

Enhanced nitrogen removal in bio-electrochemical systems by pH control

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Abstract Microbial fuel cells can be designed to remove nitrogenous compounds out of wastewater, but their performance is at present limited to 0.33 kg NO₃⁻-Nm⁻³ net cathode compartment (NCC)⁻¹d⁻¹. By maintaining the pH in the cathode at 7.2, nitrogen removal was increased from 0.22 to 0.50 kg NO₃⁻-Nm⁻³ NCC d⁻¹. Bio-electrochemical active microorganisms seem to struggle with the deterioration of their own environment due to slow proton fluxes. Therefore, the results suggest that an appropriate pH adjustment strategy is necessary to allow a sustained and enhanced biological activity in bio-electrochemical systems.

Keywords Bio-catalyzed cathode
Energy recovery
Microbial electrolysis cell
Microbial fuel cell · Wastewater

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Introduction

In bio-electrochemical systems (BESs), at least one of the anodic or cathodic reactions is microbially catalyzed (Rabaey et al. 2007). If a BES is producing electrical energy, the term microbial fuel cell (MFC) is used, whereas the term microbial electrolysis cell (MEC) is used when electrical energy is applied to drive the electrochemical reactions (Rozendal et al. 2006). Recently, there has been an increased interest in replacing abiotic cathodes with biocathodes in which living microorganisms enhance the reduction catalysis (He and Angenent 2006). With regard to the multiplicity of the possible bacterial reduction reactions, several types of biocathodes with different types of oxidants have successfully been demonstrated in microbial fuel cells or microbial electrolysis cells ranging from H_2 reduction (Clauwaert et al. 2007b; Rabaey et al. 2008) and denitrification (Clauwaert et al. 2007a; Gregory et al. 2004; Virdis et al. 2008) to dechlorination and H_2 evolution (Aulenta et al. 2008; Rozendal et al. 2008a; Shea et al. 2008). Little is known about the microbiology in biocathodes and how the biocatalysts retrieve electrons from the cathode (Clauwaert et al. 2009; He and Angenent 2006).

Combining carbon and nitrogen removal in a microbial fuel cell with biological anodes and cathodes can lead to a more energy efficient wastewater treatment. Acetate oxidation in a bio-anode can be combined with nitrate and nitrite reduction in a

biocathode, yielding a current density of the order of $60 \pm 70 \text{ A m}^{-2}$ net cathodic compartment (NCC; equivalent to a denitrification rate of $0.14 \pm 0.18 \text{ kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$) and a power production up to $10 \text{ W m}^{-3} \text{ NCC}$ (Clauwaert et al. 2007a; Viridis et al. 2008). Additionally, nitrification can be accomplished in a separate chamber, transforming the ammonium to nitrite or nitrate, while denitrification can be carried out in the cathode, as previously described by Viridis et al. (2008). In this system, the effluent of the anode was introduced to a tank for nitrification and then fed to the cathode, completing a loop. With so called "loop operated" denitrifying MFCs, a current production of $133 \text{ A m}^{-2} \text{ NCC}$ (equivalent with $0.33 \text{ kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$) was achieved. The acidity produced in the anode and nitrification tank, and conveyed to the cathode via loop operation, was assumed to partially offset the alkalinity produced by denitrification (Viridis et al. 2008). Finally, denitrification rates can be further increased by lowering of the external resistance, resulting in a lowering of the cathode potential and increase in the current density (Clauwaert et al. 2007a).

In this research, we investigated the effect of maintaining a neutral pH in the cathode of a non-loop-operated denitrifying biocathode. Second, the operation of a denitrifying biocathode in a microbial electrolysis cell (MEC) was investigated as a way to increase the denitrification rate and the efficiency as a microbial electrolysis cell (MEC), a cell voltage was investigated.

Materials and methods

Microbial fuel cell construction and operation

The MFC was made of two Plexiglas frames ($10 \times 10 \times 2 \text{ cm}^3$ per frame; 0.400 L MFC of which 0.110 L NCC). The anodic and cathodic frames were filled with graphite granules (type 00514, diameter $1.5 \pm 0.5 \text{ mm}$, Le Carbone, Belgium) and were connected to the external electrical circuitry with a graphite rod current collector (5 mm diameter, Morgan, Belgium). A cation exchange membrane (CEM) (Ultrex CMI7000, Membranes International Inc.) was used between the anodic and cathodic frame. The anode and cathode were both fed autoclaved nitrogen-purged medium in continuously ($0.79 \text{ l} \cdot \text{h}^{-1}$).

