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Ano-Cathodophilic Biofilm Catalyzes Both Anodic Carbon Oxidation and Cathodic Denitrification

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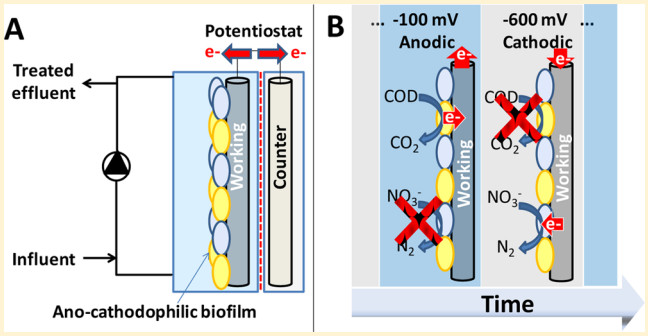
Supporting Information

ABSTRACT: Biocathodic denitrification using bioelectrochemical systems (BES) have shown promise for both wastewater and groundwater treatment. Typically, these systems involve anodic carbon oxidation and cathodic denitrification catalyzed by two electroactive biofilms located separately at an anode and a cathode. However, process efficiencies are often limited by pH drifts in the respective electrode-biofilms: acidification (pH <5.5) in the bioanode and basification (pH >8.5) in the biocathode. Here, we describe for the first time a single electroactive biofilm that acts as a bioanode and a biocathode, alternately catalyzing anodic acetate oxidation (Coulombic efficiency (CE) 85.3%) and cathodic denitrification (CE 87.3%) (−400 mV Ag/AgCl). Our results indicate that the ano-cathodophilic biofilm denitrified autotrophically using the electrode (−200 to −600 mV Ag/AgCl) as a direct electron donor. Further, the alkalinity produced from cathodic denitrification partially (19%) neutralized the acidity of the anodic reaction. Switching the electrode potential to temporarily favor either an anodic or cathodic reaction may represent a unique method for removing carbon and nitrate from contaminated liquors. This study offers new insights into the development of sustainable BES-based nutrient removal processes.



INTRODUCTION

In conventional biological nitrogen removal, ammonium is first oxidized to nitrite (NO_2^-) by autotrophic ammonium oxidizing bacteria and nitrite is further oxidized to nitrate (NO_3^-) by nitrite oxidizing bacteria. Denitrification is finally completed by heterotrophic denitrifying bacteria, which reduce both nitrite and nitrate to dinitrogen using organic carbon in the wastewater as an electron donor (reaction 1: complete denitrification with acetate as the electron donor).[1]

$$5CH_3COO^- + 8NO_3^- + 3H^+ \rightarrow 10HCO_3^- + 4N_2 + 4H_2O \quad (1)$$

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- (\text{at anode}) \quad (2)$$

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O (\text{at cathode}) \quad (3)$$

Recently, bioelectrochemical systems (BES) have been proposed as an alternative technology to achieve denitrification.[2−5] These systems make use of electroactive bacteria to catalyze anodic carbon oxidation (reaction 2) and cathodic denitrification (reaction 3) at an anode and a cathode, respectively. Such bioelectrochemical processes harness the reducing power (electrons) contained in wastewater organics directly as electricity (at the anode), which drives the cathodic denitrification reaction.[2,3,5−8] For example, Virdis et al.[3] achieved nitrate removal efficiency of 97.1% with a BES using synthetic wastewater containing a low COD/N ratio of 4.48 kg COD kg^{-1} NO_3^--N, a ratio that is much lower than typically required for heterotrophic denitrification.[9]

Typically, anodic oxidation of carbon and cathodic denitrification are catalyzed by two different electroactive biofilms located separately at the anode and cathode of a BES. Since the anodic carbon oxidation liberates protons (reaction 2) and the cathodic denitrification reaction consumes protons (reaction 3), prolonged operation of a BES inevitably leads to significant pH drift in the respective electrode-biofilms (acidification in the anodic biofilms and basification in the cathodic biofilms).[10] If not neutralized (typically by adding acid/base or pH buffers), such a pH drift severely inhibits biofilm activity and eventually the entire process stalls.[6,11,12] For instance, Clauwaert et al.[6] demonstrated that biocathodic denitrification rates in a BES could be improved 2.3-fold (1.5 vs 0.66 mM-N h^{-1}) by continuous neutralization of the electrolyte, preventing basification (>pH 8).[2,6] However, continuous pH adjustments during BES operation are not practical.[13]

To control pH drift without dosing external chemicals, Cheng et al.[11] verified a concept that allows an electroactive

