Enzymatic Oxidation of C1 Compounds in a Biochemical Fuel Cell

P L YUE and K LOWTHER
School of Chemical Engineering, University of Bath, Claverton Down, Bath BA2 7AY (U K)
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ABSTRACT

The operation of a direct biochemical fuel cell was demonstrated with sequential oxidation reactions along the methylotrophic pathway. The reactions were promoted by methanol and formate dehydrogenases. Electron transfer was facilitated by phenazine metho- or ethosulphate. The study investigates the use of carbon cloth as a high surface area electrode material. Enzymes immobilized on the electrode by physical adsorption performed better than when left in free solution. A maximum current efficiency of 36% was obtained, but stable operation could not be maintained. While both methanol and formaldehyde were suitable substrates, formic acid yielded only a very small current because the enzymes were effective at different pH conditions.

1 INTRODUCTION

It is well known that the conversion of chemical energy into electrical energy via a thermal or combustion process is inherently inefficient. The overall energy conversion is subject to the limitations of the Carnot cycle resulting in efficiencies much less than 50%. Fuel cells are theoretically much more efficient devices because they are not restricted by the Carnot cycle. A fuel cell functions by the oxidation of a suitable fuel at an anode with simultaneous transfer of electrons via an external circuit and protons to a complementary reduction reaction at a cathode. The power output of the cell is a function of the number and rate of transfer of electrons and the potential difference between the electrodes. In theory, energy conversion efficiency of well over 50% can be achieved.

In 1839, Grove [1] described the first fuel cell in which electricity was generated by supplying hydrogen and oxygen to two separate electrodes immersed in sulphuric acid. Since then many other fuels have been proposed for use in fuel cells, but they are either relatively expensive or may require operation of the cell at relatively high temperatures to achieve any worthwhile results. At present, the hydrogen–oxygen cell remains as the only fuel cell that is commercially attractive [2, 3]. The key to the utilization of other economically realistic fuels lies in more research on innovative electrocatalysis.

Few options presently exist which can exploit conventional electrocatalysis at mild physical and chemical conditions. The rapid development of space exploration in the last 25 years has suddenly spearheaded a vigorous research programme on fuel cell technology. The advantage of biological systems over the conventional chemical systems was recognized. It is conceptually feasible to develop a biochemical fuel cell (biofuel cell) which can operate under very mild conditions. A very wide range of organic and inorganic substances such as hydrocarbons, alcohols, carbohydrates, proteins, ammonia (in fact, any material which is capable of oxidation by organisms) can serve as fuels. The conversion of waste materials into electrical energy is also theoretically possible and, of course, a very attractive option. Recently Aston and Turner [4] have presented a comprehensive review on the development of biofuel cells.

The operation of a biofuel cell relies on the principles of bioelectrocatalysis, making use of materials derived from biological systems as catalysts for reactions occurring in anodic/cathodic compartments of the cell. Efficient transfer of electrons released from the oxidation/reduction reactions to the electrodes is crucial to the performance of a fuel cell.
Biological systems possess the essential quality of controlled electron transfer Potter [5] in 1911 had already demonstrated that microbial cultures could develop an electrode potential. However, the biofuel cells attempted in the 1960s were mostly of the "indirect" type. Bioelectrocatalysis was effected first with micro-organisms and later with enzymes within the anodic half-cell. The indirect mode of operation required firstly the catalytic production of electroactive substances which were then used to fuel conventional chemical fuel cells. The indirect fuel cell has the advantage of being able to function on very crude mixtures of feed, including waste materials, and the benefit of partly utilising known fuel cell technology. Unfortunately, all indirect fuel cells, perhaps with the exception of that developed much more recently by Karube et al., in 1981 [6], gave very disappointing results.

The 1970s witnessed a shift to research on "direct" biofuel cells, much of this due to the rapid advance of the science and technology of enzymes. This type of biofuel cell functions by direct transfer of electrons between organism or enzyme and the electrode, thus providing a truly biocatalytic system independent of conventional electrochemical processes. Several attempts have been made to develop direct biofuel cells [7-10] which can exploit the potential of enzymatic oxidation reactions.