A concentrated solution of sodium acetate and NaNO_3 , was continuously added ($8.5 \text{ ml} \cdot \text{h}^{-1}$) to the recirculation loop ($0.35 \text{ l} \cdot \text{h}^{-1}$) of the anode and the cathode respectively to obtain the desired volumetric COD and nitrate loading rates. Thus, the calculated nitrogen concentration in the medium entering the cathode was between 0.02 and $0.11 \text{ g}_3 \text{NO}_3 \cdot \text{l}^{-1}$, while the acetate concentration in the medium entering the anode was between 0.05 and $0.32 \text{ g COD} \cdot \text{l}^{-1}$. To minimize concentration polarization in the anode, the amount of oxidizable substrate in the anode was always twice the amount of reducible substrate in the cathode. Thus, in the case of a complete denitrification in the cathode, the coulombic efficiency in the cathode was 100% and as a consequence, the coulombic efficiency in the anode was 50% . The medium that was fed to anode and cathode contained $6 \text{ g}_2 \text{HNaPO}_4 \cdot 2\text{H}_2\text{O} \cdot \text{l}^{-1}$, $3 \text{ g KH}_2\text{PO}_4 \cdot \text{l}^{-1}$, $1 \text{ g NaHCO}_3 \cdot \text{l}^{-1}$, $0.2 \text{ g MgSO}_4 \cdot 7\text{H}_2\text{O} \cdot \text{l}^{-1}$, $0.1 \text{ g NH}_4\text{Cl} \cdot \text{l}^{-1}$, $0.0146 \text{ g CaCl}_2 \cdot \text{l}^{-1}$ and trace elements as previously described (Clauwaert et al. 2007b). All tests were performed at room temperature ($22 \pm 2^\circ \text{C}$).

Measurements

The cell voltage over a $1 \cdot \Omega$ resistor and the cathode potential were recorded every minute with a data acquisition unit (HP 34970A, Agilent) for the operation as a microbial fuel cell. In the case of operation as a microbial electrolysis cell (MEC), a cell voltage was applied with a potentiostat (PAR Bi-Stat Potentiostat, Princeton Applied Research, France; three electrode set-up). The current and cathode potential were measured every 30 s .

Polarization curves were obtained with a potentiostat at a scan rate of $0.2 \text{ mV} \cdot \text{s}^{-1}$ after an open circuit stabilization of 15 min . The ohmic cell resistance was determined with the current interrupt method (Clauwaert et al. 2007a). The potential of the cathodic electrode was monitored with a Ag/AgCl reference electrode (assumed to be 0.197 V vs. standard hydrogen electrode, SHE) (model RE-5B, BASi) and the anode potential was calculated from the cathode potential, the cell voltage applied and the ohmic cell resistance (Clauwaert et al. 2008). Overpotentials are defined as the difference between the electrode potential and the equilibrium potential of the electrode after open circuit stabilization (Clauwaert et al. 2008).

Nitrate, nitrite, and sulfate were measured as previously described (Clauwaert et al. 2007a). The presence of nitrate and nitrite in the cathode effluent indicated that not all equivalents were reduced: 5 and 3 mol electrons equivalents per mol nitrate and nitrite, respectively. Current (I , $A\ m^{-3}\ NCC$) can be expressed as an equivalent denitrification rate (D , $kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$) according to the equation: $D = 2.507 \times 10^{-3} I$ (Clauwaert et al. 2007a). A gas trap was used to quantify the gas production ($24.23\ l\ mol^{-1}$). The qualitative presence of H_2 was determined using a H_2 sensor (OPUS, Zellweger Analyt-ics, UK). pH and conductivity were measured with a Consort multimeter.

