



## A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency

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### Abstract

A microbial fuel cell containing a mixed bacterial culture utilizing glucose as carbon source was enriched to investigate power output in relation to glucose dosage. Electron recovery in terms of electricity up to 89% occurred for glucose feeding rates in the range  $0.5\text{--}3\text{ g l}^{-1}\text{ d}^{-1}$ , at powers up to  $3.6\text{ W m}^{-2}$  of electrode surface, a five fold higher power output than reported thus far. This research indicates that microbial electricity generation offers perspectives for optimization.

### Introduction

A microbial fuel cell is a mimic of a biological system in which bacteria do not directly transfer their produced electrons to their characteristic electron acceptor. Instead, the transport process is subsequently conducted over an anode, a resistance or power user, and a cathode. Thus way, bacterial energy is directly converted to electrical energy (Rao *et al.* 1976). Research on microbial fuel cells ('biofuel cells') has received increased attention as a means to produce 'green' electricity from natural substrates, such as carbohydrates. Interest has focused on three main types: heterotrophic (Delaney *et al.* 1984), photoheterotrophic (Tsujimura *et al.* 2001) and sediment cells (Tender *et al.* 2002). The first two use suspended bacterial cultures to produce the current while the third type encompasses electrodes inserted into the solid sediment matrix. However, applicability of the technology for general purposes remains low for the first two types, because of bottlenecks in both the anode and the cathode compartments and because of difficult transfer through the proton exchange membranes. The third type offers limited perspectives for civil practice because of the generally low levels of electron donor in sediments.

Anode optimization can be provided by either biological or (electro)chemical means. Studies by Bond *et al.* (2002) indicated that several existing organisms were capable of providing stable current output for biofuel cells over several days, even weeks. A recent study (Bond & Lovley 2003) showed that *Geobacter sulfurreducens* was capable of transferring quantitatively the electrons provided by acetate as the sole carbon source to the electrode. For this, the researchers attached the bacteria to the electrode surface. Yet, the power output of these cells remained lower than  $0.1\text{ W m}^{-2}$  electrode surface. Park & Zeikus (2003) reached power densities of about  $0.7\text{ W m}^{-2}$  using a graphite electrode containing  $\text{MnO}_2$  and activated sludge fed with glucose as an inoculum. Similar results were obtained in a photo-electrochemical biofuel cell (Tsujimura *et al.* 2001). In the latter case, power densities of  $0.3\text{--}0.4\text{ W m}^{-2}$  electrode surface were attained, resulting in a light yield of 2.5–4%.

Electrochemical optimization can occur through enlargement of the specific electrode surface, or the insertion of redox mediators in either the electrode or in the feeding solution. The application of redox mediators has been the subject of several studies. Different approaches exist, varying from the immobilisation of enzymes (Okawa *et al.* 1999) or organic dyes (Kubota

*et al.* 2000) onto the electrode surface to the insertion of the mediator into the matrix of the electrode. However, stable binding of the mediator to the electrode surface is difficult to achieve (Park & Zeikus 2003) both in terms of chemistry and costs. The use of costly proton exchange membranes (Ultrex costs about d' 100 per square meter) results in an expensive technology compared with anaerobic digestion.

In this work, we used a plain graphite electrode and a microbial consortium previously adapted to microbial fuel cells to investigate how the loading rate, being the amount of carbon source added per unit of reactor volume and time, affected the applicability of the system (Vandevivere & Verstraete 2001) and the metabolism of the enriched microbial consortium.