The key enzymes involved in the bioelectrochemical reactions are oxidoreductases which in theory are ideal for effective electron transfer required for oxidation/reduction reactions accompanying fuel cell operations. In practice, fast shuttling of electrons to the electrodes is far from easy to achieve. Some of the problems of electron transfer have been discussed by Hill and Huggins [11]. The principle of using a solution mediator of low molecular weight to facilitate electron transfer is well known. Plotkin et al. [8] and more recently Davis et al. [10] have examined the application of a number of mediators used in conjunction with alcohol dehydrogenases. A variety of possibilities exist for enhancing electron transfer from a redox protein to an electrode. These have been elegantly presented by Aston and Turner [4] who have also discussed the problems associated with the use of mediators. Much more research is required to study the design of electrodes, the interaction of enzyme with the electrode and the practicality of mediated systems if the potential of biofuel cells is to be realised.

The biochemist and the biochemical engineer are presented with the challenge to exploit the ever increasing availability of enzymes and enzyme immobilisation techniques to overcome some of the problems encountered in the research on biofuel cells. This paper presents an experimental study of a biofuel cell based on enzymatic oxidation reactions along the methylotrophic pathway. A carbon fabric with high surface area was chosen as the material for the electrodes. It was intended to test the potential benefits of this fabric in promoting electron transfer. Possible advantages of immobilising the enzymes on the electrode by physical adsorption were examined.

2 A METHANOL FUEL CELL

Methanol is reckoned by the chemical industry as the fuel of the future. It is non-explosive, easily transportable and produced in large quantities at economically competitive prices. Its production is expected to double within the next decade. Methanol is superior to many alternative hydrocarbon substrates as a fuel source. It is completely soluble in water and thus, unlike other gaseous or liquid hydrocarbons, additional mass transfer resistances are avoided. It can readily be derived from coal, natural gas or renewable resources and can be obtained in very pure form, thus avoiding toxicological problems.

The oxidation of methanol follows the methylotrophic pathway sequentially to form formaldehyde, formate and carbon dioxide. Each oxidation step releases two electrons, yielding a total of six electrons per molecule of methanol oxidised to carbon dioxide. The process of substrate consumption is clean and free from the pollution problems which normally accompany many conventional chemical oxidation processes.

In the present study, bioelectrocatalysis was achieved by utilising the catalytic activity of enzymes, rather than whole living microorganisms. Earlier investigations on biofuel
cells have employed both photosynthetic and non-photosynthetic micro-organisms, e.g. refs 12 and 13. More recent work has moved towards the use of enzymes as crude microbial extracts become more readily available at lowering costs. In the case of methanol fuel cells, it is probably easier to effect electron transfer using isolated enzymes than it is using micro-organisms, moreover, fuel is not required for growth. Methanol, formaldehyde, and formate dehydrogenases are commercially available. Methods have also been established for the preparation of crude or pure extracts of some of these enzymes.

The reaction paths for the oxidation of methanol to carbon dioxide are schematically illustrated in Fig. 1. Here methanol dehydrogenase (MDH) is shown to be responsible for the oxidation first to formaldehyde and then to formate. Formate dehydrogenase is required for the last reaction step of carbon dioxide evolution. Of course, formaldehyde dehydrogenase can be used for the oxidation of formaldehyde. The figure also illustrates the assistance of a mediator, phenazine methosulphate (PMS) in the shuttle of electrons. MDH oxidises methanol and reduces the mediator, the latter is then reoxidised at the anode, transferring the corresponding number of electrons. The electrons then flow through the external circuit to the cathode where oxygen is reduced. The circuit is completed by the diffusion of protons, derived from the enzymatic reactions, across the cation exchange membrane to the cathodic half-cell to react with the hydroxyl group to form water.

The dehydrogenases are external mono-oxygenases, which normally require a reduced cofactor (DH₃) such as NADH₂, which combines with the otherwise inactive apo-enzyme to give an active complex which [14] catalyses the reaction

\[ S + O_2 + DH_2 \rightarrow S-O + H_2O + D \]

Unfortunately, the high cost and instability of the cofactor make the process less attractive, unless the reducing agents can be regenerated. Regeneration can be achieved cyclically by coupling the reaction to an enzymatic reaction which requires an oxidised cofactor. Formate dehydrogenase may be suitable for this purpose and stable immobilised preparations of this enzyme have been obtained. The coupling of reaction and regeneration is represented as

![Diagram of enzymatic oxidation of methanol to carbon dioxide in a biofuel cell](image-url)
An alternative solution to the problem of the inherent instability of the reduced co-factor is to reduce the prosthetic groups of the enzyme directly, using electrochemical techniques. This approach requires systems with rapid and efficient electron transfer between enzyme active sites and electrodes. A protein can be reduced directly at the electrode, but often the reduction is irreversible. Electron transfer can be hindered by peripheral protein structures. However, by using a low molecular weight intermediate electron mediator in solution, electron transfer can be facilitated.

