INFLUENCE OF NITROGEN LIMITATION ON PERFORMANCE OF A MICROBIAL FUEL CELL

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ABSTRACT

Nitrogen limitation within biological wastewater treatment processes is a cause for concern in a number of large-scale industrial sectors. Microbial fuel cells (MFC) are an innovative technology with potential application within the wastewater treatment industry. Currently, little is known about the impact of nitrogen limitation on these systems. In this work we describe the operation of a microbial fuel cell system operating on a synthetic wastewater (acetic acid), under conditions of increasing nitrogen limitation.

Stable operation of the cell was observed under all conditions, with improved electrochemical performance (as power density) being noted as nitrogen limitation was imposed on a cell initially operated under conventional nitrogen/carbon ratio. Even under zero 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. This supposition has yet to be confirmed, a subject of ongoing work in our laboratories.

The work highlights the opportunity for continuous operation of a microbial fuel cells for wastewaters having extremely low nitrogen levels, such as is evidenced in forest industry, pharmaceuticals and petrochemical industries. Further, the indication of 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 cost and environmental savings.

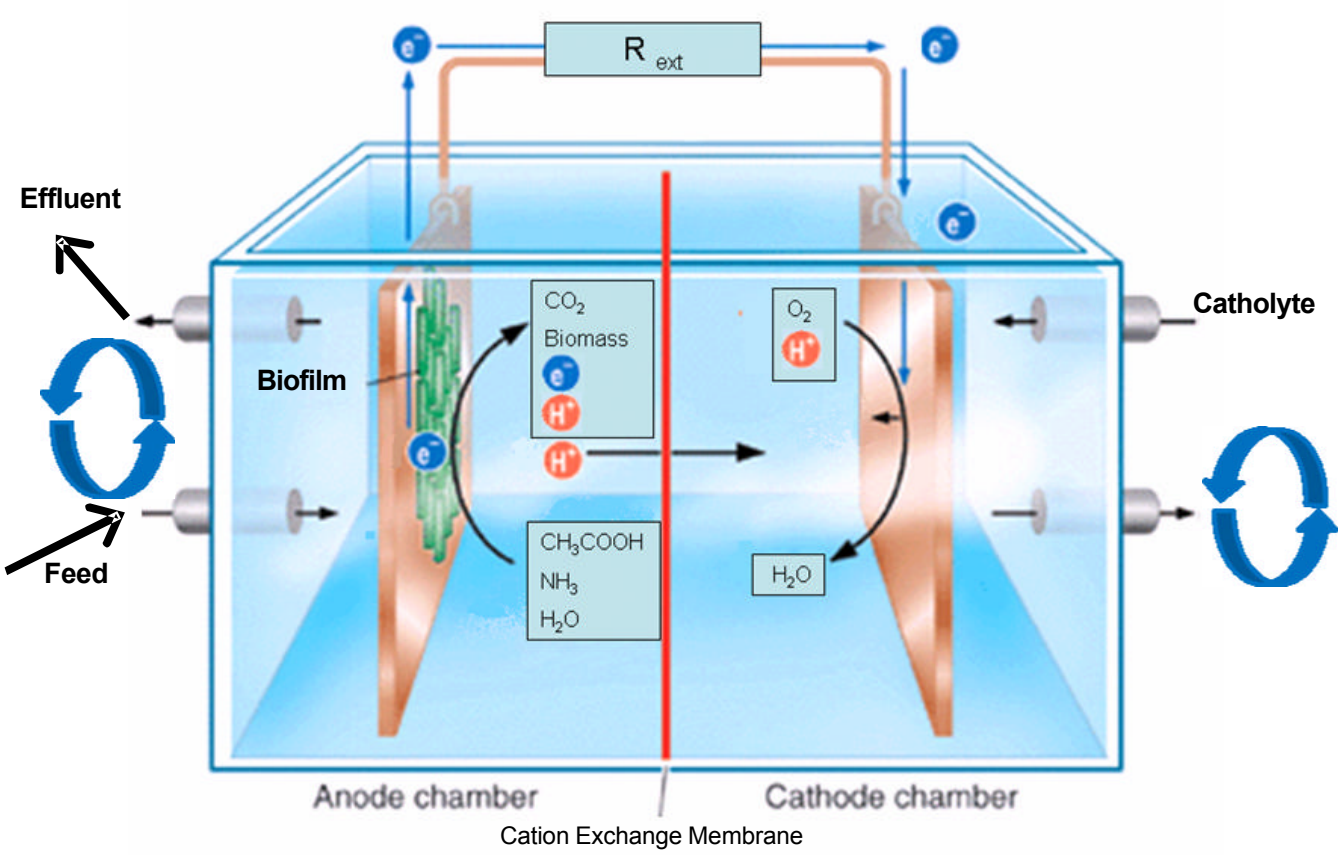
KEYWORDS

microbial fuel cell; nitrogen limitation; nitrogen fixation

1 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 organics by the cells releases electrons, which are coupled to the oxygen reduction activity via electrodes, thus generating an electrical current.

Figure 1: Schematic of microbial fuel cell



Considerable research is being conducted towards utilisation of the organic components of wastewaters as feedstocks for MFCs, and this has broadened out 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. forest industry and 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 element 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 $CH_{1.8}O_{0.5}N_{0.2}$ was found, the nitrogen making up 11.4% of the dry weight of this mean value (Nielsen et al. 2003). Under exertion of a nitrogen limitation to a growing cell culture, a range of impacts are observed, including redirection of the substrate carbon into storage polymer (glycogen or polyhydroxyalkanoate) or overproduction of extracellular polymeric substances (EPS) (Egli and Zinn 2003, Nielsen et al. 2003).

Further, under extreme nitrogen limitation, bacteria capable of biological nitrogen fixation will synthesise the nitrogenase enzyme and undertake to obtain their nitrogen requirement from atmospheric dinitrogen (Dennis et al. 2004, Hill et al. 1972). The function of nitrogen reduction is both energy consuming and requires a transfer of electrons, both factors potentially influencing the redox reactions of a microbial fuel cell.

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 content of the input feed, with all other parameters remaining constant. The hypothesis we wish to test is that if ammonium addition has an effect on microbial metabolism, and that this metabolism directly impacts on the electron transfer performance of a cell,

then manipulation of the exogenously supplied nitrogen will modify the bioelectrochemical performance of a microbial fuel cell.

2 MATERIAL AND METHODS

2.1 CELL DESCRIPTION

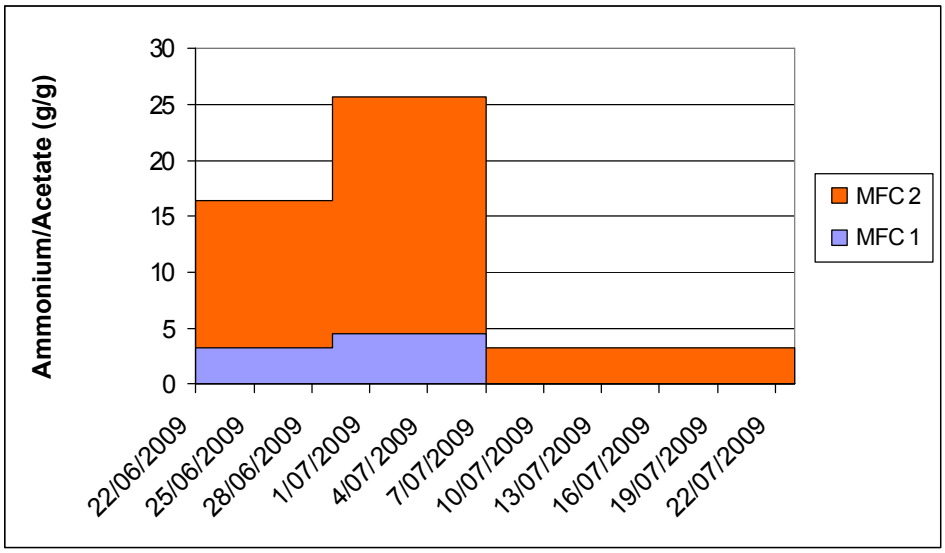
The reactor is compound by two plexi-glass compartments bolted together. The anode and the cathode have the same volume (108 cm^3) and are separated by a Cation Exchange Membrane (CEM) (CMI-7000 from Membranes International inc.). Both compartments were filled with graphite chips which were 2-4mm in diameter and have a bulk density of 0.64g cm^{-3}. The reactor 1 has a granule volume of 42.2cm^3 and surface area of 845cm^2 (considering the granules has 1.5mm radius sphere), leaving a liquid volume of 74.9cm^3 in all the anode side (tube + chamber). The reactor 2 has a granule volume of 38.4cm^3 and a surface area of 768cm^2, leaving a liquid volume of 80.2cm^3.