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biofilm to maintain a neutral pH environment while sustaining current production. They demonstrated that an anodophilic biofilm can intermittently catalyze anodic carbon oxidation and cathodic oxygen reduction when acetate and oxygen were alternately supplied to such an "ano-cathodophilic" biofilm.[11,14]

This study aimed to test whether the same principle held true for a cathode-driven denitrifying BES. Since cathodic denitrification also consumes protons (reaction 3), allowing an electroactive biofilm to alternately catalyze anodic carbon oxidation and cathodic denitrification might help reduce the demand for external chemicals to control pH. To our knowledge this is the first report demonstrating that anodophilic biofilms can denitrify using electrons derived from the same electrode, which temporarily serves as a bioanode and a biocathode in a BES. The relationship between electrode potential and the cathodic denitrification rate for such unique switchable electrophilic biofilm was also examined. This study offers new insights into the development of sustainable BES-based nutrient removal processes.

EXPERIMENTAL SECTION

Bioelectrochemical System. All experiments were conducted using a two-chamber bioelectrochemical reactor. It consisted of two identical half cells (14 × 12 × 2 cm) separated by a cation exchange membrane (surface area 168 cm^2) (Ultrex CMI-7000, Membrane International Inc.). Both half cells were loaded with identical conductive graphite granules (3−5 mm diameter, KAIYU Industrial (HK) Ltd.). This reduced the void volume of each half cell from 336 to 220 mL. Four graphite rods (5 mm diameter, length 12 cm) were used as current collectors in each half cell to enable electrical connection between the graphite granules and the external circuit.

The reactor was operated as a three-electrode system coupled to a potentiostat.[11] Only one-half cell was inoculated with bacteria and the electrode therein is termed the working electrode. The electrode in the other half cell is termed the counter electrode. The working electrode was polarized against a silver−silver chloride (Ag/AgCl) reference electrode (MF-2079 Bioanalytical Systems) at a defined potential using the potentiostat. The reference electrode was mounted in the working chamber and was embedded within the granular graphite working electrode. All electrode potentials (mV) reported in this paper refer to values against an Ag/AgCl reference electrode (ca. +197 mV vs standard hydrogen electrode [15]).

The BES process was continuously monitored and controlled using a computer program (LabVIEW). The working electrode potential and current of the BES were monitored via the potentiostat. The pH and oxidation−reduction potential (Eh) of the working electrolyte were continuously monitored using in-line Eh and pH sensors (TPS Ltd. Co., Australia), respectively. All signals were regularly recorded to an Excel spreadsheet via the computer program interfaced with a National Instrument data acquisition card (DAQ). Accuracies (less than 0.1 mV) of the voltage signals from the DAQ were regularly checked using a high precision digital multimeter (resolution 10 μV).

Process Start-up and General Operation. Returned activated sludge obtained from a secondary municipal wastewater treatment plant was used to inoculate the working chamber (ca. 2 g MLSS L^{-1}). The plant had been operated to achieve biological carbon and nitrogen removal. Unless stated otherwise, the working medium consisted of (mg L^{-1}): NH_4Cl 125, $NaHCO_3$ 125, $MgSO_4{\cdot}7H_2O$ 51, $CaCl_2{\cdot}2H_2O$ 300, $FeSO_4{\cdot}7H_2O$ 6.25, and 1.25 mL L^{-1} of trace element solution, which contained (g L^{-1}): ethylene-diamine tetra-acetic acid (EDTA) 15, $ZnSO_4{\cdot}7H_2O$ 0.43, $CoCl_2{\cdot}6H_2O$ 0.24, $MnCl_2{\cdot}4H_2O$ 0.99, $CuSO_4{\cdot}5H_2O$ 0.25, $NaMoO_4{\cdot}2H_2O$ 0.22, $NiCl_2{\cdot}6H_2O$ 0.19, $NaSeO_4{\cdot}10H_2O$ 0.21, H_3BO_4 0.014, and $NaWO_4{\cdot}2H_2O$ 0.050.[11] Sodium acetate or sodium nitrate were provided as the electron donor or acceptor, respectively. A similar medium was used as the counter electrolyte except no acetate or nitrate was added. During the initial start-up period, yeast extract was added to the working electrolyte (50 mg L^{-1} final concentration) as a bacterial growth supplement. Both the working and counter electrolytes were renewed regularly (and prior to each specific experiment as described below) to avoid accumulation of ions. A total liquid volume of 400 and 2000 mL was continuously recirculating (ca. 8 L h^{-1}) through the working and the counter half cell, respectively, via two separate external recirculation bottles (250 mL and 2 L). A gas bubbler filled with deionized water (ca. 100 mL) was installed as a one-way gas valve to prevent atmospheric air intruding into the working chamber via the recirculation bottle. Gases produced inside the working chamber were vented through the water trap to the atmosphere. The process was operated at 22 ± 2 °C.