In the methanol biofuel cell developed by Plotkin et al. [8], a bacterial methanol dehydrogenase was used. No cofactor was required and phenazine metho- or phenazine ethosulphate acted as the electron mediator. Both anode and cathode were platinum gauzes rolled into cylinders. The results suggest that further developments of the cell such as immobilisation of the enzyme and improvements in the electrode design should facilitate larger and more stable current outputs.

3 BIOFUEL CELL EFFICIENCY

The efficiency of the fuel cell may be defined as follows [15]:

\[
\text{Efficiency} = \frac{\Delta G}{\Delta H} = 1 - T \frac{\Delta S}{\Delta H} \tag{1}
\]

where \(\Delta G\) is the net change of free energy as the reaction proceeds from reactants to products, \(\Delta H\) and \(\Delta S\) are the changes in enthalpy and entropy respectively and \(T\) is absolute temperature.

For many systems, the \(T \Delta S\) term amounts to no more than 10% of \(\Delta H\), therefore making it possible to have a high efficiency of 80% - 90%. The net release of free energy is dictated solely by the reaction

\[
-\Delta G = nFE = W \tag{2}
\]

where \(F\) is the Faraday constant, \(n\) the number of electrons transferred per mole of substrate reacted, \(E\) is the net reversible potential difference of the cell, \(e\) the open-circuit voltage, and \(W\) is the maximum work available. Thus the theoretical efficiency is only achievable under reversible conditions in the cell. When the cell is driving current through an external load, not all the potential is available to do work.

Another useful measure is the efficiency with which the enzyme transduces the chemical energy of the substrate into current. Maximum current \(I_{\text{max}}\) is obtained when all the enzyme activity is realised as current

\[
I_{\text{max}} = neN_{\text{A}}A \tag{3}
\]

where \(e\) is the charge of an electron, \(N_{\text{A}}\) the Avogadro number, and \(A\) is the specific activity of enzyme in (mol product) (mg enzyme)\(^{-1}\) s\(^{-1}\). The fuel cell current efficiency \(e\) is then the percentage of enzyme activity realised as current

\[
e = \frac{I_{\text{observed}}}{I_{\text{max}}} \times 100\% \tag{4}
\]

The so-called overpotential of the cell, \(e\) the unattainable potential difference, is dependent on the kinetics of the system. There are three types of overpotential: (a) activation, (b) concentration and (c) ohmic. Activation overpotential occurs when reversible conditions are not maintained as a current is drawn from the cell.
intermediate steps in the chemical reaction or electron transfer sequence at the electrodes is then rate limiting. The overpotential may be reduced by using a more efficient enzyme which is capable of lowering the energy of activation and establishing equilibrium more rapidly. Access of electrons to the electrode may be made easier by increasing the effective area of the electrode. Concentration overpotential is caused by slow mass transfer of substrates, mediators or products to or from the electrode. Diffusional resistance may be minimised either by mechanical agitation or by bubbling an inert gas through the solution in the cell. Ohmic overpotential arises from the resistance of the bulk electrolyte, thus causing slow transport of the ionic species. Additional ohmic overpotential may also occur in the vicinity of each electrode because of changes in electrolyte concentration due to ion formation or discharge.

The electrode was washed with 0.1 M NaOH, followed by 0.01 N hydrochloric acid and distilled water. The enzyme was adsorbed on the carbon cloth electrode by soaking a piece of cloth of known weight in a solution containing predetermined amounts of the appropriate enzymes. Typically 10 ml of buffered or unbuffered solution containing 10 mg of methanol dehydrogenase was used and the cloth incubated overnight at 0 °C. Bovine serum albumin, a protein standard, was used to evaluate the adsorbance of enzyme on the electrode. After the protein was adsorbed on a piece of carbon cloth under the same conditions as those used for immobilising the dehydrogenases, the protein was removed from the cloth by elution using distilled water and NaOH solution. The eluted protein concentration was measured by the method of Warburg and Christian [16]. The method involved measuring the optical density of an appropriately diluted protein at both 260 μm and 280 μm using a spectrophotometer.