## Materials and methods

### Set-up

The assembly comprised Plexi-glass elements that were bolted together. Four cells were connected into one block, thus giving rise to four cathodes and anodes. Two cell blocks were operated in parallel to enable comparison. The anode cells had a total volume of 45 ml, of which 40 ml was liquid phase. To this anode compartment, different amounts of glucose as electron donor (expressed in COD equivalents) were dosed and allowed to react for certain time periods. Overall, the dosage was expressed in terms of volumetric loading rate ( $\text{g COD l}^{-1} \text{d}^{-1}$ ). Plain graphite electrodes (Morgan) were inserted into both the cathode and anode compartments ( $50 \text{ cm}^2$ ), and externally connected using a plain graphite rod. The proton exchange membrane (Ultrex, Membranes International Inc.) was incubated in 2% (w/v) NaCl for 3 h prior to use. For the cathode, a 100 mM phosphate buffer was prepared and enriched with 100 mM of potassium hexacyanoferrate to optimize electrode-oxygen charge transfer (Park *et al.* 2000). The cathode compartments were continuously aerated at a minimum dissolved oxygen level of  $6 \text{ mg l}^{-1}$ . The two used set-ups were installed on a shaker (60 rpm) at  $28^\circ \text{C}$ . The tests were monitored for 6 d consecutively; samples (5 ml) were taken every day and concomitant volume correction was subsequently performed.

### Bacteria

The mixed culture used for the experiments was obtained as a result of an enrichment transfer procedure over a period of five months at this laboratory, initiated

with anaerobic sludge originating from a methanogenic reactor at a potato processing company. Fuel cell biofilm bacteria were repetitively used to inoculate the subsequent biofuel cells in order to obtain a microbial selection. For this, biofilms were scraped off the electrodes and suspended in sterile nutrient broth, to be brought into a new sterile reactor. After five consecutive biofilm enrichments, a biofuel cell was operated for five weeks, with a weekly refreshment of the medium but re-use of the suspended bacteria (centrifugation and subsequent resuspension). The bacteria were fed at a rate of  $1 \text{ g glucose l}^{-1} \text{d}^{-1}$ , and always purged with nitrogen gas until anoxic prior to use. During this selection, current densities and conversion efficiencies, initially of the order of  $0.2 \text{ W m}^{-2}$  and 5% respectively, gradually increased. For the experiments described below, the contents of the whole anode compartment were harvested, centrifuged and resuspended in nutrient broth (Oxoid). The experiments were initiated by adding 20 ml nutrient broth and 20 ml cell suspension ( $8.6 \log \text{ c.f.u. ml}^{-1}$ ) to the anode compartment with a headspace of 5 ml. Before the start of the experiment, the anode compartment was purged with  $\text{N}_2$ . Microbial biomass was determined as the COD of the suspended solids.

### Electrochemical monitoring

Measurements of the power output were performed using an Agilent HP 34970 data acquisition unit. Every 30 s, a full channel scan was performed and the data stored. External system resistance  $R$  was maximum  $100 \Omega$ , current was deducible as  $I = V \times R^{-1} = Q \times t^{-1}$  (Equation (1)) with  $I$  the current (A),  $V$  the voltage (V),  $Q$  the charge (C) and  $t$  time (s). Power output of the cells was calculated as  $P$  (Watt) =  $I \times V$  (Equation (2)) Energy production can then be expressed as  $E$  (Joule) =  $P \times t$  (Equation (3)). The relation between charge and chemical oxygen demand (COD) was calculated as  $Q = 96485 \text{ (C mol}^{-1}) \times \text{COD (g)/32 (g mol}^{-1}) \times 4 \text{ (mol}^{-1})$  (Equation (4)). Theoretical comparisons were made on the basis that 1 mol glucose can provide 2860 kJ for complete conversion into  $\text{CO}_2$  and  $\text{H}_2\text{O}$ .