Establishing Anodophilic Biofilm at the Working Electrode. After inoculation with activated sludge the working electrode was maintained at a constant potential of −300 mV for about 10 days to establish an anodophilic biofilm on the graphite granules.[11] From day 4 onward the pH of the working electrolyte was controlled at 7 ± 0.2 by feedback dosing NaOH (2 M) or HCl (2 M) in the recirculation bottle, to overcome anodic acidification. Renewal of the working electrolyte was performed at day 7 and 9 when the system had shown signs of substrate depletion (anodic current declined to <10% of the previous maximum level). During each medium renewal, the working chamber was flushed and occasionally backwashed (backward flow of liquid) with approximately 2 L fresh medium to remove planktonic cells.

To verify whether an effective anodophilic biofilm had been established after the start-up period, anodic Coulombic efficiency (i.e., the percentage of electrons recovered as anodic current from the electron donor substrate, here acetate) of the anodic reaction (at electrode potential of −300 mV) was determined.[16] The affinity of the biofilm for the anodic potential was also measured to obtain the relationship between anodic current and electrode potential and the results are described separately in the Supporting Information (SI) (Figure S1-3).[17]

Effect of Acidification on the Anodic Activity of the Anodophilic Biofilm. To test the effect of electrolyte acidification, the anodic current produced by the established biofilm was recorded with or without active control of the electrolyte pH. Prior to the experiment, the working chamber of the BES was flushed with 2 L fresh medium and steady state (baseline current close to zero) was maintained thereafter in the absence of acetate. After steady state was reached, concentrated sodium acetate solutions (0.83 mmol acetate, 277 μL 3M) were injected every two hours into the working electrolyte over an 80 h period to trigger periodic anodic current production by the biofilm. The electrolyte was actively maintained at about pH 6.8 throughout the experiment by feedback dosing NaOH (2 M), except during 8−40 h where pH control was inactivated to allow electrolyte acidification. The working electrode was poised at −400 mV throughout the run.

Evaluating the Ability of the Anodic Biofilm to Drive Cathodic Denitrification. *Nitrate-Spike Experiments.* To test whether the established anodic biofilm could denitrify using electrons obtained from the same working electrode, two separate nitrate-spiking experiments were conducted. The first trial was conducted (after the previous acidification study) using the actively metabolizing anodophilic biofilm and the electrolyte pH was controlled at 6.8−7.5 by dosing NaOH (2 M) or HCl (2 M). Prior to the experiment the working chamber was flushed, backwashed and then filled with fresh medium free of acetate and nitrate. After steady state was reached, sodium acetate (3.3 mmol) was added to trigger the expected anodic current response. Once the current had declined to its original, low background level, sodium nitrate (3.0 mmol) was added to test if the biofilm could now drive cathodic current production. Since the counter electrolyte was not supplied with acetate or other organic electron donors, the reducing equivalents (electrons) required for nitrate reduction at the working electrode were derived from water splitting reactions at the counter electrode. The consumption of OH^- and H^+ at the working chamber was also recorded over the course of this experiment.

The second trial was conducted in a similar fashion, except with the following modifications: (i) the electrolyte pH was not controlled in order to observe to what extent it would increase with cathodic denitrification; (ii) atmospheric air was allowed to intrude into the working recirculation bottle headspace to enable trace amounts of oxygen to dissolve into the working electrolyte. This step was to ensure complete oxidation of carbon (here acetate) before the subsequent nitrate injection. Hence, any observable nitrate reduction would be most likely be driven by the cathodic reaction; (iii) concentration profiles of acetate, nitrate and nitrite were obtained to verify the relationship between current production, acetate oxidation and nitrate reduction. The working electrode was poised at −400 mV in both experiments.

Basification Effect of Cathodic Denitrification on Cathodic Current Production. In poorly pH-buffered environments cathodic denitrification is known to cause detrimental basification of the electroactive biofilm.[6] To study the effect of basification the nitrate-driven cathodic current was recorded with or without active control of the electrolyte pH. Prior to the experiment the working chamber was flushed with 2 L of fresh medium and steady state was maintained thereafter in the absence of both acetate and nitrate. After steady state was reached concentrated sodium nitrate solution (0.2 mmol) was added to the working electrolyte every three hours over a 50 h period to trigger periodic cathodic current production by the biofilm. The electrolyte was actively maintained at about pH 7 throughout the experiment by feedback dosing 2 M HCl, except from 20 to 43 h where pH control was terminated to permit electrolyte basification. The working electrode was poised at −400 mV.