The enzyme activities were determined by measuring the oxygen depletion with a dissolved oxygen meter at 30 °C. A chart recorder was used to record the dissolved oxygen concentration. 1 ml of the appropriate buffer solution that was 2.5 mM with respect to the substrate was placed in the oxygen cell and stirred by aeration until saturated with oxygen. Then 10 μl of enzyme solution was added and the oxygen depletion measured.

Experiments were performed to study the operation of the fuel cell using methanol or formaldehyde or formic acid as substrate. In the case of formaldehyde, which is notoriously difficult to handle, refrigerated samples and micropropette tips were used to minimise losses. However, the actual concentration of formaldehyde was not determined. The enzymes used were methanol and formate dehydrogenases, supplied by Sigma Chemical Company Ltd. The enzymes were either in free solution or immobilised by adsorption on the electrode. The effects of gassing rate and pH on cell performance were examined. The conditions of each series of experiments are listed in Table 1. The operating cell voltage and current output were measured by a digital multimeter and recorded on a chart recorder. The refer-
ence external load was 10 Ω for all measurements.

5 RESULTS AND DISCUSSION

The activities of the enzymes, methanol and formate dehydrogenases (MDH and FDH), expressed in terms of oxygen uptake, are summarized in Table 2. Phenazine methosulphate and ethosulphate are clearly more effective mediators than tetrazolium. The PMS and PES molecules are more mobile and should have easier access to the active sites on the enzymes. Although PMS is less stable than PES, it was chosen for the present experiments because of its slightly higher activity with both dehydrogenases.

The enzyme MDH could use either methanol or formaldehyde as substrate with PMS or PES as the mediator. Enzyme activity with the former substrate appeared to be slightly higher. It should however be noted that possible losses of formaldehyde had not been accounted for. The formate dehydrogenase FDH was active with formic acid as the substrate, but again, only when PMS or PES was the mediator. The value of activity for MDH with methanol as the substrate and PMS as the mediator was 0.32 μmol min⁻¹ mg⁻¹. Plotkin et al. [8] obtained a value of 0.40 μmol min⁻¹ mg⁻¹ under similar conditions with PES as the mediator.

Results on protein adsorption capacity showed that the carbon fabric used retained 70 mg of protein (bovine serum albumin per gram of cloth). The manufacturer’s estimate was of the order of 100 mg (g)⁻¹. The pretreatment of the cloth was thought to be severe enough to prepare the surface for satisfactory adsorption. It is quite possible that other pretreatment methods may be employed to raise the adsorption capacity further. However, optimisation studies were not considered to be necessary at present, as long as sufficient adsorption of enzymes was achieved. According to the adsorbance capacity obtained here, it was therefore assumed that the physical adsorption method used was able to immobilise all the dehydrogenases introduced. This assumption may well be invalid, but would give the most conservative estimates of the current efficiency.

The first series of experiments was designed to examine the feasibility of operating a fuel cell with carbon fabric electrodes using methanol as substrate. The enzymes, methanol and formate dehydrogenases (MDH

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TABLE 1
Experimental conditions

<table>
<thead>
<tr>
<th>Experimental series number</th>
<th>Substrate (0.02 mM)</th>
<th>Enzyme (5.6 mg MDH, 7.1 mg FDH)</th>
<th>Buffer (0.2 M HBO₃, 0.05 M NH₄Cl)</th>
<th>Mediator (2.08 mg)</th>
<th>Gas flow rate (ml s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>MDH, FDH in free soln</td>
<td>Yes</td>
<td>PMS</td>
<td>0.334</td>
</tr>
<tr>
<td>2a</td>
<td>Methanol</td>
<td>MDH, FDH adsorbed</td>
<td>Yes</td>
<td>PMS</td>
<td>0.167 - 0.583</td>
</tr>
<tr>
<td>2b</td>
<td>Methanol</td>
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<td>0.334</td>
</tr>
<tr>
<td>2c</td>
<td>Methanol</td>
<td>MDH, FDH adsorbed</td>
<td>No</td>
<td>PMS</td>
<td>0.334</td>
</tr>
<tr>
<td>3a</td>
<td>Formaldehyde</td>
<td>MDH, FDH adsorbed</td>
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<td>PMS</td>
<td>0.167 - 0.583</td>
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<tr>
<td>3b</td>
<td>Formaldehyde</td>
<td>MDH, FDH adsorbed</td>
<td>Yes</td>
<td>PMS</td>
<td>0.334</td>
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<tr>
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<td>Formaldehyde</td>
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<td>PMS</td>
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<tr>
<td>4a</td>
<td>Formic acid</td>
<td>FDH adsorbed</td>
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<td>PMS</td>
<td>0.334</td>
</tr>
<tr>
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<td>Formic acid</td>
<td>FDH adsorbed</td>
<td>No</td>
<td>PMS</td>
<td>0.334</td>
</tr>
</tbody>
</table>

