417 mW m^{-2} with the maximum voltage output of 0.3 V at the external resistance ($R_{ext.}$) of 800 Ω. The series connection produced the steady voltage of ~0.50 V for the MEC and provided the energy of 0.28 kWh m^{-3} for nitrogen removal over the 10-day period. Therefore, the integrated MFC-MEC system is capable of accelerating anammox with the power produced from wastewater, and achieving self-sustained high-rate nitrogen removal at low energy and carbon cost.

Please add Table 2 here

Significance of accelerating anammox in the integrated MFC-MEC system for wastewater treatment plants

Low growth rate of anammox bacteria has posed a major obstacle for the acceptance of anammox in wastewater treatment plants. An innovative solution as explored in this study by expediting anammox in the integrated MFC-MEC system without solely relying on traditional anammox process, and thus expanding anammox applications. The simultaneous removal of ammonium and nitrite under anoxic condition clearly indicated the occurrence of anammox in MECs, given the previous anammox MFCs studies shared the similar inoculation/operational protocols, had the same ammonium/nitrite trend as this study and confirmed the existence of anammox bacteria.[39-42] By supporting MECs with the electric power generated in MFCs treating wastewater, the integrated system well utilized the unique feature of MFCs (long-term power production stability but low power output) and achieved the self-sustained anammox with higher nitrogen removel rate (8.2 mg-N L^{-1} d^{-1}) than conventional anammox (6.8 mg-N L^{-1} d^{-1}). Although the reation rate was limited in the batch-mode system compared with conventional continuous flow BNRs (μ_m value, Table 2)，the integrated MFC-MEC system was more energy efficient and required much less carbon sources than conventional BNRs.

Conclusion

Novel integrated MEC-MFC was developed to accelerate anammox without external power consumption and alleviate the dependence on slow growing anammox bacteria. Batch-mode tests clearly showed more than 85 % of nitrogen was removed in the anammox MEC within 10 days while only 62 % of nitrogen was removed in conventional anammox. The simulation of the modified Nernst-Monod model revealed the enhancement of half-velocity constant (K_s) and the maximum specific utilization rate (μ_m) in the anammox MFC over conventional anammox. The power supply of MECs intrigued the electron transfer from ammonium to anode, leading to a higher anammox rate. MFCs as the power supply to MECs achieved the self-sustained wastewater treatment, and well applied the low power output of MFCs. By saving energy consumption, avoiding carbon requirement, and accelerating anammox in a single unit, the integrated MFC-MEC possess a great potential for self-sustained advanced nitrogen removal in wastewater treatment plants.

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Caption list of tables and figures.

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Fig. 5 ORP (a) and pH (b) changes in anammox MEC and Controls before and after of experiments.

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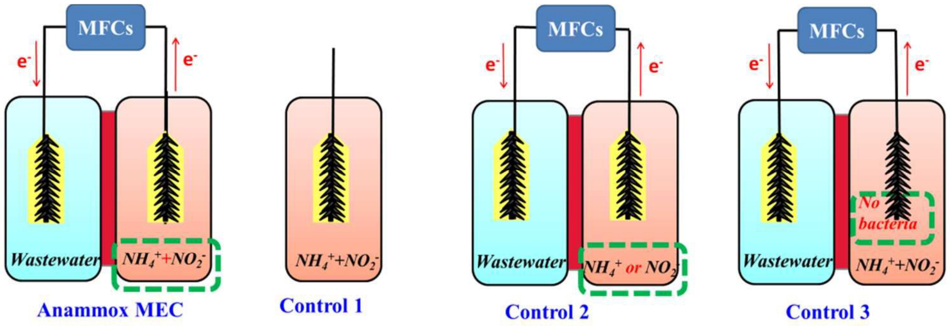
Table 1. Values of kinetic parameters derived from kinetics models (Eqs.1-4).