Electrodes rods provide the contact between the graphite chips and the external circuit and allowed the electron transfer from the anode to the cathode through an external resistance. The external resistance was maintained at 10ohm to permits an important 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 (Freguia et al. 2007, Logan et al. 2006). In our work, we wanted to show the rapid adaptation of the cell response to a nitrogen limitation.

2.2 OPERATIONAL CONDITIONS

The anolyte was recirculated continuously using a peristaltic pump at a rate of 1.5L/h. The medium (6g/L Na_2HPO_4, 3g.L KH_2PO_4, 0.05g.L $MgSO_4$, 0.025g.L $CaCla_2$, trace element) was supplemented with sodium acetate (500-800 mg/L) and ammonium chloride (0-50mg/L). The medium was under an intermittent feed, at 4.5 minutes off, 6 seconds on, at an average rate of 10mL/h. The ammonium on acetate ratio during the experiment is described in Figure 2, noting that the ammonium input to MFC1 feed was zero from 7/07/09.

Figure 2: Ammonium/Acetate ratio during the experiment (MFC1 ratio during 07 month was zero)



The catholyte was a phosphate buffer (6g.L Na_2HPO_4, 3g.L KH_2PO_4). The concentration was the same as in the anode side in order to limit the cations diffusion through the membrane. The solution was air sparged in a vessel and recycled continuously at a rate of 2L/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 10Ω resistor was recorded every minute.

2.3 CHEMICAL ANALYSIS

Daily sampling of anolyte feed and discharge for measurement of Chemical oxygen demand (COD, via *Standard methods-APHA 1998*), total organic carbon (TOC, highTOC II, Elementar Analysensysteme 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 NukolTM column (0.53 µM ID) ramped from 30ºC to 150ºC. Butan-1-ol was used as an internal standard. The same analytical procedure allowed measurement of the low molecular weight alcohols, methanol and ethanol.

2.4 REACTIONS

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

$$0.5CH_3COOH + (1 - 1.48Y_X)\,H_2O + 0.18Y_XNH_3 \rightarrow (1 - Y_X)\,CO_2 + (4 - 4.17Y_X)\,H^+ + (4 - 4.17Y_X)\,e^- + Y_XCH_{1.75}O_{0.52}N_{0.18} \quad (1)$$

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 from the equation (1) the electron balance assuming that the test conditions do not allow any methanogenesis reaction (Freguia et al. 2007).

$$0 = \gamma s.\Delta S - 3{,}600Q/F - 4.17\Delta X \quad (2)$$

In the equation, ΔS is the substrate consumption (units C-mmol), γs is the degree of reduction of the substrate (4 for acetic acid), Q is the charge transferred through the external resistance (mCoulomb), F is the Faraday constant, ΔX is the biomass growth (C-mmol)