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TABLE 2
Enzyme activities

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Mediator</th>
<th>Activity (O₂ uptake) (μM min⁻¹ mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDH</td>
<td>Methanol</td>
<td>PMS</td>
<td>0.32</td>
</tr>
<tr>
<td>MDH</td>
<td>Formaldehyde</td>
<td>PMS</td>
<td>0.29</td>
</tr>
<tr>
<td>MDH</td>
<td>Methanol</td>
<td>PES</td>
<td>0.28</td>
</tr>
<tr>
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<td>Formaldehyde</td>
<td>PES</td>
<td>0.25</td>
</tr>
<tr>
<td>MDH</td>
<td>Methanol</td>
<td>Tetrazolium</td>
<td>0.098</td>
</tr>
<tr>
<td>MDH</td>
<td>Formaldehyde</td>
<td>Tetrazolium</td>
<td>0.084</td>
</tr>
<tr>
<td>FDH</td>
<td>Formic acid</td>
<td>PMS</td>
<td>0.143</td>
</tr>
<tr>
<td>FDH</td>
<td>Formic acid</td>
<td>Tetrazolium</td>
<td>0.092</td>
</tr>
</tbody>
</table>
and FDH), were placed together in the anodic compartment and left in free solution. The gas flow rate in the anode half-cell was set at 0.334 ml s\(^{-1}\). No current was produced before the enzymes were introduced. An average current of 0.90 mA at a cell voltage of 65 mV was obtained from six sets of experiments. Results were reproducible to within a standard deviation of ±6%. When FDH was added to the cell, subsequent to the addition of MDH, the results showed a barely noticeable increase in the current output. It was concluded that the enzyme MDH was effective for methanol oxidation and that a biofuel cell could be operated based on enzyme catalysis by MDH using carbon cloth electrodes.

In the second series of experiments, the enzymes were immobilised on the carbon cloth anode by physical adsorption as described earlier. A buffer solution of pH 9.5 was used in the anode half-cell. The gas flow rate in the anode compartment was varied from 0.167 to 0.583 ml s\(^{-1}\) to study the effect of agitation. Results of the variation of current with gas flow rate are shown in Fig. 2. The cell current output rose by 18% when the gas flow rate doubled from 0.167 to 0.334 ml s\(^{-1}\). The effect of mixing levelled off at a further increase of gassing rate. The results showed that the solution in the anode compartment should be mixed sufficiently to minimise mass transfer effects on the performance of the fuel cell. At the gassing rate of 0.334 ml s\(^{-1}\), the average current obtained from six sets of experiments with the enzymes immobilised on the electrode was 138% higher than that when the enzymes were in free solution, thus proving the advantage of immobilisation.

The fuel cell was then operated at the fixed gassing rate of 0.334 ml s\(^{-1}\) over a period of 1 h. The time history of cell current output against a resistance of 10 Ω was recorded. Curve a in Fig. 3 shows that the current output was stable for about 20 min of operation and then dropped from 2.14 mA to 1.57 mA (i.e., a reduction of 26 7%) in 1 h. The effect of pH was studied using the same electrode with enzymes immobilised on the carbon cloth by adsorption. Distilled water at pH 7, rather than the buffer solution at pH 9.5, was introduced into the cell. Values of cell current output over 1 h are shown (as curve b) in Fig. 3. Although the initial current generated was nearly the same in both cases, the deterioration of current commenced earlier (after about 10 - 15 min) when no buffer was used. The current developed, levelled off to 0.67 mA (about a third of the initial value) after 60 min.

