Bioelectrochemical nitrogen removal as a polishing mechanism for domestic wastewater treated effluents

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ABSTRACT

Addition of an external carbon source is usually necessary to guarantee a sufficiently high C/N ratio and enable denitrification in wastewater treatment plants (WWTPs). Alternatively, denitrification processes using autotrophic microorganisms have been proposed i.e., with the use of H₂ as electron donor or with the use of cathodic denitrification in bioelectrochemical systems (BES), in which electrons are transferred directly to a denitrifying biofilm. The aim of this work was to investigate and demonstrate the feasibility of applying an easy-to-operate BES as a polishing mechanism for treated secondary clarified effluent from a municipal WWTP, containing low levels of organic matter, buffer capacity and low concentrations of remaining nitrate. In the proposed system, nitrogen removal rates (0.018–0.121 Kg N m⁻³ d⁻¹) increased with the nitrogen loading rates, suggesting that biofilm kinetics were not rate limiting. The lowest energy consumption for denitrification was 12.7 kWh Kg N⁻¹, equivalent to 0.021 kWh m⁻³ and could be further reduced by 14% by adding recirculation circuits within both the anode and cathode.

Key words | autotrophic denitrification, bioelectrochemical systems, cathodic denitrification, nitrogen removal

INTRODUCTION

Increasing worldwide population and related industrial/human activities have long been known to cause serious environmental damage due to the unsustainable use of natural resources. Amongst the environmental problems related to worldwide overpopulation, the discharge of domestic and industrial wastewater containing nitrogen compounds straight into rivers and seas are of special concern. Nitrogen is considered a limiting nutrient, due to the low concentrations usually found in natural environments, which generally determines the growth and spatial distribution of organisms in waterways (Howarth & Marino 2006). Although natural systems are able to auto-depurate part of these nutrient discharges without significantly impacting the equilibrium of the waterway, a highly increased nitrogen discharge is likely to cause eutrophication especially in coastal areas, leading to excessive growth of planktonic algae (algal blooms) and consequently to ecosystem destruction mainly due to the depletion of dissolved oxygen (DO) (Howarth et al. 2011; Braig et al. 2013).

Nitrogen in domestic wastewaters is encountered mainly in the form of ammonium (Gray 2004). Thus, technologies for its removal commonly comprise an aerobic activated sludge treatment for organic matter removal and ammonium oxidation to nitrate, coupled to an anoxic denitrification step able to reduce nitrate to nitrogen gas while also removing organic matter (Smith & Solley 2002). Although urban wastewater treatment is a common practice, the discharge of nitrogen still occurs to a certain extent, since the process is not able to achieve 100% removal. The inability of some wastewater treatment plants (WWTPs) to completely remove N can be attributed to imbalances in the C/N ratio during the denitrification step (Smith & Solley 2002), when the proportion of organic matter (electron/carbon donor) is not sufficient to sustain cell requirements for biological heterotrophic denitrification. Because of that, methanol or other carbon sources can be added to guarantee an ideal C/N ratio and enable the heterotrophic denitrification step (Spellman 2008).

Thus, although some WWTPs are able to considerably reduce the concentration of N in effluents, a discharge of diluted streams (clarified effluent from a municipal WWTP) still containing low concentrations of nitrogen...
(≈5 mg N L⁻¹) can still be expected. Considering that big WWTPs may have the capacity to treat approximately 200 ML d⁻¹ (200 x 10⁶ L day⁻¹), the referred N concentration represents a total discharge of 1,000 Kg N/day, still potentially causing significant disturbance to receiving waterways. In fact, the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (2000) point out that levels of NO₃⁻ (NO₃⁻·N + NO₂⁻·N) ranging from 1 to 30 µg L⁻¹ are already considered trigger values to slightly–moderately disturbed aquatic systems in some tropical areas of Australia. Those values are highly dependent on the level of environmental protection of the aquatic ecosystem and average rainfall, which may considerably influence the licensed discharge limits.