### Chemical analysis

Samples were filtered through a syringe  $0.22 \mu\text{m}$  filter unit (Millex Corp.). For analysis of the volatile fatty acids, an extraction in diethyl ether was performed. The samples were analysed with a capillary FID (flame ionization detector) GC 8000 Carlo Erba

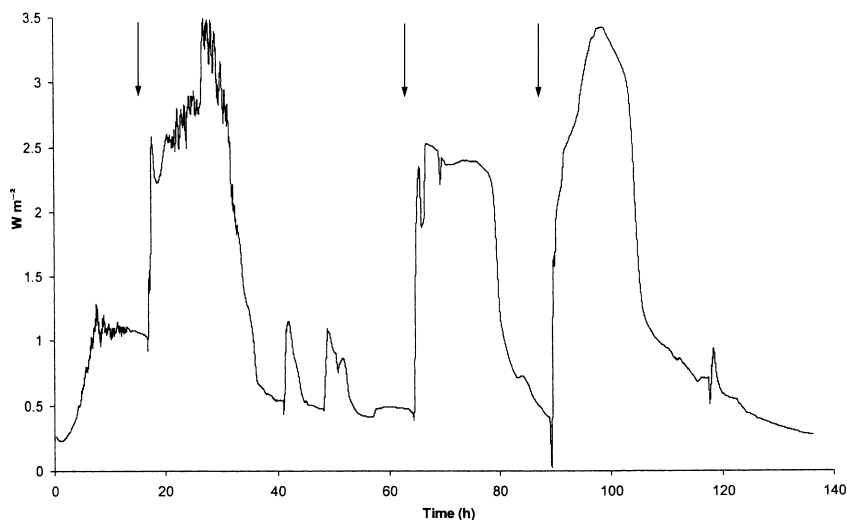


Fig. 1. Power output ( $\text{W m}^{-2}$  electrode surface) of a biofuel cell during the 6 d test period. The addition of glucose is indicated by the arrows. The smaller intermediate peaks are caused by replenishment of the medium.

Instruments (Wigan, UK), connected to a computer. The column used was an Alltech (Deerfield, USA) EC-1000 (30 m, I.D.: 0.32 mm,  $d_f$ : 0.25  $\mu\text{m}$ ). The temperature was controlled at 135  $^{\circ}\text{C}$  for the isotherm oven and 200  $^{\circ}\text{C}$  for the detector and the injector.  $\text{N}_2$  was used as the carrier gas at 3  $\text{ml} \times \text{min}^{-1}$ . Samples were diluted 10 times in deionised water and studied for monosaccharides using ion chromatography (Dionex, Carbopac1 column with borate and amino trap, Pulsed Amperometric Detection ED40). Bacterial concentrations were determined turbidometrically at 580 nm and converted to biomass-COD using a previously obtained calibration curve. Gas chromatography (Intersmat IGC 120 MB) was used for determination of  $\text{CO}_2$  and  $\text{CH}_4$  in the headspace.  $\text{H}_2$  was measured using a Microtox exhaled hydrogen monitor (GMI). COD measurements were made according to the dichromate method (Greenberg *et al.* 1992).

## Results

The startup of the power production, expressed as  $\text{W m}^{-2}$  electrode surface, for a period of 18 h is depicted in Figure 1. Then the cell was dosed with 0.08 g COD, corresponding to 2 g glucose  $\text{l}^{-1}$ , thus representing a loading rate of 2 g glucose  $\text{l}^{-1} \text{d}^{-1}$  from 18 to 42 h. After 42 h all glucose had disappeared as verified by ion chromatography (not shown). The net power output based on glucose was calculated as the average increase over the baseline. For the dose of 2 g COD  $\text{l}^{-1}$