The next series of experiments used formaldehyde as the substrate. The enzymes MDH and FDH were adsorbed on the carbon cloth electrode by the same method. The mediator in the anode was PMS. The effects of gassing rate and pH were studied as in the previous set of experiments. The behaviour and performance of the fuel cell are very similar for both substrates. Again, a rapid rise in cell current output was observed when the flow rate was increased to about 0.334 ml s\(^{-1}\). Subsequent increase in flow rate brought the cell current to the asymptotic value of

![Fig 2 Effect of gas flow rate on current output for methanol oxidation](image)

![Fig 3 Variation of current output with time for methanol oxidation curve a, with buffer, curve b, without buffer](image)
about 2.2 mA. The results show that MDH is effective for the enzymatic oxidation of formaldehyde, thus suggesting that the oxidation reaction is not the rate limiting step. What governs the cell performance is probably the limiting rate of electron transfer, which is largely determined by the coupling of the mediator to the enzymes responsible for the redox reaction steps. There are, of course, electrochemical techniques available where the hydrodynamics can be controlled, e.g., the rotating disc method, thus allowing the rate-limiting step to be tested. Nevertheless, the present results confirm that mixing is essential in reducing mass transfer effects.

The variation in cell current output with time at the fixed flow rate of 0.334 ml s⁻¹ is shown in Fig. 4 for two values of pH (9.5 and 7). In general, the behaviour of the fuel cell was similar, though not identical, to that when methanol was used as the substrate. When no buffer was used, the current output declined more sharply, dropping rapidly to less than 0.06 mA in 20 min, thus confirming that the anodic pH should be higher than neutral for the enzymatic oxidation of methanol and formaldehyde by MDH.

The reverse was observed when formic acid was used as the substrate in the last set of experiments. The reaction was catalysed by FDH which was adsorbed on the carbon cloth anode. The current produced in this case was much smaller. Figure 5 shows the variation of current as a function of gas flow rate with and without the buffer solution. Without the buffer, the current output was about 0.06 mA, which was the level of cell current produced after an hour of operation with methanol or formaldehyde as substrate. When the buffer was used, the maximum current produced was only 0.03 mA. The results show that not only has FDH less than half the activity of MDH, as seen in Table 2, but also that in the present study the last oxidation step in the methylltroph pathway does not enhance the methanol fuel cell performance.

The inactivity of the methanol or formaldehyde fuel cell to maintain its maximum current output for a long period may be related to some localised denaturing of MDH adsorbed on the electrode. On the other hand, there is evidence that the instability problem with biofuel cells is related to the dealkylation of the mediator to its synthetic precursor [9]. However, in the present study further addition of mediator after the onset of decline in current did not produce any effect on the fuel cell output. Further research is required to study how these problems interact with the performance of the carbon cloth electrode.

The fuel cell efficiency, as defined in eqn (4) for the four series of experiments, is shown in Table 3. When the enzymes were in free solution, an efficiency of 16% was achieved, but about 36% efficiency was obtained for MDH-based reactions with the enzyme immobilised on the electrode. The FDH-catalysed reaction only yielded an efficiency of 1%.

Fig. 4 Variation of current output with time for formaldehyde oxidation: curve a, with buffer; curve b, without buffer.

Fig. 5 Effect of gas flow rate on current output for formic acid oxidation: curve a, with buffer; curve b, without buffer.
TABLE 3
Current efficiencies

<table>
<thead>
<tr>
<th>Experimental series number</th>
<th>Current efficiency ε (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.0</td>
</tr>
<tr>
<td>2</td>
<td>35.6</td>
</tr>
<tr>
<td>3</td>
<td>36.2</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

6 CONCLUSIONS

The present study has demonstrated the potential of using a carbon cloth as the electrode material in a biofuel cell based on the enzymatic oxidation of C_1 compounds. Oxidation reactions catalysed by methanol dehydrogenase can produce currents with up to 36% efficiency when the enzyme is immobilised on the electrode by physical adsorption. Other enzyme immobilisation techniques currently being studied may lead to further improvements on current efficiency. Adequate mixing of the solution in the fuel cell should be used to reduce mass transfer effects.

It is postulated that either localised denaturing of enzyme or the instability of mediator may be the cause of decline in the fuel cell output. Optimisation studies which couple the most efficient and stable mediator with the best technique of enzyme immobilisation are needed if the potential of the carbon cloth electrode is to be more fully exploited.

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