Overcoming the need for carbon-source addition to achieve full denitrification, some processes using autotrophic microorganisms have been proposed (Watanabe et al. 2001). Overall, autotrophic denitrification can be advantageous due to lower biomass formation relative to heterotrophic systems (an important factor in some systems, since it reduces clogging the reactors), and due to the fact that no organic matter needs to be added, which avoids further carbon contamination into watercourses (van Rijn et al. 2006). These processes have long been reported to occur in the presence of hydrogen gas as electron donor and CO₂ as a carbon source (Kurt et al. 1987; Szekeres et al. 2001). However, the fact that H₂ has low solubility in water still poses a drawback for its applicability, as it does not dissolve in sufficient quantities to be used by the microbial community (Ergas & Reuss 2001).

As an alternative to sparging H₂ in the water column, H₂ can be generated electrochemically by applying a sufficiently low cathodic potential in an electrochemical system. Since hydrogen is generated in situ, its delivery to a denitrifying community is enhanced, thus increasing the removal efficiency of nitrogen compounds (Huang et al. 2013). Similarly, denitrification can also be achieved in a bioelectrochemical system (BES) where electrons are fed directly to the denitrifying organisms, without the need for intermediate H₂ generation (Gregory et al. 2004). Therefore, BES has been demonstrated as a promising technique for nitrogen removal from waters lacking sufficient carbon source, such as groundwater and drinking water (Pous et al. 2013a).

Although BES denitrification has been well demonstrated with synthetic media, real wastewaters have often much lower conductivity (Puig et al. 2012) and lower buffer capacity than that added to the synthetic media, which could lead to a sharp rise in pH at the cathodic biofilm because of the insufficient diffusion of protons – and consequently limit the denitrification process. Therefore, the aim of this work is to investigate and demonstrate the feasibility of applying an easy-to-operate BES to polish a diluted stream containing low levels of organic matter, low buffer capacity and low concentrations of remaining nitrate from secondary treatment in an activated sludge process. More specifically, this work focuses on: (1) identifying the maximum achievable denitrification rates in an upflow membraneless system when operated with this type of diluted stream; (2) identifying the energy consumption of the proposed system; and (3) identifying the ion transfer mechanisms between the cathode and the anode, and whether the use of different mixing conditions could improve the performance of the system (i.e. current generation and energy consumption).

MATERIAL AND METHODS

Reactor setup and operation

The reactor used for the experiments comprised an upflow membraneless cylinder reactor approximately 31 cm high, with a 64 cm² cross-section area (2 L total volume capacity) in which graphite granules (EC-100, Minus 3/8 inch by 10 U.S. Standard Mesh–10 mm by 2 mm-Graphite Sales, Inc., USA) were used as both anode and cathode materials. The cathodic granules were placed across the entire cross-section of the cathodic region (5 cm depth and 64 cm² cross-section area, totalizing approximately 320 mL), at a distance of 3 cm from the bottom of the reactor, allowing uniform inflow water distribution (Figure 1). The cathodic granules occupied approximately 50% of the volume within the cathodic region, leaving a liquid volume of approximately 160 mL (net cathodic volume – NCV). Anodic granules – abiotically operated by splitting water and generating oxygen – were contained in a plastic mesh basket with internal volume of approximately 200 mL (7 cm long and 28 cm² anodic cross-section) and placed at the upper portion of the reactor, with an anode–cathode gap of 7.5 cm. Similarly to the cathode, the anodic granules occupied approximately 50% of the volume within the anodic basket, leaving a liquid volume of approximately 100 mL (net anodic volume – NAV). A graphite rod (National CMG rods 74-5671-00 3/16x 12) was inserted within the granules as a current collector in each electrode. In this proposed setup, the projected surface area of the electrodes thus corresponds to the cathodic and anodic...
cross-section areas (64 and 28 cm² respectively). Media recirculation was initially applied within the cathode electrode only (at 9 L day⁻¹/C₀), to avoid mass transfer limitations, except when running specific recirculation tests. A Ag/AgCl (saturated KCl) reference electrode (RE 0) was inserted on the lower portion of the anode–cathode gap, controlling the cathodic potential at −0.9 V vs Ag/AgCl (corresponding to approximately −0.703 V vs standard hydrogen electrode – SHE), with a VMP3 potentiostat (Bio-Logic, France).