The Impact of Cathodic Polarization on Cathodic Denitrification. A kinetic experiment was conducted to examine the relationship between electrode potential, current and cathodic denitrification rate. Prior to the experiment the working chamber was thoroughly flushed with 4 L of fresh medium under open circuit conditions (i.e., no potential was applied) to remove any residual acetate or other organic carbon sources. The chamber was then refilled with fresh medium and a steady state was established with an initial electrode potential of −600 mV. After reaching steady state, sodium nitrate solution (3M) was injected into the working electrolyte to obtain a nitrate concentration of approximately 4 mM. The electrode potential was varied between 0 and −600 mV in a stepwise manner. Changes to potential were made only when the current appeared steady, which was typically attained after 30 min. Working electrolyte samples (0.5 mL) were collected every 10−15 min and quantified for nitrate to establish denitrification rates with each increase in potential. Denitrification rates were obtained from linear regression curves. The nitrate concentration throughout the experiment ranged from 2 to 4 mM, and this was confirmed to be sufficient to assume zero-order nitrate reduction kinetics. The resulting current and denitrification rates (mM h^{-1}) were plotted against electrode potentials. A control was run in the absence of nitrate.

Alternate Supply of Acetate and Nitrate to the Ano-Cathodophilic Biofilm. To validate the concept of using a single ano-cathodophilic biofilm for alternating the catalysis of anodic carbon (acetate) oxidation and cathodic denitrification, an experiment was conducted to allow the biofilm to alternately receive acetate (2.4 mmol) and nitrate (1.2 mmol) in a sequential manner. Electrolyte pH was maintained at ≥6.8 by feedback dosing 2 M NaOH. The working electrode was poised at −400 mV over a period of about 100 h. The consumption of NaOH was recorded to determine actual OH^- demand, and compared with the theoretical OH^- demand assuming that 7 mol OH^- is required per 8 mol of electrons produced as current (reaction 2, i.e. $7H^+/8e^-$). Coulombic efficiencies of both the anodic acetate oxidation and the cathodic denitrification were estimated as the ratio (%) of the recovered anodic/cathodic charges and the theoretical charges produced assuming 8 mol electrons could be released per mol of acetate added (reaction 2) and 5 mol electrons could be accepted per mol of nitrate added (reaction 3).[8] This assumption of five electrons accepted in denitrification defines the theoretical maximum coulombs (578.9 coulombs) that the cathode could possibly donate under the fully anoxic conditions in this experiment. Conversion of electrons to Coulombs (C) was based on Faraday's constant (96 485 C·mol $electron^{-1}$).

Chemical Analysis. Liquid samples collected from the BES were immediately filtered through a 0.22 μm filter (0.8/0.2 μm Supor Membrane, PALL Life Sciences) upon collection and were stored at 4 °C prior to analysis. Acetate, nitrite (NO_2^--N) and nitrate (NO_3^--N) were analyzed using a Dionex ICS-3000 reagent free ion chromatography (RFIC) system equipped with an IonPac AS18 4 × 250 mm column. Potassium hydroxide was used as an eluent at a flow rate of 1 mL min^{-1}. The eluent concentration was 12−45 mM from 0 to 5 min, 45 mM from 5 to 8 min, 45−60 mM from 8 to 10 min and 60−12 mM from 10 to 13 min. Column temperature was maintained at 30 °C. Suppressed conductivity was used as the detection signal (ASRS ULTRA II 4 mm, 150 mA, AutoSuppression recycle mode).

RESULTS AND DISCUSSION

Acclimatization of Anodophilic Biofilm and its pH Sensitivity. To demonstrate whether a single electroactive biofilm could catalyze both anodic carbon oxidation and cathodic denitrification we first established an anodophilic biofilm on the graphite working electrode, which was poised at a potential of −300 mV as described above (Figure 1). Anodic current began to evolve two days after inoculation (Figure 1A). As the anodic current gradually evolved, anolyte acidification intensified (Figure 1B, 48−96 h). To verify whether this limited

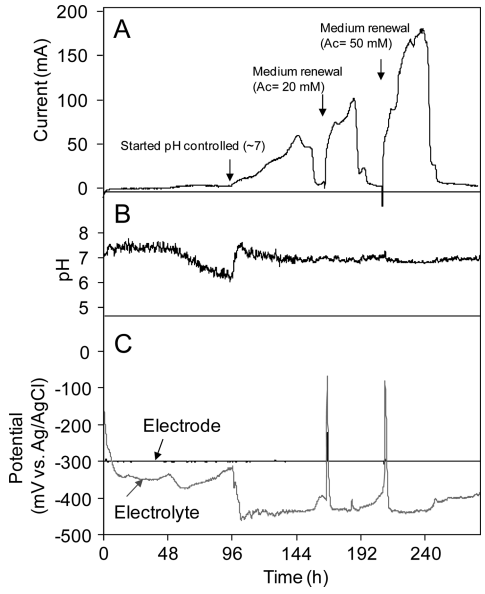

Figure 1. Evolution of anodic current during the initial acclimatization of the electroactive biofilm. Activated sludge was inoculated at time zero.

