Influence of nitrogen limitation on performance of a microbial fuel cell
P. Belleville, P. J. Strong, P. H. Dare and D. J. Gapes

ABSTRACT

We describe the operation of a microbial fuel cell (MFC) system operating on a synthetic wastewater (acetic acid), under conditions of increasing nitrogen limitation. Two MFCs were operated under feed conditions which spanned a range of TKN/COD values of 1.6–28 mg/g. Stable operation was observed in all cases, even when no ammoniacal nitrogen was added to the cell. Improved electrochemical performance (measured as power density, W/m²) was observed as nitrogen limitation was imposed on the cells. Even with no ammonium addition, continuous function of the cell was maintained, at levels consistent with operation at balanced nutrient supplementation. The work has implicated biological nitrogen fixation as a potential source of nitrogen within the MFC. Whilst this hypothesis has yet to be confirmed, the work highlights the opportunity for continuous operation of microbial fuel cells utilising wastewaters with extremely low nitrogen levels, present in pulp and paper, pharmaceutical and petrochemical industries. Further, the described increases in some of the electrochemical indices (e.g. power density) under application of nitrogen limitation may provide a new approach to increasing fuel cell performance. Finally, the lack of any need to add supplemental nitrogen to a MFC-based wastewater treatment technology holds potential for significant financial and environmental savings.

Key words | microbial fuel cell, nitrogen fixation, nitrogen limitation

INTRODUCTION

Microbial fuel cell (MFC) technology provides an innovative approach for direct conversion of the energy contained within organic materials into electricity. This is accomplished by physically separating the electron accepting (oxygen reducing) activity from the electron donating (oxidation of organics) activity of the microbial cell (Figure 1). Consumption of organic compounds by the bacterial cells releases electrons, which are coupled to the oxygen reduction activity via electrodes, thus generating an electrical current.

Considerable research is being conducted towards utilisation of the organic components of wastewaters as carbon sources for MFCs, and this has broadened to include aspects such as nitrogen and sulphur removal. Whilst investigations span an increasingly wide range of configurations and operational environments, there appears to be opportunity for considering the impact of nutrient limitation on MFC performance.

Two factors make this investigation pertinent: one applied and one biochemical. Firstly, a nitrogen-deficient environment is relevant to a number of industrial wastewaters (e.g. pulp and paper, petrochemical and pharmaceutical wastewaters), the work thus addresses a critical issue for expansion of this technological approach into these sectors.

From a biochemical perspective, nitrogen is a key nutrient in biological metabolism, providing an essential precursor for synthesis of protein, nucleic acids and cell wall polymers. In a comparison of the elemental composition of a number of microbial species, an average composition of CH₁₈O₁₀₅N₀.₂ was found, the nitrogen making up 11.4% of the dry weight of this mean value (Nielsen et al. 2003). A range of impacts are observed as nitrogen limitation is imposed upon a growing cell culture, including converting carbon into storage polymers (glycogen or polyhydroxyalkanoate) or overproduction

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When under extreme nitrogen limitation, bacteria capable of biological nitrogen fixation will synthesise the nitrogenase enzyme in order to utilise atmospheric dinitrogen (Hill et al. 1972; Dennis et al. 2004). However, nitrogen reduction can potentially influence the redox reactions of a microbial fuel cell, as it is both energy-consuming and requires a transfer of electrons.

Given that the function of an MFC is dictated by the metabolism of the associated microbial cultures, redirection of metabolism via changes in nitrogen assimilation may significantly impact on the electrochemical performance. The aim of this work is to describe the operation of laboratory-scale MFCs, exposed to a regime of nitrogen limitation, expressed by manipulation of the ammonium/organic carbon ratio of the input feed, with all other parameters remaining constant. The hypothesis we wish to test is that the manipulation of the exogenously supplied nitrogen will modify the bioelectrochemical performance of a microbial fuel cell, due to its direct effect on microbial metabolism end electron transfer.

**MATERIAL AND METHODS**

**Cell description**

Two reactors (MFC1 and MFC2) were constructed from plexi-glass compartments (108 cm³ volume each) that were bolted together sandwiching a Cation Exchange Membrane (CEM, CMI-7000, Membranes International Inc, USA.) between each compartment. Both compartments were filled with graphite chips with 2–4 mm diameter and a bulk density of 0.64 g cm⁻³. The MFC1 had a granule volume of 42.2 cm³ and surface area of 845 cm² (calculated on the assumption that the granules were equivalent to 1.5 mm radius spheres), leaving a liquid volume of 74.9 cm³ in all the anode side (tube + chamber). The MFC2 had a granule volume of 38.4 cm³, a surface area of 768 cm², leaving a liquid volume of 80.2 cm³.