The cathode electrode was inoculated with biomass obtained from a previously operating denitrifying BES. The reactor was initially fed for 30 days (growth phase) at a flow rate of approximately 1 L d⁻¹/C₀ with synthetic media containing 0.1 g L⁻¹ MgSO₄·7H₂O, 15 mg L⁻¹ CaCl₂·2H₂O, 1 g L⁻¹ NaHCO₃, 20 mg L⁻¹ NO₃⁻N, 19.4 mg L⁻¹ NaH₂PO₄, 5.84 mg L⁻¹ NH₄Cl, 19 mg L⁻¹ KCl and trace elements solution as previously described (Lu et al. 2006). The medium was previously sparged with N₂ for 30 minutes to remove oxygen. After this period, the clarified secondary effluent from a WWTP was used as feed.

The raw wastewater was tested (n = 4) for the presence of NO₃⁻N, NO₂⁻N and NH₄⁺-N total organic carbon (TOC), total inorganic carbon (TIC), volatile fatty acids (VFA) and alcohols. Previously to analysis, all samples were filtered through a 0.22 μm membrane filter. Dissolved nitrogen species were analysed via flow injection analyser (FIA). TOC and TIC were analyzed using a high-temperature combustion (680 °C/720 °C) method with platinum beads.

Figure 1 | BES reactor scheme. RE 0: reference electrode connected to the potentiostat, controlling the system via a three electrode setup; RE 1–4: additional reference electrodes 1–4 used for potential losses measurements with the use of a multimeter; AN: graphite granules anode electrode; CAT: graphite granules cathode electrode; C1 and C2: recirculation circuits 1 and 2 respectively; SP: liquid sampling port.
and Pt mesh as catalyst and near infrared detector (NIRD) for carbon dioxide (Shimadzu TOC-L CSH Total Organic Carbon Analyser with TNM-L TN unit). VFA and alcohols were determined by gas chromatography using a flame ionization detector (FID, Agilent Technologies 7890A GC System), a polar capillary column (DB-FFAP with 30 m × 0.53 mm × 1.0 μm) and a deactivated Uniliner (Restek Drilled Uniliner, hole on top, PN 21055) with phosphoric acid treated glass wool for on-column injections.

The characterization indicated the presence of 2.5 ± 0.5 mg L⁻¹ NO₃⁻N, 0.2 ± 0.1 mg L⁻¹ NH₄⁺-N, 36 ± 5 mg L⁻¹ TIC and 9.0 ± 0.7 mg L⁻¹ TOC. Concentrations of VFA and alcohols (ethanol, propanol, butanol, acetic acid, propionic acid, iso-butyric acid, butyric acid, valeric acid, iso-valeric acid and hexanoic acid) were below the detection limit of the method at all times, confirming that only non-readily available organic matter was present in the wastewater. DO was also monitored in the inlet of the BES reactor using a DO probe (SevenGo pro™), whereas N₂ was assumed to be a result of nitric oxide (NO) generation as assumed previously demonstrated (Richardson et al. 2009; Virdis et al. 2009; Puig et al. 2012), whereas N₂ was assumed to be a result of complete denitrification happening in the system and calculated as all unaccounted nitrogen in liquid samples.

Four reference electrodes (additionally inserted into the water column of the reactor and which were independent of the potentiostatic controlled potentials) enabled measurement of losses of potential in different regions of the reactor with the use of a multimeter (Fluke 179 True RMS). Reference electrodes were placed (1) on top of the anode, (2) below the anode, (3) just above the cathode and (4) below the cathode, as indicated in Figure 1. The losses of potential were measured at different mixing conditions, each of which was set as a different combination of presence/absence of feed and recirculation circuits 1 (within the anodic and gap areas) and 2 (within the cathodic area), as indicated in Figure 1. The recirculated circuits were set at approximately 9 L h⁻¹ and the tested mixing conditions were as follows: (1) full mixing: feed + circuit 1 + circuit 2; (2) feed + recirculation circuit 2; (3) feed only, no recirculation circuits operating; (4) no feed, no recirculation circuits operating. Each mixing condition was kept for 1 hour at a flow rate of 14.4 L d⁻¹ before each measurement of losses.