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

$$E_{coulombic} = \frac{MI}{F\gamma_s q\Delta COD}$$

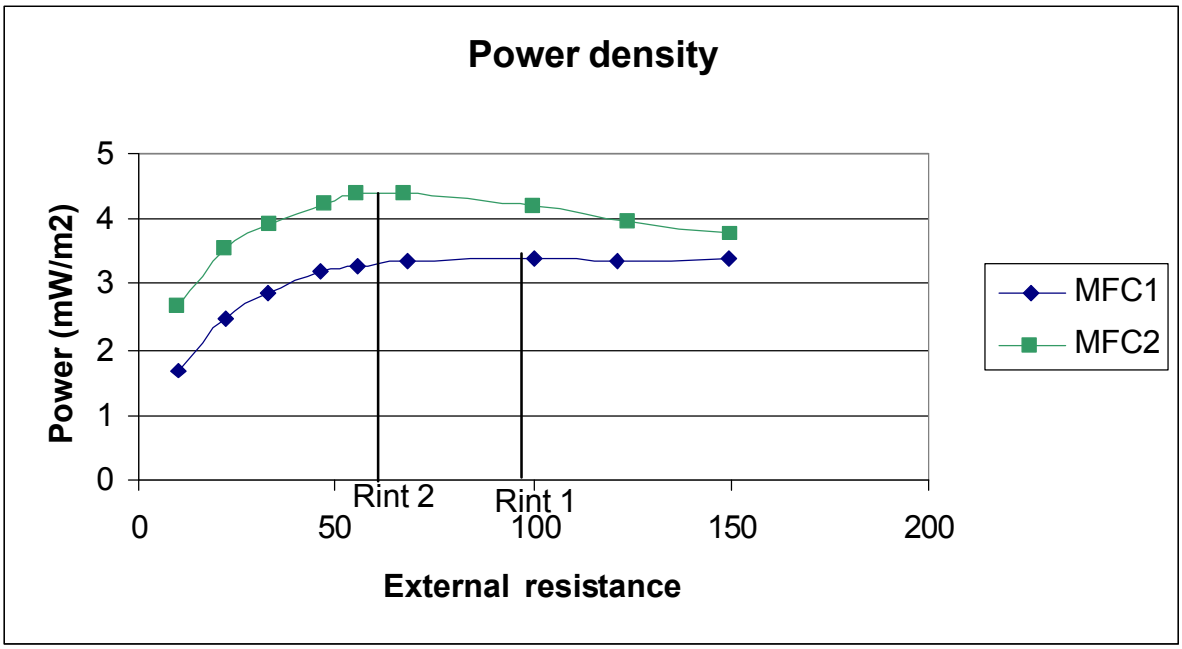
M is the molecular weight of the oxygen (32g/mol), I is the current A, F is the Faraday constant, γs is the degree of reduction of the substrate (4 for acetic acid), q is the volumetric influent flow rate (L/s), ΔCOD is the substrate consumption as COD(g/L)

3 RESULTS

3.1 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 when 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 Ω and 68 Ω for MFC1 and MFC2, respectively. Similarly, the open circuit voltage was found to average 401mV and 402mV for MFC1 and MFC2, respectively.

Figure 3: Mean daily current and power output throughout experimental period



3.2 CARBON REMOVAL

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

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

	MFC1			MFC2		
Feed N/C (g/g)	4.5	3.2	0	26	16	3.3
Tot CODin	369 (58)	533 (44)	474 (52)	351 (47.2)	553 (121)	517 (44)
Tot CODout	147 (35)	280 (114)	235 (46)	64 (24.4)	208 (60.4)	188 (37)
Tot COD removal	222 (58)	251 (340)	243 (37)	287 (59)	330 (232)	326 (46)
Tot TOCin	488 (25)	320 (140)	458 (32)	252 (86)	382 (509)	445 (38)
Tot TOCout	173 (72)	131 (99)	175 (39)	29 (17)	103 (66)	144 (33)
Tot TOC removal	284 (61)	189 (203)	285 (24)	223 (73)	272 (390)	306 (28)

3.3 POWER PRODUCTION

The power production was measured every minute during the experiment, and a summary of this performance over the period of the experimental work is presented in Figure 4, plotted as power output from the cell under the 10Ω external resistance. 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

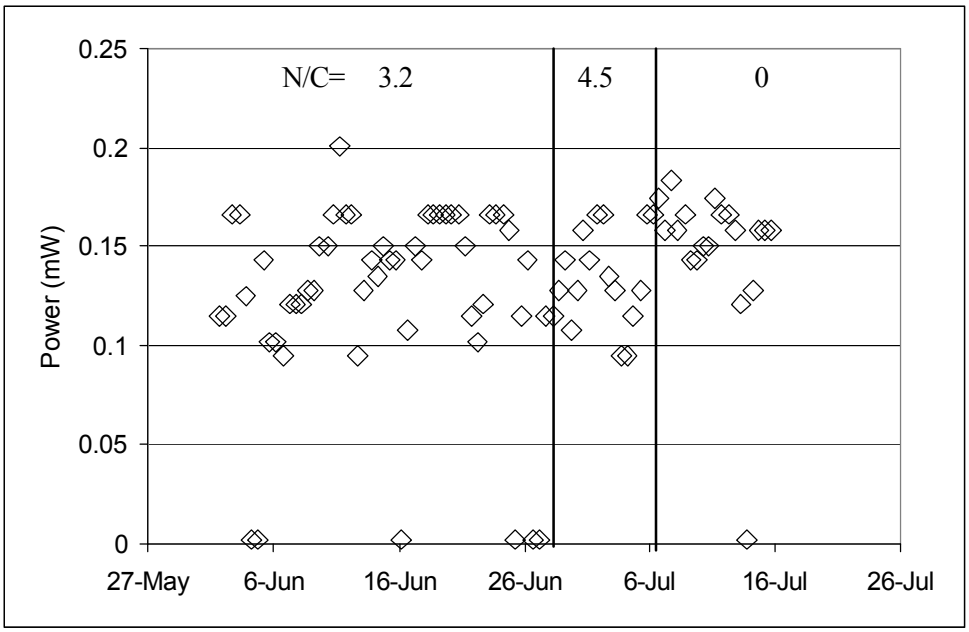
from disruptive changes (cleaning, feed composition changes, pump failures) was rapid, with normal power production resuming in 2-3hrs these disruptions.