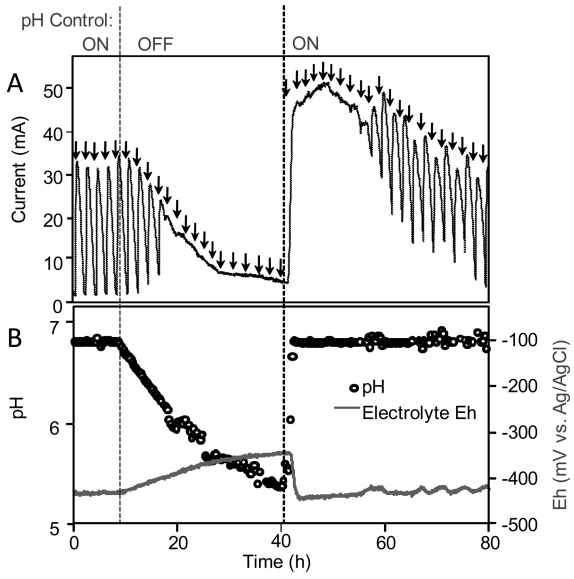

Figure 2. Current production by the established anodic biofilm receiving a regular supply of acetate with and without active pH control (~6.8). Notes: solid black arrows indicate supplies of 0.83 mmol acetate; the biofilm-electrode was maintained at a potential of −400 mV vs Ag/AgCl.

the onset of anodic activity, the anolyte was neutralized with sodium hydroxide. Anodic current production resumed immediately, until the substrate (acetate) became limiting. Replenishments of substrate and the removal of planktonic cells thereafter resulted in immediate resumption of anodic current production, indicating that an anodophilic biofilm was established on the electrode (Figure 1A). More detailed characterization of the anodic activity of the established biofilm is provided in the SI (Figure S1-4).

Acidification Inhibited Anodic Activity. Since the anodic microbial oxidation of acetate releases protons, prolonged current production resulted in acidification (pH < 5.4) of the electrolyte when no active pH correction was implemented (Figure 2). As long as the electrolyte was neutralized the biofilm stayed metabolically active for anodic current production (Figure 2B and SI Figure S4). Such a pH sensitivity of anodophilic biofilms has been described by others.[7,11] However, the ability of an anodophilic biofilm to catalyze denitrification, and subsequently neutralize the electrolyte pH, has not been tested.

The Anodophilic Biofilm Could Denitrify Using Electrons from the Same Electrode. Nitrate-spiking tests were conducted with the established anodophilic biofilm to test whether it could denitrify using electrons derived from the same working electrode (Figure 3). Cathodic current was produced almost instantly after the addition of nitrate (Figure 3A, E). This is surprising because prior to this test the biofilm was not acclimatized for cathodic denitrification. The negative current observed in Figure 3E prior to acetate and nitrate addition was probably due to cathodic oxygen reduction as air was allowed to intrude into the working chamber.[11] Nevertheless, chemical measurements confirmed the cathodic current production was associated with denitrification in the absence of organic carbon (acetate), indicating that nitrate was reduced using the working electrode as electron donor (Figure 3H).

Since abiotic hydrogen production was unlikely to occur at the set potential of −400 mV,[2,4,18] the observed cathodic current was most likely driven by the biofilm via autotrophic