Electrodes rods provided the contact between the graphite chips and the external circuit, allowing electron transfer from the anode to the cathode through an external resistance. This resistance was maintained at 10 ohm to permit a positive growth yield in the anode side. Indeed, several authors have shown that the low external resistance allows, with a set current, a higher anode potential, increasing the potential difference between the electrode and the biofilm and therefore increasing the bacterial yield (Logan et al. 2006; Freguia et al. 2007). In our work, we wanted to show the rapid adaptation of the cell response to a nitrogen limitation.

**Operational conditions**

A synthetic wastewater was prepared as the anolyte feed material to the MFCs. The medium (6 g/L Na₂HPO₄, 3 g/L KH₂PO₄ 0.05 g/L MgSO₄, 0.025 g/L CaCl₂, trace element) was supplemented with sodium acetate (350–550 mg/L) and ammonium chloride (0–50 mg/L). The medium was under an intermittent feed, at 4.5 minutes off, 6 seconds on, at an average rate of 10 mL/h. This anolyte (termed feed in this work) was recirculated continuously using a peristaltic pump at a rate of 1.5 L/h.

Three operational phases were conducted throughout the experimental period, with each phase defined by a change in feed composition, as defined by the nitrogen/acetate ratio. Chemical Oxygen Demand (COD) and Total Kjeldahl Nitrogen (TKN) are used as the base measurement of the organic and nitrogenous components within the system; the operational phases are summarised in Figure 2 and Table 1. It is noted that that the ammonium input to MFC1 feed was zero from day 15, and the measurement of a positive TKN (mean value 0.75 mg/L) reflects analytical sensitivity and the presence of any nitrogenous contamination in the makeup constituents. The spread of ratios reflects the plan to span ranges of TKN/COD which would be considered nitrogen balanced against nitrogen limited conditions. The modifications to the feed COD reflects a development of the thinking around the optimal method for obtaining nitrogen limitation. Concerns from Phase 2 results that MFC2 might become carbon limited led to the increase of feed COD back to the original 500 mg/L target of Phase 1 (see Figure 2).
The TKN/COD ratio of MFC1 was kept low throughout the experiment, to enable description of a cell operated continuously under nitrogen limited conditions.

The catholyte (fluid within the cathode compartment) was a phosphate buffer (6 g.L Na$_2$HPO$_4$, 3g . LK H$_2$PO$_4$). The concentration was the same as in the anode side in order to limit ionic diffusion through the membrane. The catholyte solution was oxygenated by external sparging with air and recycled continuously at a rate of 2 L/h.

Daily measurements of pH were recorded, for both anolyte and catholyte solutions, with the catholyte being replaced when pH greater than 7.5 was recorded. Voltage across a 10$\Omega$ resistor was recorded every minute.

**Chemical analysis**

Anolyte feed and effluent was sampled for analysis of Chemical oxygen demand (COD, via Standard methods-APHA 1998), total organic carbon (TOC, highTOC II, Elementar Analysen-systeme GmbH, Germany), and volatile and fatty acids (VFA). These latter measurements involved pH correction of the filtered sample using formic acid, and subsequent analysis using a capillary gas chromatograph fitted with a flame ionisation detection (HP 5890A, Hewlett Packard, USA). The column used was a 30 m NukoTM column (0.53 mm ID) ramped from 30°C to 150°C. Butan-1-ol was used as an internal standard. The same analytical procedure allowed measurement of the low molecular weight alcohols, methanol and ethanol. Nitrogen was measured as Total Kjeldahl Nitrogen (TKN), based on a Kjeldahl digest and colorometric quantitation.

**Reactions**

The description of the cells are based on the acetate oxidation reaction (Freguia et al. 2007)

$$\begin{align} 
0.5CH_3COOH + (1 - 1.48Y_X) H_2O 
+ 0.18Y_X NH_3 \rightarrow (1 - Y_X) CO_2 + (4 - 4.17Y_X) H^+ 
+ (4 - 4.17Y_X) e^- + Y_X CH_3O_0.52N_0.18 
\end{align}$$

In the equation, $Y_X$ represents the growth yield (C-mmol biomass/C-mmol substrate). This growth yield corresponds to the part of the energy (electrons) consumed into the biofilm to ensure the maintenance of a viable electrophilic bacterial community.