### Calculations

Energy consumption was calculated as described elsewhere (Saleem et al. 2011). Cell voltage (ΔE_{cell}) was measured by the potentiostat. Losses of potential across the liquid phase in the anodic, gap and cathodic regions were measured as ΔE_{an} = RE1–RE2, ΔE_{gap} = RE2–RE3, ΔE_{cat} = RE3–RE4, where ΔE is the electrolyte’s voltage measured between

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**Table 1 | Days of operation and applied flow rate before each sampling time**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Flow rate (L d⁻¹)</th>
<th>Time range (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5</td>
<td>0–4</td>
</tr>
<tr>
<td>2</td>
<td>2.16</td>
<td>4–12</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>12–18</td>
</tr>
<tr>
<td>4</td>
<td>3.85</td>
<td>18–19</td>
</tr>
<tr>
<td>5*</td>
<td>7.2</td>
<td>37.0–37.16</td>
</tr>
<tr>
<td>6*</td>
<td>7.2</td>
<td>37.16–37.2</td>
</tr>
<tr>
<td>7*</td>
<td>14.2</td>
<td>37.2–37.26</td>
</tr>
<tr>
<td>8*</td>
<td>14.2</td>
<td>37.26–37.32</td>
</tr>
</tbody>
</table>

Asterisks indicate samples were taken at the same day, after running the reactor at the selected flow rate for at least three times the HRT.
two of the additional reference electrodes (RE). Pseudo-
ohmic resistances (PR) were then calculated according to
Ohms’ law (PR = ΔE/I), where PR (Ω) is the electro-
lyte’s resistance of each region and I is the current (A)
flowing through the device. The use of the term pseudo-
ohmic resistance herein acknowledges that the overall resis-
tance across the electrolyte might be lower than ohmic
resistances itself, thus this term represents the total elec-
trolyte resistance.

RESULTS AND DISCUSSION

Denitrification from clarified secondary effluent in the
BES reactor

Figure 2 highlights the results of experimental phases 1 to 8.
The nitrogen loading rate increased with increasing flow
rates, from 0.027 Kg N m⁻³ d⁻¹ in the beginning of the
experiments to 0.221 Kg N m⁻³ d⁻¹ at phase 8. Nitrogen
removal rates (0.018–0.121 Kg N m⁻³ d⁻¹) increased con-
sistently to the increasing nitrogen loading rates, indicating
the biofilm was able to respond well to the increasing
loads (Figure 2(a)). Nitrogen removal rates presented
herein are within the ranges reported in previous BES
studies (Clauwaert et al. 2007; Puig et al. 2011; Gregoire
et al. 2014), despite the lack of membrane (commonly used
in BES to separate cathodic and anodic reactions), the
absence of organic matter as electron donor and the low
conductivity (<2 mS cm⁻¹) of the wastewater feeding the
system proposed herein. In fact, it has previously been
demonstrated that cathodic denitrification is possible in
low ionic strength streams, although removal rates can be
affected by low conductivity and low buffer capacity (Puig
et al. 2012).