Results

A microbial fuel cell with an acetate-oxidizing bio-anode and nitrate-reducing bio-cathode was reactivated after a hibernation period of 3 months during which no substrate was added to the electrode compartments. 5 days after reactivation with an external resistor of $1\ \Omega$ and a nitrate loading rate of $0.137\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$, the current density increased up to $55\ A\ m^{-3}\ NCC$. The nitrate loading rate was increased to $0.228\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$, while a complete nitrate and nitrite removal was established, as also confirmed by the current density of $89 \pm 2\ A\ m^{-3}\ NCC$. This corresponds with a calculated denitrification rate of $0.223\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$ (5 mol electrons per mol N for a complete denitrification). When the nitrate loading rate was further increased to $0.342\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$, only $80 \pm 3\%$ of the nitrate/nitrite reduction equivalents were removed. The current density was $108 \pm 4\ A\ m^{-3}\ NCC$, corresponding to $79 \pm 3\%$ of the current density that would have occurred in the case of a complete denitrification. At this loading, the pH in the cathode effluent was 8.3 while the pH of the influent medium was 7.2. For subsequent nitrate loading conditions, ranging from 0.228 to $0.684\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$, the anode and cathode media were amended with NaOH and HCl, respectively, to maintain the influent pH (7.2 ± 0.1) in the anode (7.2 ± 0.1) and cathode effluent (7.1 ± 0.1). While the nitrate loading rate was increased from 0.228 to $0.456\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$, the current density, the denitrification rate (Fig. 1) and the gas production

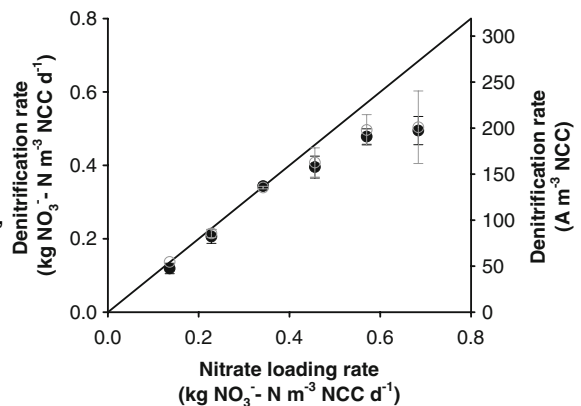


Fig. 1 The denitrification rate as a function of the nitrate loading rate when the cell was operated as a MFC ($R_{ext} = 1.1\ \Omega$). The closed circles represent the current density expressed as a denitrification rate ($0.2\ mol\ nitrogen\ per\ mol\ electrons$ for a complete denitrification) and the open circles represent the denitrification rate based on the concentration of nitrate and nitrite in the cathode effluent. The weight line indicates a 100% coulombic efficiency in the cathode and a complete denitrification to N_2

rate (Table 1) were further increased. From a nitrate loading rate of $0.456\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$ and higher, denitrification was incomplete as nitrate and nitrite were detected in the cathode effluent (Fig. 1).

The increasing cathode potentials correlated with increasing current densities when operated with an external resistor of $1\ \Omega$ (Fig. 2). Polarization curves performed at different nitrate loading rates confirmed the improved MFC performance and showed that the cathode overpotentials were lowered with increasing current densities (Fig. 3). From these polarization curves, the external resistance for maximal power production can be determined (Aelterman et al. 2006). This optimal resistance decreased from $48\ \Omega$ at a loading rate of $0.137\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$ to $21\ \Omega$ at a loading rate of $0.228\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$ and further decreased to $11\ \Omega$ at a loading rate of 0.456 and $0.684\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$. The ohmic cell resistance, as determined using the current interrupt method, was $1.8 \pm 0.5\ \Omega$.

In a next phase, the cell was operated as a MEC to enhance the denitrification rate at a nitrate loading rate of $0.684\ kg\ NO_3^- - N\ m^{-3}\ NCC\ d^{-1}$, which was incomplete when operated as a MFC. At an applied voltage of $-0.2\ V$, the cathode potential was $-0.469 \pm 0.016\ V$ versus SHE and the current

Table 1 Overview of the specific gas production rate and the cell (MFC) with an external resistance of $1\ \Omega$ or as a equivalent rate of nitrogen removed to the gas phase based on microbial electrolysis cell (MEC) operated with an applied cell the gas production rate for the cell operated as a microbial fuel voltage of -0.2 or -0.4 V

Nitrate loading rate ($\text{kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$)	Test duration (h)	Gas production rate ($\text{L gas l}^{-1} \text{ NCC d}^{-1}$)	Nitrogen removal rate based on the gas production rate ($\text{kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$)
0.137 (MFC $1.1\ \Omega$)	714	0.107 ± 0.027	0.124 ± 0.031
0.228* (MFC $1.1\ \Omega$)	284	0.174 ± 0.032	0.201 ± 0.037
0.342* (MFC $1.1\ \Omega$)	186	0.212 ± 0.043	0.246 ± 0.050
0.456* (MFC $1.1\ \Omega$)	443	0.324 ± 0.068	0.375 ± 0.079
0.570* (MFC $1.1\ \Omega$)	253	0.393 ± 0.012	0.455 ± 0.014
0.684* (MFC $1.1\ \Omega$)	299	0.431 ± 0.037	0.498 ± 0.043
0.684* (MEC -0.2 V)	141	0.415 ± 0.037	0.48 ± 0.043
0.684* (MEC -0.4 V)	85	0.342 ± 0.091	0.396 ± 0.105