Symbol	Description	Value	Unit
$K_{SNH4+\ MEC}$	half-velocity constant of ammonium in anammox MEC	4.11	mM
$K_{SNO2-\ MEC}$	half-velocity constant of nitrite in anammox MEC	2.33	mM
$K_{SNH4+\ ana}$	half-velocity constant of ammonium in Control 1	3.47	mM
$K_{SNO2-\ ana}$	half-velocity constant of nitrite in Control 1	3.4	mM
$\mu_{mNH4+\ MEC}$	maximum specific utilization rate of ammonium in anammox MEC	0.38	mmol g^{-1}VSS h^{-1}
$\mu_{mNO2-\ MEC}$	maximum specific utilization rate of nitrite in anammox MEC	0.3	mmol g^{-1}VSS h^{-1}
$\mu_{mNH4+\ ana}$	maximum specific utilization rate of ammonium in Control 1	0.18	mmol g^{-1}VSS h^{-1}
$\mu_{mNO2-\ ana}$	maximum specific utilization rate of nitrite in Control 1	0.2	mmol g^{-1}VSS h^{-1}

Table 2. Comparison of anammox MEC, conventional anammox and BNR regarding energy consumption and saving.

	Nitrification and denitrification in conventional BNR[57]	**Conventional anammox**[26-29]	**Anammox MEC powered by MFC**
Oxygen consumption (mole per mole of NH_4^+)	2	0.75	0.75
Energy production (kWh m^{-3})	–	–	0.28
Energy consumption (kWh m^{-3})	0.505	0.19	0.19
Energy saving (kWh m^{-3})	-0.505	-0.19	+0.09
COD requirement (mg L^{-1} per mg-N L^{-1})	3.5-5	–	–
μ_m (mmol g^{-1}VSS h^{-1})	1.21-9.86	0.09-3.74 (0.18-0.2 in Control 1, this study)	0.3-0.38



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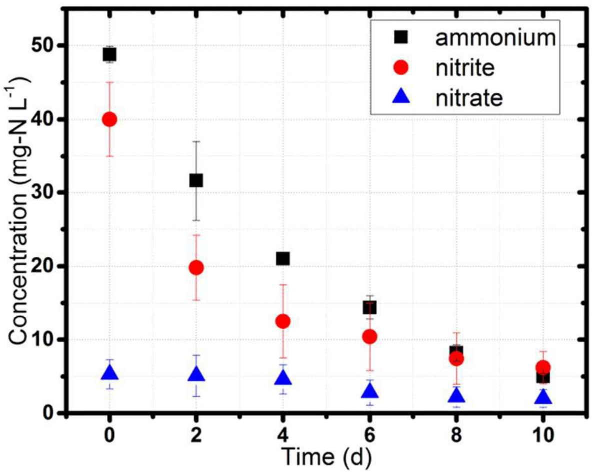

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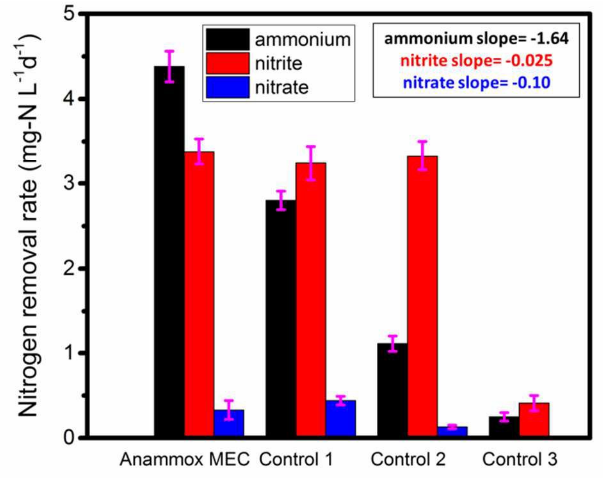

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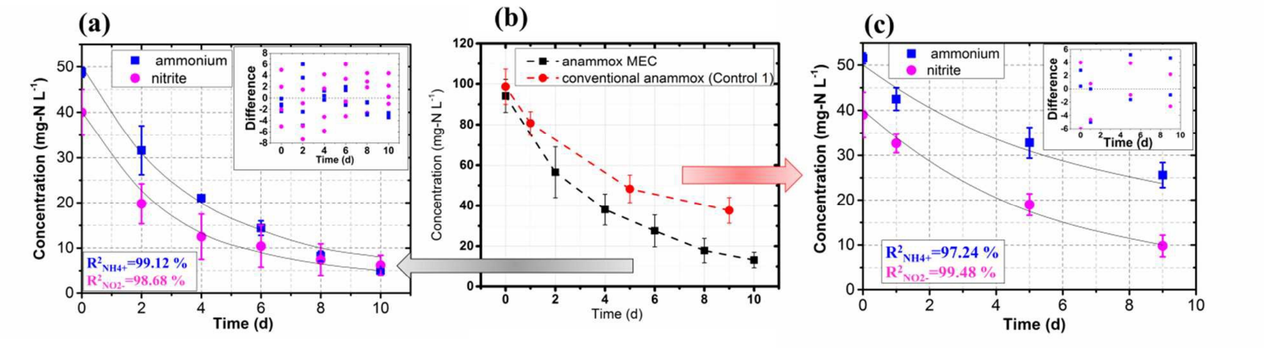