Similarly to the increasing N removal rates with increas-
ing flow rates, current generation tended to increase from
phase 1 until phase 5 (4.0 to 5.7 mA), but decreased slight-
ly from phase 6, possibly indicating that the biofilm was start-
ing to be washed out at the fast flow rates. As indicated in
Table 2, the nitrate concentration of the wastewater entering
the reactor was very low, as was expected as a (treated)
secondary clarified effluent. However, concentration of
nitrogen compounds in the effluent leaving the BES
decreased even more, to values lower than 1 mg L⁻¹ at all
times. In the 19th day of operation in phase 4, the rather
smaller concentration of nitrogen species entering the reac-
tor–which is also reflected in the slightly decreased
nitrogen loading rate compared to the previous phase,
despite the faster flow rate, (Figure 2(a)) – explains the
very low (or nearly zero) concentration of dissolved nitrogen
species that were detected in the effluent in phase 4. From
phase 5 onwards, the percentage of nitrate found in the efflu-
ent – compared to that of the total influent nitrogen – started
to increase again, which corroborates with the hypothesis
that the denitrifying biofilm was reaching its maximum
capacity at the tested conditions (7.2 L d⁻¹ flow rate).
Accordingly, a further increase in flow rate to 14 L d⁻¹ led
to a rather higher percentage of nitrate which was not
removed.

Cathodic denitrification have already been previously
demonstrated in BES, whereby electrons could be promptly
delivered from an inert surface (poised at certain redox
potentials) to microorganisms performing nitrate, nitrite
and/or nitrous oxide reduction (Clauwaert et al. 2007;
Virdis et al. 2009; Desloover et al. 2011). However, the
applied cathodic potential in the present study is more
negative than that from the cited published studies (and
more negative than the theoretical potential for hydrogen
evolution, −0.41 V vs SHE) and it is possible that bio-
catalized hydrogen formation may be occurring at a certain
extent, as previously demonstrated for carbon-based cathode
materials (Rozendal et al. 2007; Jourdin et al. 2015).
Nitrite was present at very low concentrations in the inflow and not detected in the outflow of the reactor. Furthermore, analysis of the greenhouse gases N₂O and CH₄ also indicated negligible amounts of both compounds (<0.5% N₂O-N generated from influent nitrogen and <0.01 μM CH₄ being generated at all times) (data not shown). Ammonium concentration tended to increase slightly in the beginning of the experiments (phase 1), indicating that dissimilatory nitrate reduction to ammonium (DNRA) was happening to a certain extent. The occurrence of DNRA was previously shown to be related to the ratio of available electron donors (i.e. H₂) to that of nitrate, as well as to electron uptake capacity by the biofilm (Sander et al. 2015). Thus, since the hydrogen produced was likely not fully consumed at the beginning of the experiment (which is supported by the low coulombic efficiency – data not shown), it could have stimulated DNRA to occur, indicating that the biofilm in the present study had not yet reached a steady-state condition during the first experimental phase. Interestingly, the ammonium entering the reactor started being consumed from phase 2. It is possible that small concentrations of oxygen in the influent may be enabling sequential nitrification and denitrification (Goel & Flora 2005; Liang et al. 2013) to occur in different portions of the cathode bed. However, it is worth noting that the ammonium concentration in the influent of phase 4 was already very small, thus justifying the nearly zero concentration detected in the effluent (Table 2).

The pH of the clarified wastewater entering the reactor (6.9 ± 0.1) tended to increase slightly after the cathodic zone due to denitrification/reduction reactions (Figure 3(a)). An important aspect to consider is the fact that the BES in the present study was operating with no additional buffer capacity to the amount already contained in the treated wastewater. However, the system was proven to work as a N-polishing mechanism, indicating that the buffer naturally contained in treated effluent was sufficient to neutralize the alkalinity generated by cathodic denitrification.