to the 40 ml liquid in the anode compartment, representing 1588 J available energy, the energy production as electricity corresponds according to Equation (3) with  $2.2 \text{ W m}^{-2} \times 0.005 \text{ m}^2 \times 24 \text{ h} \times 3600 \text{ s h}^{-1} = 950 \text{ J}$ . Hence, the peak recorded between 18 and 42 h represents an energy recovery as electricity of 65%. The period 42 to 66 was used for sampling. At 66 and at 90 h this feeding protocol was repeated without waiting for glucose analysis. In a similar way a series of test runs (each with two fuel cells in parallel) were performed with doses between 0.5 and 5 g  $\text{l}^{-1} \text{d}^{-1}$ , each administered 3 times (Table 1). The feedings that corresponded to 1.5;2.5;3 and 3.5 g  $\text{l}^{-1} \text{d}^{-1}$  received a pH correction to exclude an influence of the pH drop on the performance. The mass balances of these experiments, expressed as mg COD, are given in Table 2. The total VFA concentrations decreased compared to the initial concentration for loading rates up to 3 g  $\text{l}^{-1} \text{d}^{-1}$ . This was due to the decrease in acetate and propionate concentrations. Only butyric acid concentrations increased in reactors with a feeding of more than 3 g  $\text{l}^{-1} \text{d}^{-1}$ , the changes in concentration at lower feeding rates were minor. Maximum power values of about 3.6  $\text{W m}^{-2}$  were attained. The smaller peaks in between the feed peaks were caused by use of the carbon source present in the nutrient broth, which was added after sampling to correct for reactor volume.

The gas compositions showed that  $\text{H}_2$  remained below 500 ppmv for all reactors (data not shown) corresponding to a maximum of 0.003% of the COD being converted to hydrogen. The final uncorrected

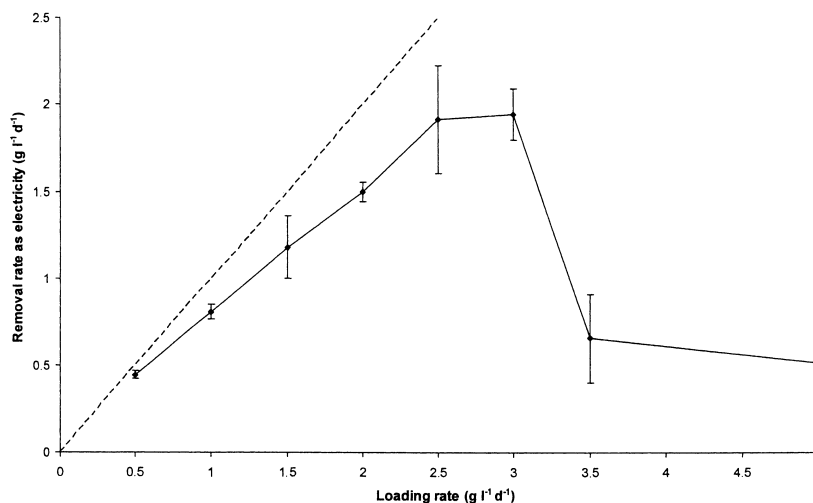


Fig. 2. Removal rate of glucose to electricity for different volumetric loading rates. The removal rate increases up to a loading rate of 3 g COD  $l^{-1} d^{-1}$ . The straight line corresponds to 100% carbon source to electricity conversion.

Table 1. Coulombic glucose to electricity conversion efficiency. The electricity produced was converted to 'Produced coulombs' using Equation (1), the added coulombs were calculated according to Equation (3).

Experiment n°	Loading rate (g COD $l^{-1} d^{-1}$ )	Produced coulombs ( $n = 2$ )	Added coulombs	Coulombic efficiency (%)
1	0.5	2991 ± 25	3369	89 ± 4
2	1	3391 ± 28	4192	81 ± 4
3	1.5	3096 ± 394	3917	79 ± 12
4	2	4022 ± 20	5355	75 ± 3
5	2.5	3618 ± 486	4712	77 ± 12
6	3	3282 ± 591	5049	65 ± 5
7	3.5	1009 ± 391	5358	19 ± 7
8	5	792 ± 19	7662	10 ± 2

Table 2. Microbial fuel cell mass balance and final reactor parameters. Changes were calculated relative to the initial concentration at  $t = 0$ . All values were expressed as COD, either by using Equation (3) for electricity conversion, or by calculating the stoichiometric amount of oxygen required for the oxydation of the components (biomass, VFA, glucose) to  $CO_2$  and  $H_2O$ .