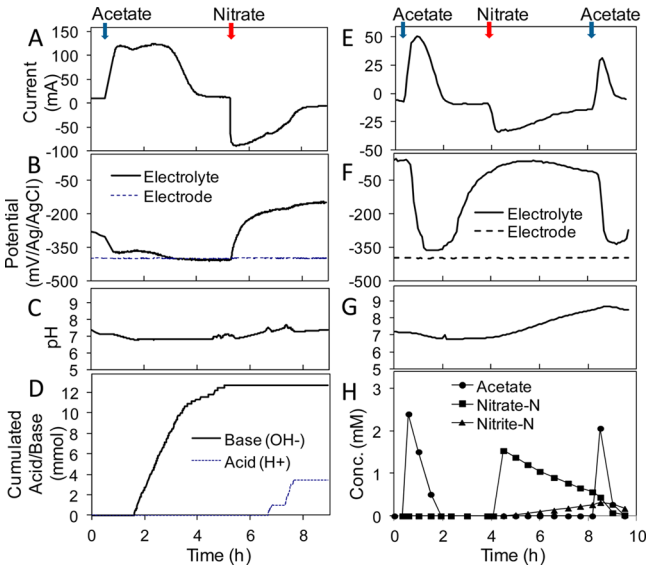

Figure 3. Effect of nitrate on the electrochemical activity of the established anodophilic biofilm. [A-D]: pH controlled at 6.8−7.5 by dosing 2 M NaOH/HCl; acetate added at 0.8 h (8.25 mM); nitrate added at 5.2 h (7.5 mM-N); [E-H]: pH uncontrolled; aerobic headspace maintained to ensure complete oxidation of acetate (3.5 mM) before nitrate addition (2 mM-N). Working electrode was poised at −400 mV vs Ag/AgCl in both runs.

denitrification.[5,19,20] The result also indicated that the acidity produced from anodic acetate oxidation could be neutralized by the alkalinity produced from the cathodic reaction (Figure 3C, G).

Basification Inhibited Cathodic Denitrification. Cathodic denitrification leads to localized electrolyte basification, which is known to suppress the activity of denitrifying cathodic biofilms.[6] To better understand the impact of basification on biofilm activity, the nitrate-induced cathodic current was recorded with or without active control of the electrolyte pH

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(Figure 4). When the electrolyte pH was actively controlled at around 7 the biofilm responded to the regular nitrate spikes

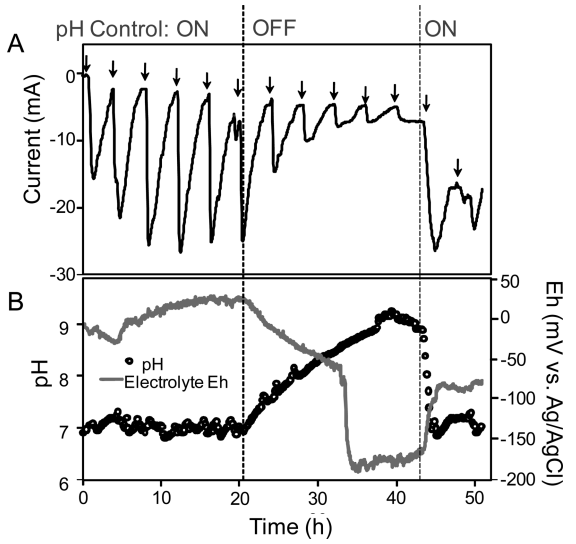

Figure 4. Cathodic nitrate-driven current production by the "ano-cathodophilic" biofilm with or without active pH control (~pH 7, with 2 M HCl). Solid black arrows indicate supplies of 0.20 mmol NO_3^--N (100 μL of 2 M sodium nitrate); the biofilm-electrode was maintained at a potential of −400 mV vs Ag/AgCl.

with cathodic current production. When no pH control was used, basification occurred (pH >8) leading to a gradual decline in cathodic current. Since nitrate was regularly replenished, the decline in cathodic current was attributed to electrolyte basification rather than nitrate limitation. Correcting the pH back to neutral immediately reinstated the cathodic activity (Figure 4), supporting the hypothesis that the established biofilm preferred a neutral environment for acquiring electrons from the electrode for denitrification.[6]

Cathodic Denitrification Rate Depended on Electrode Potential. Given that the graphite electrode acted as an electron donor during denitrification at the ano-cathodophilic biofilm, its potential should determine how much energy the microorganisms could gain from the reaction.[8] In theory, cathodic denitrification would be more thermodynamically favorable at a lower (more negative) electrode potential. Figure 5 shows that when the electrode potentials were more positive than −200 mV, only low or negligible levels of current and denitrification were obtained, indicating that the microorganisms struggled to obtain electrons from the electrode for nitrate reduction. Decreasing the potential from −200 to −600 mV increased both the cathodic current and denitrification rate (Figure 5).[2,5,19] This result suggests that the observed denitrification was predominately driven by the biocathode rather than endogenous reducing equivalences from the biofilm. In the absence of nitrate (and oxygen), current stayed low throughout the potential range tested, suggesting that cathodic hydrogen production was insignificant. Therefore, hydrogenotrophic denitrification was unlikely to have played a role and it is likely that the ano-cathodophilic biofilm denitrified using the electrode as a direct energy source.