As indicated in Figure 3(b), the cell voltage increased over time (with increasing flow rates), which is in accordance with the current profile as indicated in Figure 2(a). It is understandable that the voltage of the system generally achieved values higher than 2.2 V, due to the low applied cathodic potential. The application of a fixed cell voltage
(0.9 V) in a previous study has been shown to lead the cathodic potential to reach only −0.1 V vs SHE during nitrate reduction (Lee et al. 2013), which is much higher than that applied herein (−0.7 V vs SHE). Interestingly, however, the energy consumption per kilogram of N removed was in fact decreasing over time (from 72.5 to 12.7 kWh Kg N⁻¹), due to the increasing nitrogen removal rates achieved when using the same system, which was reflected as higher coulombic efficiency at latter phases or, in other words, smaller loss of electrons at faster feed rates. Although energy consumption is still higher than that required for operation of other nitrate-removing technologies such as activated sludge itself (approximately 2.3 kWh Kg N⁻¹)(Mulder 2003), a low inflow nitrogen concentration is intrinsically difficult to remove partly due to bacterial kinetics (Vasiliadou et al. 2006). In addition, although some WWTPs are able to considerably reduce the concentration of N in effluents, a discharge of diluted streams still containing low concentrations of nitrogen (2–10 mg L⁻¹ N) can still be expected, due to imbalances in the C/N ratio during the denitrification step (Smith & Solley 2002), when the proportion of organic matter (electron/carbon donor) is not sufficient to sustain stoichiometric requirements for biological denitrification. Even if the influent C/N ratios could theoretically be enough to sustain full denitrification in a WWTP, very high aerobic-to-anoxic recirculation ratios are required to achieve high nitrogen removal efficiencies. The nitrate concentration in the effluent decreases with increasing recirculation ratios. However, this comes at the price of increased energy consumption for pumping and increased return of DO to the anoxic tank. In addition, at high recirculation ratios, a sizeable portion of the influent organic matter is oxidised in the anoxic tank by DO coming back with the aerobic-to-anoxic recycle stream, before it can be used as an electron donor for denitrification. This may lead to carbon insufficiency and the need to dose chemical oxygen demand (COD) in the form of methanol, ethanol or acetate to guarantee full denitrification.

**Losses of potential and pseudo-ohmic resistances**

As demonstrated, although the removal of nitrate from artificial wastewaters can be achieved with the use of BES, the application of this technology to the treatment of waters containing low conductivities faces another challenge (Pous et al. 2013b). As previously reviewed by Oliot et al., the flux of ions between the electrodes in electrochemical systems is driven mainly by migration (electrostatic potential gradient dependent on electrolyte resistivity) and convection (fluid agitation/flow) (Oliot et al. 2016). Therefore, in the absence of agitation, the electrolyte conductivity/resistivity will play a key role in the transfer of ions (migration) between cathode and anode. Because of that, many studies have been done in order to understand and minimise those losses (Zhao et al. 2006; Rozendal et al. 2008; Borole et al. 2010). When the water undergoing treatment has a low conductivity, limited electron and proton transfer due to increased ohmic resistances can occur, causing a reduction in current production, lower conversion of NO₃⁻ to N₂ and higher accumulation of intermediates (Puig et al. 2012).

In the present study, calculated total electrolyte’s pseudo-ohmic resistance (i.e., total electrolyte resistance) ranged from 127 ± 3 to 152 ± 2 Ω, and varied between different regions of the reactor. Current generation and the electrolyte’s pseudo-ohmic resistance within the cathode and anode areas were greatly affected by mixing conditions (Figure 4(a) and 4(b)). When applying both recirculation

![Figure 4](https://iwaponline.com/wst/article-pdf/76/11/3150/210897/wst076113150.pdf)

*Figure 4 | (Bio)electrochemical response at different recirculation regimes. (a) Current generation (mA); (b) cathodic, anodic and gap pseudo-ohmic resistances. Recirculation: (1) feed + circuit 1 + circuit 2; (2) feed + circuit 2; (3) feed only; (4) no feed, no recirculation.*
circuits 1 and 2 together with the feed in the first hour of the experiment, the resistance was kept to a minimum of 43 ± 1 and 34 ± 1 Ω within anode and cathode respectively. The resistance then increased stepwise by sequentially interrupting recirculation within the different regions until reaching a maximum of 51.0 ± 0.5 and 49.30 ± 0.02 Ω at the anode and cathode respectively by the end of the test. The total resistance of the system was therefore decreased by 13.6 ± 1.1% with the application of all recirculation circuits, compared to that of feed only. This decrease in pseudo-ohmic resistance in the system with added recirculation circuits confirms that convection plays an important role together with migration in the transport of ions within those regions. However, although the resistance of the gap area was shown to be the highest of all regions, it seemed not to be affected by recirculation conditions. Thus, the improvement of the reactor’s performance with added recirculation was likely due to transport of ions to/from the biofilm and electrode’s surface. Furthermore, as gas is expected to be the end product of both denitrification (N₂) and anodic abiotic reactions (generating O₂), improvement of the transport of ions could be due to bubble removal, exposing again the electrode’s surface to the contact with the liquid phase.