* Continuous pH adjustment applied in anode and cathode

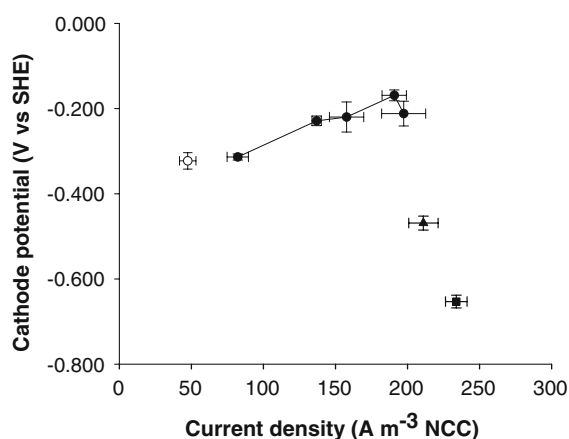


Fig. 2 The cathode potential in function of the current density. The empty circle represents the cathode potential in the case of $0.137\ \text{kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$ (pH not adjusted, MFC $1.1\ \Omega$), the closed circles represent the cathode potentials in the case of 0.228 to $0.684\ \text{kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$ (pH adjusted, MFC $1.1\ \Omega$), the closed triangle (pH adjusted, $0.684\ \text{kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$) and the closed square (pH adjusted, $0.684\ \text{kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$) represent the case where the cell was operated as a microbial electrolysis cell with an applied cell voltage of -0.2 and -0.4 V, respectively

density was $21 \pm 10\ \text{A m}^{-3} \text{ NCC}$ (Fig. 2). This MEC current density was equivalent with a calculated denitrification rate of $0.52 \pm 0.026\ \text{kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$. Based on the presence of nitrate and nitrite in the cathode effluent, the calculated denitrification rate was $0.49 \pm 0.043\ \text{kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$ ($72 \pm 6\%$ reduction equivalents removed), while it was $0.480 \pm 0.043\ \text{kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$ based on the gas production rate (Table 1). The

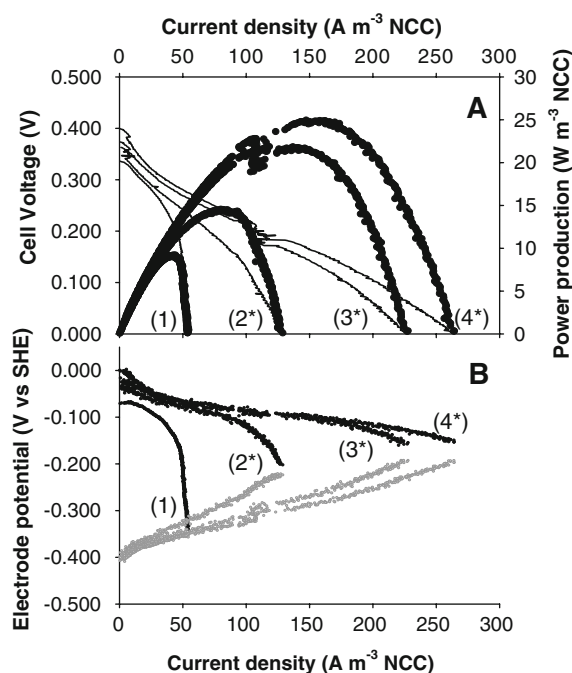


Fig. 3 The polarization curves ($0.2\ \text{mV s}^{-1}$) of the MFC operated with a $1.1\ \Omega$ external resistor with a nitrate loading rate of (1) 0.137 , (2) 0.228 , (3) 0.456 and (4) $0.684\ \text{kg NO}_3^- \cdot \text{Nm}^{-3} \text{ NCC d}^{-1}$. a Cell voltage (line) and power production (closed circles) in function of the current density b The cathode potential (black dots) and the anode potential (grey dots) in function of the current density. The asterisk indicates a continuous pH adjustment applied in anode and cathode

applied cell voltage on the MEC was changed to -0.4 V, yielding a cathode potential of -0.653 ± 0.015 V versus SHE, and a current density of