Experiment	Amount COD added (mg)	$\Delta$ Biomass (mg COD)	VFA (mg COD)	Electricity (mg COD)	Total (mg COD)	Recovery (%)	Final pH
1	262	18 ± 11	-28 ± 27	232 ± 47	222 ± 55	85 ± 21	6.5 ± 0.9
2	326	71 ± 5	-13 ± 5	263 ± 60	321 ± 60	99 ± 19	6 ± 0.7
3	304	53 ± 9	-22 ± 9	200 ± 31	231 ± 34	76 ± 11	7.1 ± 0.6
4	416	70 ± 1	- 1 ± 10	312 ± 32	381 ± 34	92 ± 8	5.2 ± 0.2
5	366	59 ± 14	- 6 ± 16	234 ± 38	287 ± 44	78 ± 12	7.2 ± 0.5
6	392	52 ± 4	28 ± 9	255 ± 46	335 ± 47	85 ± 12	6.2 ± 0.6
7	416	51 ± 7	51 <sup>a</sup>	78 ± 30	180 ± 31	43 ± 7	6.4 ± 0.4
8	595	94 ± 1	89 ± 21	61 ± 29	244 ± 36	41 ± 6	4.7 ± 0.1

<sup>a</sup> Data from one single reactor.

reactor pH (experiments 1, 2, 4 and 8) was lower for higher loading rates, and total VFA concentration increased concomitantly. This accumulation of the VFA and lower pH values can have a negative influence on bacterial activity. Figure 2 shows the removal rates ( $\text{g glucose l}^{-1} \text{d}^{-1}$ ) for the different reactors. Inhibition could be observed at a feeding rate of 3.5 and  $5 \text{ g l}^{-1} \text{d}^{-1}$ , with concomitant accumulation of volatile fatty acids. In the latter conditions of overloading, transfer of electrons to the electrode decreased to about 15% conversion.

## Discussion

During the initial repetitive transfers of an anaerobic microbial culture from an anode compartment to a new compartment, the conversion of the carbon source towards electricity increased. Since oxygen was absent in the medium, and no methane formation was observed, this could be attributed to electron transfer by the bacteria to the electrode. The resulting culture maintained an efficient glucose-electricity conversion rate up to a volumetric loading rate  $B_V$  of  $3 \text{ g l}^{-1} \text{d}^{-1}$  (Figure 2). The influence of the pH on the conversion appeared to be of no significant importance in the obtained range (Table 2). Decrease of the bacterial external electron transfer was observed for glucose loading rates of  $3.5 \text{ g l}^{-1} \text{d}^{-1}$  and higher. This transfer deficiency generally resulted in an increased bacterial growth, higher total VFA concentrations and a lower pH (Table 2). The results indicate that the restriction caused the bacteria to accumulate VFA, mainly butyric acid, up to  $1905 \pm 414 \text{ mg l}^{-1}$  for the  $5 \text{ g l}^{-1} \text{d}^{-1}$  loaded reactors. The latter suggests a shift of the metabolism of the bacteria from outwardly oxidative (production of  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{H}^+$  and electricity) to fermentative electron flow (fermentation to volatile fatty acids).

In a biofuel cell, bacteria drain off their electrons to the anode. The better the cell functions, the lower the biomass yield generally is. The low biomass changes observed (Table 2) are representative of anaerobic conversion (Vandevivere & Verstraete 2001). However, no clear relationship between cell performance and biomass generation could be established in this experimental setup.

The current bottleneck for application of these systems is apparently not the conversion efficiency, but the conversion rates. In this work, an optimum of  $3 \text{ g COD l}^{-1} \text{d}^{-1}$  was obtained. Such a rate is low compared to other reactor systems implemented for current

green energy applications such as anaerobic digestion, where feeding rates in the range of  $5$  to  $25 \text{ g l}^{-1} \text{d}^{-1}$  are possible (Vandevivere & Verstraete 2001). This paper indicates that rates can be improved by selecting for adapted anodophilic microbial consortia, but significant technical improvements are needed to make biofuel cells perform at competitive industrial rates.

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