We can deduce the electron balance from the Equation (1), assuming that the test conditions do not allow any methanogenic reactions (Freguia et al. 2007).

$$0 = \gamma_S \Delta S - 3.600Q/F - 4.17\Delta X$$

where $\Delta S =$ substrate consumption (C-mmol), $\gamma_S =$ degree of reduction of the substrate (4 for acetic acid), $Q =$ charge

**Table 1** | Substrate removal performance of MFCs (95% Confidence interval in brackets). Data in mg/L unless specified

<table>
<thead>
<tr>
<th>Phase</th>
<th>Feed N/COD (g/g)</th>
<th>MFC1</th>
<th>MFC2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5.5</td>
<td>2.6</td>
</tr>
<tr>
<td>1. Tot CODin</td>
<td></td>
<td>369 (58)</td>
<td>533 (44)</td>
</tr>
<tr>
<td>2. Sol CODout</td>
<td></td>
<td>147 (35)</td>
<td>280 (114)</td>
</tr>
<tr>
<td>3. Tot TOCin</td>
<td></td>
<td>128 (21)</td>
<td>197 (15)</td>
</tr>
<tr>
<td>4. Tot TOCout</td>
<td></td>
<td>74 (12)</td>
<td>132 (52)</td>
</tr>
<tr>
<td>5. COD removal [1–2]</td>
<td></td>
<td>222 (58)</td>
<td>292 (221)</td>
</tr>
</tbody>
</table>
transferred through the external resistance (mCoulomb), 

\[ F = \text{Faraday constant}, \Delta X = \text{biomass growth (C-mmol)} \]

The coulombic efficiency of the cell has been determined throughout the experiment using the following relation:

\[ E_{\text{coulombic}} = \frac{MI}{Fq\Delta\text{COD}} \]

\[ M = \text{molecular weight of oxygen (32 g/mol), I = current (A), F = Faraday constant, q = volumetric influent flow rate (L/s), } \Delta\text{COD} = \text{substrate consumption as COD (g/L)} \]

**RESULTS**

**Cell characteristics**

To characterise the intrinsic electrochemical function of the cells, open circuit voltage and internal resistance were regularly measured. The cells reached a stable current production within 2 days of changes to feed composition. The internal resistance of each cell could be deduced from the maxima of a plot of power output as a function of the external resistance (one example given in Figure 3), and averaged 98 Ohms and 68 Ohms for MFC1 and MFC2, respectively. Similarly, the open circuit voltage was found to average 401 mV and 402 mV for MFC1 and MFC2, respectively.

**Carbon removal**

The organic substrate removal measurements in each reactor are described in Table 1. The cell performance as measured by TOC or the COD showed a similar pattern, with MFC2 showing higher removals (290–330 mg/L as COD) than MFC1 (220–250 mg/L as COD). Changes to the ammonium input (as measured by the N/COD ratio) provided no significant change in removal performance within the cells.

**Power production**

Overall power production across the 10 Ohm external resistance (presented as daily averages in Figure 4) provides a good representation of the overall performance of the MFCs throughout the experimental period. The figure does show some significant variations, many of which are result of changes to catholyte, cleaning procedures and cuts to the input feed supply. Operationally, the cells recovery from disruptive changes (cleaning, feed composition changes, pump failures) was rapid, with normal power production resuming within 2–3 hrs of these disruptions.

Figure 4 indicates that the power output remained relatively constant for MFC1, the cell under greatest nitrogen limitation throughout the experiment. In MFC2, ammonium concentration did appear to influence the power output.

This is better described in Figure 5a, where power output (normalised to the anode surface area) is plotted as a function
of N/COD ratio. For MFC2, reducing the N/COD ratio to less than 5 mgN/gCOD increased the power output by approximately 60%, compared with the high-ratio values. The significance of this change was confirmed from a linear regression of power against N/COD. The regression was significant ($R^2 = 0.74$), with a non-zero slope ($-0.034 \text{mW.g/}[\text{mg.m}^2]$, p value $= 6 \times 10^{-7}$) being calculated. The regression for MFC1 was also significant, again showing a negative correlation between power and N/COD (slope $= -0.67 \text{mW.g/}[\text{mg.m}^2]$, p value $= 0.0009$).