$234 \pm 7 \text{ A m}^{-3} \text{ NCC}$ (Fig. 2) (equivalent with denitrification rate of $0.586 \pm 0.019 \text{ kg NQ}^{-}\text{-Nm}^{-3} \text{ NCC d}^{-1}$). Based on the nitrate and nitrite removal in the cathode effluent, the calculated denitrification rate was $0.503 \pm 0.049 \text{ kg NQ}^{-}\text{-Nm}^{-3} \text{ NCC d}^{-1}$ ($74 \pm 7\%$ reduction equivalents removed), while it was $0.396 \pm 0.105 \text{ kg NQ}^{-}\text{-Nm}^{-3} \text{ NCC d}^{-1}$ based on the gas production rate (Table 1). No H_2 was detected in the gas produced when the cell was operated as a microbial electrolysis cell. When the cell was operated with an applied voltage of -0.4 V and without nitrate in the cathode (pH 7.2), the current density was $3 \text{ A m}^{-3} \text{ NCC}$ at a cathode potential of -0.765 V versus SHE. The chemical hydrogen evolution rate at this potential was thus in this case maximum $2.7 \text{ g H}_2\text{-Nm}^{-3} \text{ NCC d}^{-1}$, which equals a stoichiometrical denitrification capacity of $0.008 \text{ kg NQ}^{-}\text{-Nm}^{-3} \text{ NCC d}^{-1}$.

When the nitrate loading was further increased to $0.798 \text{ kg NQ}^{-}\text{-Nm}^{-3} \text{ NCC d}^{-1}$ with an applied cell voltage on the MEC of -0.4 V , no further increase in current density or denitrification rate could be established. The cathode potential was $-0.596 \pm 0.041 \text{ V}$ versus SHE. The pH of the cathode effluent was 6.3 ± 0.1 and the pH in the anode effluent was 7.6 ± 0.1 .

Discussion

In this research, we have successfully demonstrated that the biocathodic denitrification rate can be significantly increased by continuous neutralization of the bulk pH in the cathode, assuming that the proton diffusion and migration from anode to cathode through the cation exchange membrane is negligible. In this way, we could establish a denitrification rate up to $0.50 \text{ kg NQ}^{-}\text{-Nm}^{-3} \text{ NCC d}^{-1}$ whereas in previous studies, where no continuous pH adjustment was performed (NL mode), the denitrification rate was limited to approximately $0.18 \text{ kg NQ}^{-}\text{-Nm}^{-3} \text{ NCC d}^{-1}$ (Clauwaert et al. 2007a; Viridis et al. 2008). These data seem to confirm the hypothesis that an acidified cathode influent allows an enhanced denitrification rate in the case of a loop operation (Viridis et al. 2008). However, in the study of Viridis and co-workers, pH data were not reported. A pH between 7 and 8 has been identified as an optimal pH for denitrification systems (Knowles 1982; Kurt et al.

1987). In our system, with a nitrate loading rate of $0.228 \text{ kg NQ}^{-}\text{-Nm}^{-3} \text{ NCC d}^{-1}$, incomplete denitrification and an effluent pH of 8.3 was observed without adjustment for alkalinity produced by denitrification. When the effluent pH was adjusted to near 7, complete denitrification was achieved. However, alkalinity produced in denitrification could benefit the biological reduction of oxidized contaminants that require higher pH. A high cathode pH was shown to enhance perchlorate reduction in a biocathode acclimated to biological nitrate reduction (C. Shea, W. Verstraete et al. submitted).

The dosing of acid and base to the cathode and anode electrolyte, respectively, which results in a higher electrolyte conductivity (from 9.4 to 13.1 mS cm^{-1} in the cathode), did not result in a lower measured ohmic cell resistance. Also a higher conductivity has, to the authors knowledge, not been associated with a higher denitrification activity (Visvanathan et al. 2008; Yang et al. 1995). Therefore, an enhanced denitrification activity was unlikely to be caused by the increase in conductivity, but rather by the pH control effect.