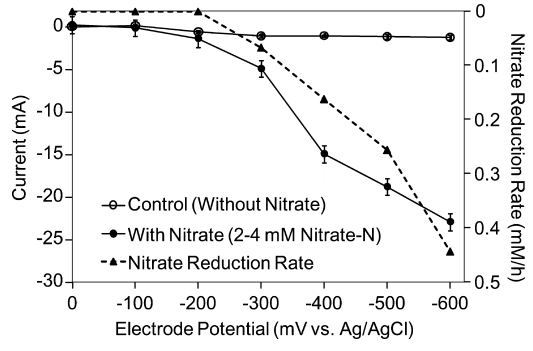

Figure 5. Dependency of cathodic current and denitrification rate of the "ano-cathodophilic" biofilm on electrode potential.

Alternating Current Driven by the "Ano-Cathodophilic Biofilm" Receiving an Alternating Supply of Acetate and Nitrate. After prolonged nitrate exposure (over two weeks), the working electrolyte was refreshed with nitrate-free medium and the anodic activity of the biofilm was verified by acetate addition. The result showed that the biofilm could immediately produce an anodic current again. Hence, the anodic activity of the biofilm was conserved without being compromised by the cathodic denitrification reaction. Henceforth, the established biofilm was regarded as "ano-cathodophilic".

To further demonstrate the concept of using a single electroactive biofilm for alternating catalysis of anodic carbon (acetate) oxidation and cathodic denitrification, we repeatedly provided the ano-cathodophilic biofilm with alternating supplies of acetate and nitrate (Figure 6). Clearly, the biofilm could reproducibly catalyze an alternating flow of electrons using acetate and nitrate as electron donor and acceptor, respectively (Figure 6A).

Coulombic efficiencies (CE) of both anodic acetate oxidation and cathodic denitrification were up to 85 and 87%, respectively (Table 1). Such high CE values reveal that both the acetate oxidation and denitrification were predominately

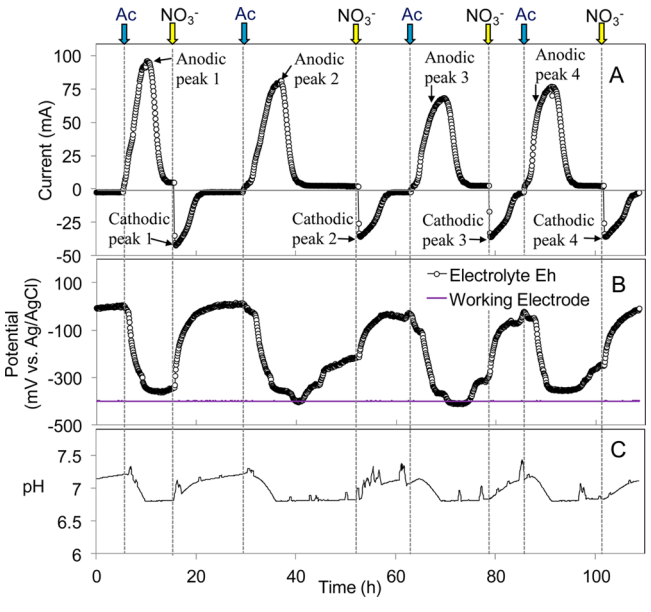

Figure 6. Alternating catalysis of anodic acetate oxidation and cathodic denitrification by the "ano-cathodophilic" biofilm. Electrolyte pH was maintained at ≥6.8 by feedback dosing 2 M NaOH. Anodic current was triggered by adding sodium acetate (2.4 mmol) and cathodic current was triggered by adding sodium nitrate (1.2 mmol). Working electrode poised at −400 mV vs Ag/AgCl.

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Table 1. Coulombic Efficiencies (CE) for the Alternating Anodic and Cathodic Current Catalyzed by the Ano-Cathodophilic Biofilm, And the Associated Reduction of Alkali Demand (Data from Figure 6)

(A) anodic phase	charges recovered as anodic current	anodic CE	theoretical OH^- demand[a]	actual OH^- demand	alkali saving
peak	Coulombs	%	mmol	mmol	%
1	1589.7	85.8	14.4	12.9	10.7
2	1672.3	90.3	15.2	12.2	19.5
3	1485.1	80.2	13.5	11.1	17.5
4	1570.3	84.8	14.2	10.2	28.3
average (±S.D)	1579.3 (76.8)	**85.3** (4.1)	14.3 (0.7)	11.6 (1.2)	19.0 (7.3)
(B) cathodic phase	charges recovered as cathodic current		cathodic CE	theoretical OH^- supply[b]	
peak	Coulombs		%	mmol	
1	506.3		87.5	6.30	
2	523.7		90.5	6.51	
3	483.3		83.5	6.01	
4	507.7		87.7	6.31	
average (±S.D)	505.3 (16.6)		**87.3** (2.9)	6.28 (0.21)	