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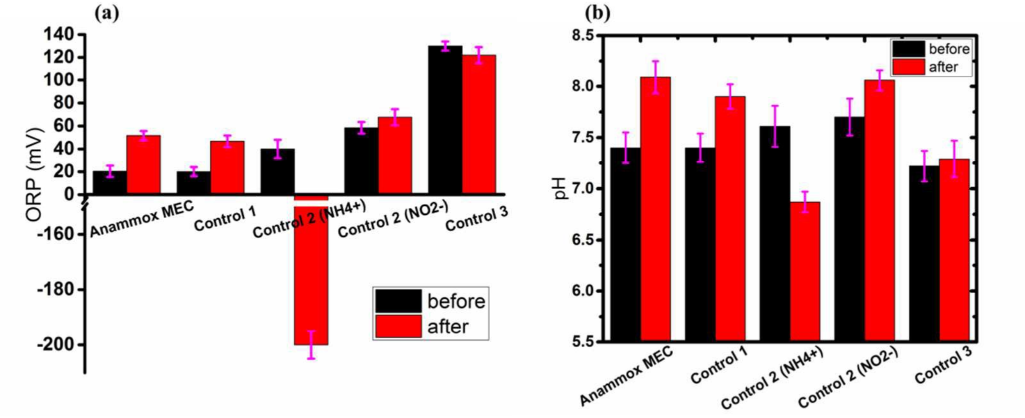

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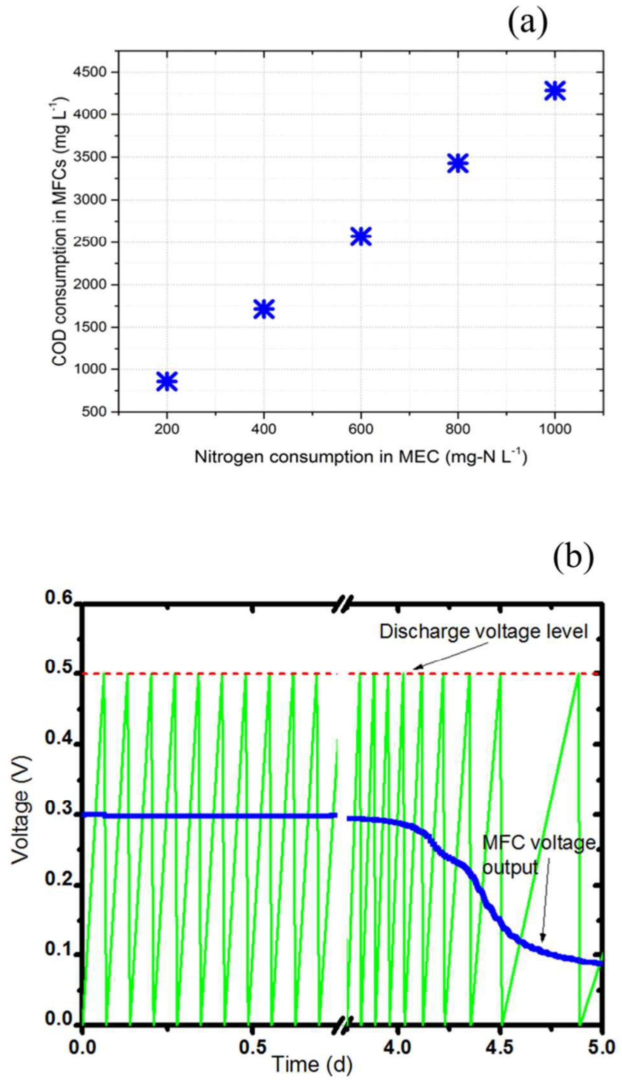

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