In this study, the denitrification rate was assessed in three different ways. First, the current production gives an accurate measurement of the amount of electrons that are consumed in the denitrification process. Secondly, from the removal of nitrate and nitrite in the effluent, the nitrogen removal rate can be calculated. If the denitrification rate calculated from the current production (assuming 5 mol electrons per mol nitrogen) is lower than the denitrification rate calculated from the removal of nitrate and nitrite, it may have been production. In this research, $\text{NO}_2\text{-N}$ measurements were conducted, so $\text{NO}_2\text{-N}$ production cannot be conclusively excluded. However, there was no such discrepancy observed in this research (Fig. 2). Thirdly, the denitrification rate could be estimated by measuring the gas production. This measurement does not allow a distinction between N_2 and N_2O formation, but it has an indicative value (Table 1).

When the MFC was operated with a fixed resistor of 1.1Ω , it was found that the cathode potential increased with increasing current densities (Fig. 2), as previously observed (Clauwaert et al. 2007a). It was most likely that the decreasing concentration overpotentials, due to the higher nitrate fluxes to the cathode, accounted for this increase in the cathode potential. From the polarization data (Fig. 3B), it can be seen that at a

nitrate loading rate of $0.137 \text{ kg NO-Nm}^{-3} \text{ NCC d}^{-1}$, the cathode overpotentials sharply increased in the proximity of the short circuit current. These cathode overpotentials tended to decrease at higher nitrate loading rates in the cases where the pH was controlled, and no sharp increase in cathode overpotentials was observed in the case of $0.684 \text{ kg NO-Nm}^{-3} \text{ NCC d}^{-1}$ near the short circuit point. Since in this case the denitriPcation was incomplete, the presence of microbial electrolysis cell conditions can only be nitrate and nitrite in the bulk solution most likely explains the absence of large concentration overpotentials. Since the pH was not adjusted in the case of a nitrate loading rate of $0.137 \text{ kg NO-Nm}^{-3} \text{ NCC d}^{-1}$, it can also not be excluded that there was an additional effect of a higher pH at the cathode to the sharp increase of the cathode overpotentials near the short circuit current point.

The cell was operated as a microbial electrolysis cell to verify if the cathodic denitriPcation could be further enhanced. The decrease in cathode potential by applying an additional voltage with the potentiostat means that this additional voltage was mainly resulting in higher cathode overpotentials (Fig. 2). It seems that the microorganisms in the biocathode could not exert the higher driving force of a low cathode potential to enhance the denitriPcation rate. At this point, it remains unclear what the limiting factors are for a biocathodic denitriPcation higher than $0.5 \text{ kg NO-Nm}^{-3} \text{ NCC d}^{-1}$. There must be either intrinsic or environmental factors limiting a further bio-catalytic conversion. Indeed, a pH neutral operation is still no guarantee that there exist no pH gradients between the bulk cathode electrolyte and the cathode interface (Clauwaert et al. 2008). However, at a nitrate loading rate of $0.798 \text{ kg NO-Nm}^{-3} \text{ NCC d}^{-1}$, the pH in the cathode was 6.3 ± 0.1 and this higher proton concentration in the bulk solution most likely lowered the pH near the cathode electrode without enhancing the denitriPcation rate. The pH could not be determined in the proximity of the cathode electrode to verify this and if these pH gradients between the electrode and the bulk would not occur, the cathode was operated outside the optimal pH range for denitriPcation (pH 7.0–8.0).

No H_2 was detected and the theoretical denitriPcation rate based on chemically produced hydrogen was calculated to be $0.008 \text{ kg NO-Nm}^{-3} \text{ NCC d}^{-1}$ with a cathode potential of -0.765 V versus SHE. Moreover, when nitrate was dosed, the cathode

potential was 0.1 V higher, so the denitriPcation based on H_2 oxidation was negligible.

Operating a MFC with a denitrifying biocathode for an optimal volumetric power density will often result in an incomplete denitriPcation. If a complete denitriPcation is preferred over optimizing the volumetric power density, operation near short circuit current conditions will be desirable. Operation under these conditions can only be justified if it results in an enhanced denitriPcation activity since -0.4 V applied with a power supply implies and electrical energy input of 3.8 kWh kg N completely denitriPced.

The results obtained in this research indicate that an enhanced denitrifying bio-catalytic activity requires appropriate pH neutralizing actions since the bio-electrochemical active microorganisms tend to deteriorate their own environment. Continuous pH adjustment in anodes and cathodes of BES is most likely economically unfavorable. Unless an acid waste stream containing nitrate is present, the bio-electrochemical system needs to be operated with a membrane that allows a significant higher proton flux than achievable at present (if possible) (Rozendal et al. 2008b), a loop operation mode (Virdis et al. 2009) or a membrane-less operation, if the latter does not inhibit the current production due to short cut conversion.

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