[a]The theoretical OH^- demand was estimated assuming 7 mol OH^- required per mol of acetate oxidized anodically under a poorly pH buffered environment; [b]The theoretical OH^- supply was estimated assuming 6 mol OH^- produced per mol of nitrate reduced cathodically to N_2; 19.2 mmol of electrons (1852.5 Coulombs) were added as acetate to trigger the anodic current production; 6 mmol of electrons (578.9 Coulombs) could in theory be accepted by the added nitrate for each cathodic peak.

catalyzed by the ano-cathodophilic biofilm using the attached graphite electrode as either an electron acceptor or donor. While some of the protons released from the anodic acetate oxidation were neutralized with NaOH, the alkalinity produced by cathodic denitrification compensated for about 19% of the total alkalinity demand of the anodic reaction (Table 1). It is worth noting that in theory a lower "actual OH^- demand" of 8.02 (i.e., 14.3−6.28 mmol OH^-) instead of 11.6 mmol OH^- should be obtained (Table 1). If this was the case (e.g., due to an imprecision in the actual alkali usage), then a higher alkalinity saving of 43.9% (i.e., 6.28/14.3 × 100%) could in theory be achieved in the experiment. Nevertheless, proper stoichiometric tuning between the supplies of acetate and nitrate is expected to further reduce the external alkalinity demand and should be the subject of future studies.

Implication and Perspectives. From a wastewater treatment perspective, a sufficient degree of alkalinity should be available to neutralize the acidity produced from the anodic reaction in a BES. In principle, if the alkalinity demand (anodic carbon oxidation) and supply (cathodic denitrification) are stoichiometrically matched, the bioelectrochemical treatment should require no external chemicals for pH control. However, domestic wastewater streams often do not contain the required alkalinity and as such an external supply of alkalinity is necessary.[13,21] Our finding suggests that by allowing the biofilm to alternately catalyze cathodic denitrification, not only could nitrogen removal be achieved but also the alkalinity produced could partially compensate the alkalinity requirement of the anodic carbon oxidation. Recently, a rotatable-electrode BES configuration was proposed to exploit the capacity of ano-cathodophilic biofilms for wastewater treatment without the need of dosing chemicals for active pH control.[14,22] It may be worth testing whether this unique configuration can facilitate the ano-cathodopilic biofilm to achieve denitrification.

Furthermore, our results indicate that the catalytic behavior of the ano-cathodophilic biofilm on the graphite electrode was greatly influenced by the electrode potential; that is, at −600 mV it was thermodynamically unfavorable for the electrode to drive anodic acetate oxidation but favorable for cathodic denitrification, whereas at a potential of −100 mV it was thermodynamically unfavorable for cathodic denitrification but favorable for the anodic reaction (Figure 5 and SI Figure S2). This suggests that the ano-cathodophilic biofilm may be used for selective catalysis of either carbon oxidation or denitrification by switching the electrode potential between −100 and −600 mV (Figure 7). It would be worth testing whether the ano-cathodophilic biofilm might still prefer using the electrode at −100 mV as an electron acceptor to oxidize carbon even in the presence of nitrate.[23]

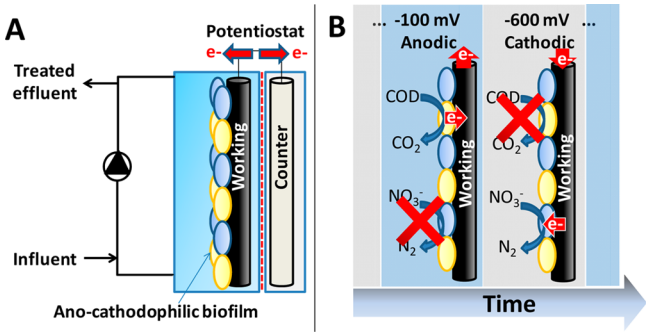

Figure 7. Selective biocatalysis of either anodic carbon oxidation or cathodic denitrification by switching electrode potential of an electrode associated with an "ano-cathodophilic biofilm".

In conclusion, the present findings substantiate previous observations that a single electroactive biofilm could be used to operate both a bioanode and a biocathode in bioelectrochemical systems.[11,14,22,24,25] It is the first example that an ano-cathodophilic biofilm could also drive cathodic denitrification, but whether the same group of bacteria in the biofilm could catalyze bidirectional electron flow is uncertain and hence further research is warranted.

■ ASSOCIATED CONTENT

Supporting Information

Anodic acetate oxidation and alkali consumption properties of the established electrophilic biofilm; relationship between anodic current and electrode potential for the established

biofilm. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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