The averaged 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/C ratio. For MFC2, reducing the N/C ratio from 25 to 3 gN/gAcetic acid increased the power output by 60%.

The coulombic conversion efficiency, as a function of applied N/C ratio, is provided in Figure 5b. Efficiencies of 30-50% were observed for the two cells, with no impact of nitrogen limitation observed for this parameter, due to the statistical uncertainty associated with the data.

Figure 4: Power density curves for a)MFC1 and b)MFC2 over the timeframe of the experimental work

a)



b)

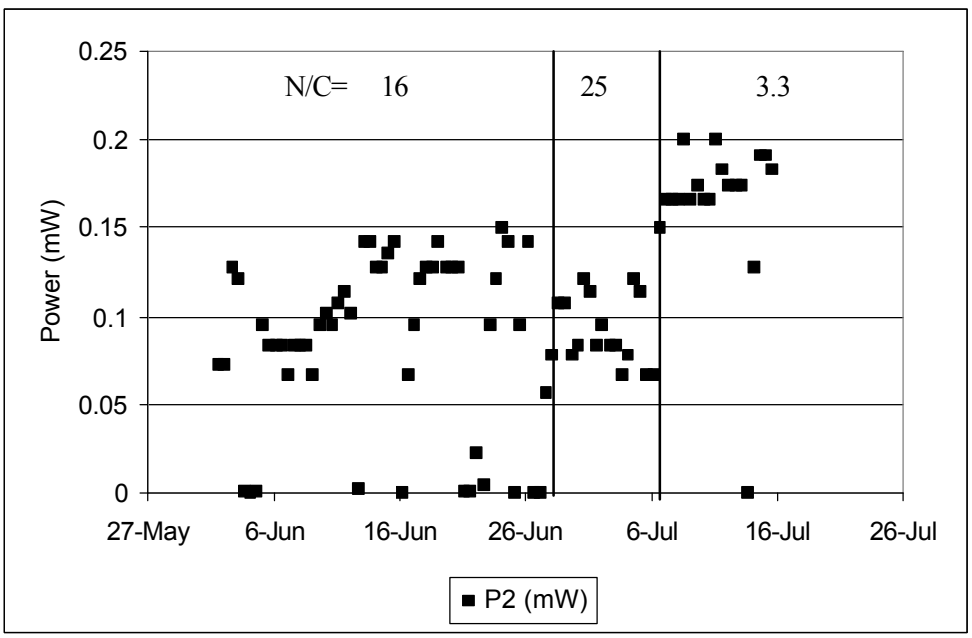
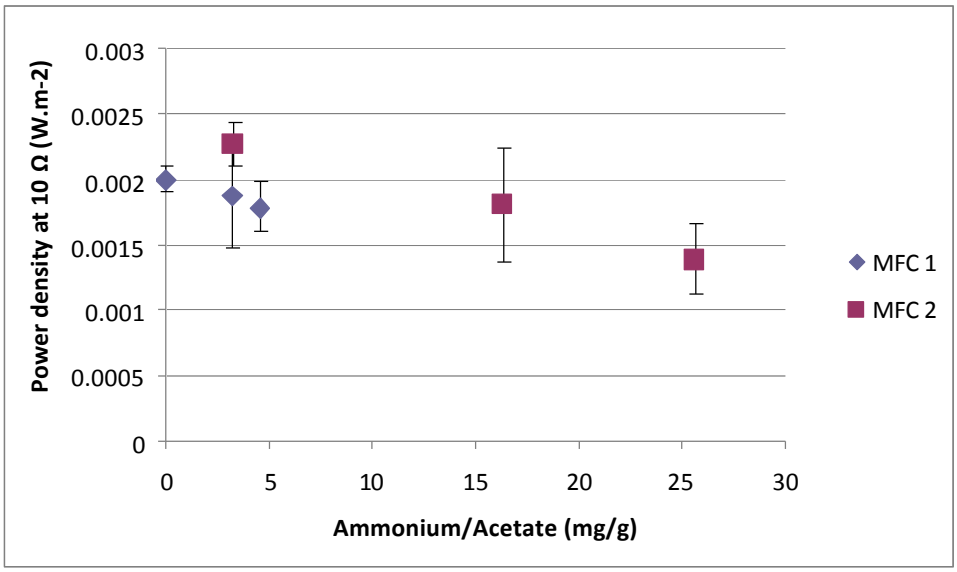