The coulombic conversion efficiency, as a function of applied N/COD ratio, is provided in Figure 5b. Efficiencies of 30–50% were observed for the two cells, with linear regression revealing no significant impact of nitrogen limitation observed for this parameter.

### DISCUSSION

The experimental results indicate that consistent performance of a heterotrophic microbial fuel cell can be maintained, under the constraint of increasing limitation of ammonium as a nitrogen source, supplied for biological growth (Table 1 and Figure 4). Further, decreases in ammonium levels appear to have enhanced the power output from the cell. The data collected thus far indicates that this increase has been at the expense of cell growth (discussed below). At around 2–4 W/m$^3$ anode volume, the specific power output from these cells was relatively low in comparison with other air-based cells described in the literature (e.g. 12.7 W/m$^3$. Liu et al. 2005 or 65–83 W/m$^3$. Clauwaert et al. 2007). In contrast, the coulombic efficiency, at 50–50% for both cells, was comparable with literature (Logan et al. 2006). Clearly, optimisation of the cell in use is required, including modifications to the liquid recirculation patterns, which show clear evidence of short-circuiting, and hence inefficient use of anode surface area. Nevertheless, this relative performance between MFCs is not relevant to the findings of the work, where our focus has been on the impact of ammonium changes to the functional performance of the MFC.

Under the extreme condition of no ammonium addition in the reactor feed to MFC1, the cell performance was maintained. This is corroborated in literature, where Clauwaert et al. (2007) studied of an acetate-fed MFC operated for 7 months without any ammonium-nitrogen addition. Nitrogen fixation is implied by these authors, and ecological studies have found numerous bacterial genera capable of biological nitrogen fixation present in microbial fuel cell communities (e.g. Kim et al. 2004). In contrast, we have described MFC operation across a range of ammonium limitation, down to a level where nitrogen fixation can also be implied. This work has not made confirmation of nitrogen fixation, which requires a specific experimental approach (e.g. acetylene reduction assay or isotope labelling experiments) to eliminate the description of a false-positive result. For example, small amounts of ammonia may be present in laboratory air, which could be utilised to sustain microbial growth, if it were absorbed into the reactor solutions. This is particularly pertinent to MFCs, as growth yields (and hence nitrogen requirements) can be low (Clauwaert et al. 2007).

Irrespective of the potential nitrogen source, our work highlights the opportunity for continuous operation of microbial fuel cells for wastewaters having extremely low nitrogen levels. One specific example of this would be pulp and paper sector wastewaters, which are characterised by COD:N ratios of 100:0.5 (by weight) or less (this being equivalent to N/COD ratio of 5, as described in the current work). Such nitrogen deficient wastewaters have been demonstrated to support the controlled growth of nitrogen fixing microorganisms within activated sludge, system (Dennis et al. 2004). Further, the increases in some of the electrochemical indices (e.g. power output) as a function of nitrogen limitation is a
source of interest, potentially providing a new approach to increasing MFC performance.

We found no impact of nitrogen limitation on the intrinsic parameters of the cells (open circuit voltage, internal resistance). All other conditions being equal (pH, temperature) there is no change in the cell overpotentials (anode and cathode) throughout the experiment. Thus, the increase in electron transfer, with no significant change in substrate consumption, under nitrogen limitation implies a decrease in biomass growth. Confirmation of this supposition is a focus of our ongoing work.

CONCLUSIONS

We have provided evidence to confirm the hypothesis that ammonium limitation has an effect on the electron transfer performance of a microbial fuel cell. Effective performance was observed, with improved electrochemical performance (as power density) being noted as nitrogen limitation was imposed on a cell initially operated under conventional nitrogen/carbon ratio.

Whilst still requiring formal confirmation, the work has implicated biological nitrogen fixation as a potential source of nitrogen within the MFCs operated under low nitrogen conditions.

Whilst much effort remains to realise practical application of MFC technology, the work highlights the opportunity for continuous operation of microbial fuel cells for wastewaters having extremely low nitrogen levels, such as is evidenced in pulp and paper, pharmaceutical and petrochemical industries. Given the large size of these industries, there is real value in pursuit of MFC applications for such sectors. Furthermore, the indication of increases in some of the electrochemical indices (e.g. power density) may provide a new approach to increasing fuel cell performance. Finally, avoiding nitrogen supplements to a MFC-based wastewater treatment technology holds potential for significant financial and environmental savings.

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REFERENCES