Energy consumption based on the amount of nitrogen removed was only calculated for the experimental phases 1–8, and tended to decrease over time of operation (Figure 3(b)). Although no liquid samples were analysed during the short term recirculation tests, calculated energy consumption based on volume of treated effluent when applying the best operational conditions herein (full recirculation) indicated a total consumption of 0.027 ± 0.001 kWh/m³. However, 13.5 ± 0.4% of this energy consumption was in fact due to losses of potential within cathode–anode gap (0.0037 ± 0.0003 kWh/m³, corresponding to a total cell voltage loss of 0.334 ± 0.013 V). Thus, as this region of the reactor does not play a role on the reactions, the total energy spent could be reduced by decreasing the distance between anode and cathode electrodes to a minimum. However, although oxygen concentrations of the effluent (after the anodic zone) were generally very low in the present study, care should be taken to avoid oxygen back diffusion from the anode to the cathode in case oxygen starts being generated in the counter electrode. The fact that denitrifying microbes can use different electron acceptors has an important impact on the current study, because oxygen is a very good electron acceptor: $\text{Eo}^\prime = \frac{1}{2} \text{O}_2/\text{H}_2\text{O} = +0.82 \text{ V} \text{ vs SHE (Madigan & Brock 2009)}$. As such, in the presence of oxygen, it is likely that denitrification reactions are suppressed and therefore nitrogen removal efficiencies would drop if aerobic conditions occurred. In addition, oxygen crossover could also lead to an increase in electron transfer, as oxygen can be easily be reduced at the cathode, thus increasing current generation (and energy consumption) without increasing denitrification activities. Moreover, although considerable electrolyte’s voltage losses within both anode and cathode have also occurred, reducing the size of those electrodes is not viable as this will greatly affect the performance of the system.

Since oxygen was removed from the wastewater feeding the reactor in the present work, further studies should also include the effects of different oxygen concentrations on the reactor’s behaviour. It has been previously shown that an adapted cathodic biofilm performing simultaneous nitrification and denitrification is still able to reduce nitrate with oxygen levels up to 5.73 mg L⁻¹ O₂ due to differences in bacterial community structure at a microscale, where organisms present at the outer biofilm layer were able to reduce oxygen concentration before nitrate could be reduced by the denitrifiers located in the inner layers (close to the electrode) (Virdis et al. 2011). However, we hypothesize herein that the effect of oxygen eventually present in real wastewaters may be minimized in the proposed setup by depleting oxygen within initial (lower) portions of the cathode biofilm, thus still enabling denitrification at low oxygen levels at the upper portions of the biofilm.

Alternative methods for improving the system’s performance may include (1) the use of different electrode materials and (2) the use of a more specialised microbial community. Although the use of different materials will not reduce ion transfer losses in the liquid phase, they may provide bigger surface areas for attachment/growth of more biomass and could improve nitrogen removal rates. Furthermore, using a more specific microbial community which is able to perform direct electron transfer using more positive cathodic potentials, as previously shown (Virdis et al. 2009), will enable further energy savings due to a lower overall applied cell voltage. Therefore, these effects should be subject of investigation in future studies.

**CONCLUSIONS**

The proposed technology presents an easy-to-operate system which was able to perform denitrification with negligible accumulation of the intermediate nitrous oxide, at the maximum nitrate removal rate of $0.121 \text{ Kg N m}^{-3} \text{ d}^{-1}$. The application of recirculation circuits can improve
electrochemical response and save up to 14% of energy consumption of the system, by improving ion transfer between cathode and anode.

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