Figure 5: Impact of nitrogen limitation on (a)power density and (b)coulombic conversion efficiency

a)



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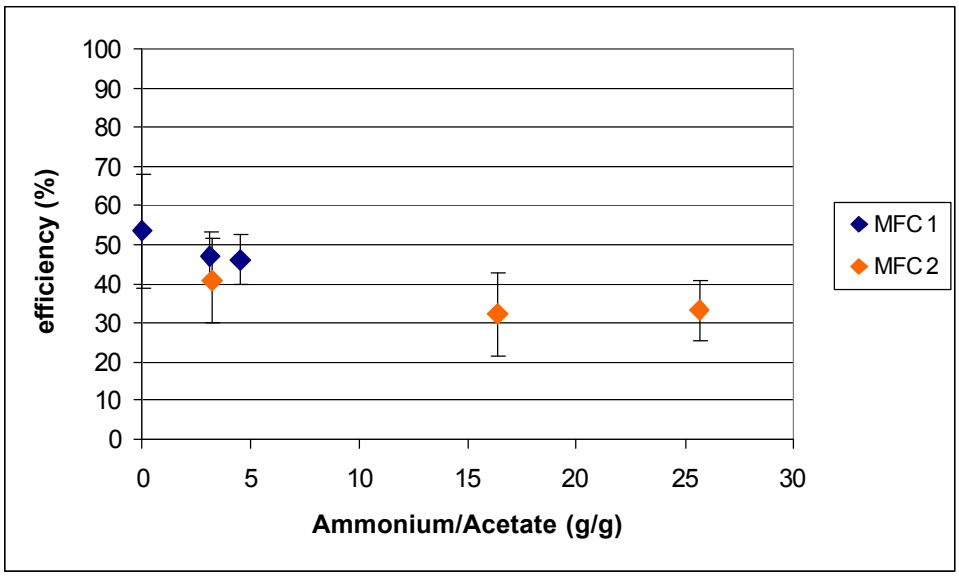

4 DISCUSSION

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 (see below discussion). 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 30-50% 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. One other author has made a study of an acetate-fed MFC operated for 7 months without any

ammonium-nitrogen addition (Clauwaert, et al. 2007). Nitrogen fixation is implicated by these authors, and reference made to ecological studies which 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 implicated. 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). Such work is currently the subject of attention within our laboratory.

Irrespective of the potential nitrogen source, our work highlights the opportunity for continuous operation of a 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, and have been demonstrated to support the controlled growth of nitrogen fixing microorganisms within an engineered, 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 thus 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 implicates a decrease in biomass growth. Confirmation of this supposition is a focus of our ongoing work.

5 CONCLUSIONS

The current work has demonstrated microbial fuel cell operation under increasing limitation for ammoniacal nitrogen. 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.

The work has implicated biological nitrogen fixation as a potential source of nitrogen within the MFC. This supposition has yet to be confirmed, a subject of ongoing work in our laboratories.

The work highlights the opportunity for continuous operation of a microbial fuel cells for wastewaters having extremely low nitrogen levels, such as is evidenced in forest industry, pharmaceuticals and petrochemical industries. Further, 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, the lack of any need to add supplemental nitrogen to a MFC-based wastewater treatment technology holds potential for significant cost and environmental saving.

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