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Microbial Fuel-Cells

Electricity Production from Carbohydrates

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ABSTRACT

Microbial fuel cells containing *Proteus vulgaris* and oxidation-reduction ("redox") mediators were investigated. The bacteria were chemically immobilized onto the surface of graphite felt electrodes, which supported production of continuous electric current and could be reused after storage. A computer-controlled carbohydrate feed system enabled the cell to generate a constant output with improved efficiency compared to the performance obtained with single large additions of fuel. The response to additions of substrate when immobilized bacteria were used was faster than that achieved with freely suspended organisms. This is attributed to the advantageous mass-transfer kinetics resulting from the proximity of the immobilized bacteria and the electrode surface.

Index Entries: Microbial fuel cell; mediator; immobilization; computer control; bioanode.

INTRODUCTION

In parallel with a renewed interest in conventional fuel cells, the biofuel cell has recently attracted attention as a means of interfacing a biological energy source with conventional electrochemical technology (*1–5*). The "direct" microbial fuel cell (*see* Fig. 1) is particularly promising, since it can be used to extract electrons from oxidation of a naturally occurring fuel, such as a carbohydrate with high efficiency. The mode of action of

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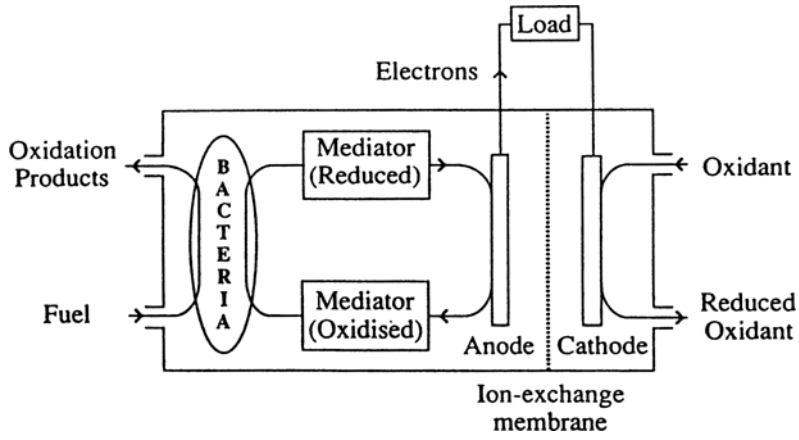


Fig. 1. Schematic diagram showing the principal features of a microbial fuel cell.

this device depends on the use of intact microorganisms as integral mini-reactors capable of performing a range of electrogenic enzymatic transformations. Inside the electrode compartment of the electrochemical cell, the organisms act as a source of electrons, initially trapped as reduced intermediates from the degradation of substrate, but available for transfer to the anode. This transfer is effected by a redox mediator that diverts electrons from the reduced intermediates and, via the anode, to the external circuit, which is completed by a suitable cathode. For the oxidation of glucose, the anode reaction is written effectively below:

$$C_6H_{12}O_6 + 6H_2O \longrightarrow 6CO_2 + 24e^- + 24H^+ \quad (1)$$

Mediator action results in a dramatic increase in the current and electrical yields obtainable (*6,7*). The facility for a substantial degree of (or even complete) oxidation is one of the important advantages that the present approach to biomass-energy conversion holds over other methods.

Studies using *Proteus vulgaris* as a catalyst and thionine as an electron-transfer mediator showed that, for limited quantities of fuel added as a single "feed," about 50% of the glucose substrate may be oxidatively converted to electricity (*8*). (This is a "real" figure in the context of Eq. 1 above, and it should be noted that the figure is not higher largely because the microbial catabolism is incomplete, rather than from any electrochemical considerations.) In the case of sucrose, coulombic yields approaching 100% of the theoretical maximum were obtained (*9*). The electronic charge obtained from the oxidation of the substrate was calculated by the integration of current vs time plots. The coulombic efficiency was defined as the percentage charge obtained compared to the theoretical charge obtainable from the complete oxidation of the substrate, according to Eq. 1. The potential for utilization of carbohydrate waste products has also been

demonstrated in *Escherichia coli* fuel cells using lactose and reconstituted milk whey (*10*), which ran continuously for periods up to 90 d with little apparent limitation by biological activity.

The present studies were carried out with several objectives in mind. First, using small-capacity cells as in previous work, the authors wished to investigate the potential for constructing integrated bioelectrodes in which the microorganisms were immobilized on the surface of (or within) the electrodes. This was seen as a desirable design modification that might improve the efficiency of the system, particularly in relation to the kinetics of mass transfer in the region of the anode surface. It was also of considerable interest to examine further the potential for regulating the electrical output and the efficiency of the cells through the application of microprocessor control (*11*). The efficiency of operation has great economic importance in view of the various potential applications (e.g., continuous running of small vehicles/boats without the necessity of recharging batteries, small power generators for remote locations not having main electricity supplies, on-site effluent treatment concomitant with power generation in sugar refineries, dairies, and so forth).

EXPERIMENTAL METHODS

The general features of the microbial fuel cell are shown in Fig. 1, and the cell is formally represented as shown below:

Anode compartment	*Cathode compartment*
Carbon electrode (−)	Carbon electrode (+)
Carbohydrate	Potassium ferricyanide
Bacteria	or
Mediator	Platinized carbon electrode
in pH 7.0 buffer	O_2 (air),
	in pH 7.0 buffer

Ion-exchange membrane

Electrodes

The anode material used was a graphite felt (RVG4000, Le Carbone). The electrodes measured 0.5×4.5×4.5 cm and weighed 1 g. This electrode material was chosen because of its low resistance (1.5 Ω/electrode) and its advantageous physical structure, which consists of an open network of interwoven fibers allowing free access of organisms and mediator to the electrode surface. The electrode structure provides a large surface area (0.47 m^2g^{-1}, by BET nitrogen adsorption) for a relatively small volume, enabling a high concentration of immobilized bacteria to be achieved. For short-term experiments, the cathode was of reticulated

vitrious carbon (RVC, Hitemp Materials, London), used in conjunction with potassium ferricyanide solution (50 m*M*). The ferricyanide becomes reduced to ferrocyanide, providing a relatively nonpolarizable electron sink for charge produced in the anode. However, when current is produced over an extended period of time, the ferricyanide concentration falls, leading to a change in the cathode potential as a result of the change in redox ratio. Consequently, in long-term experiments, an air cathode of platinized carbon paper (PCP, Prototech, MA, 4.5×4.5 cm, $<1\ \Omega$) was used in concentrated buffer to provide a more stable potential provided by the well-known oxygen electrode reaction in which O_2 is reduced to OH^-. (In principle, at pH 7, this should provide a cathode potential of 0.7–0.8 V; in practice, potentials lower than this are obtained, and there is also a limitation of cell voltage obtainable in this configuration, arising from a small degree of permeation of oxygen into the anode compartment. These factors were not important, however, for the purposes of these experiments.) Platinum wire contacts were attached to the electrodes with a conducting epoxy resin (Ablebond, 84-1LMI). To aid initial wetting, the electrodes were soaked in ethanol and then boiled in water to remove adsorbed ethanol. Electrodes were cleaned and stored in hydrochloric acid (0.1 *M*), and thoroughly rinsed in water before use.

Preparation of Bacteria

On the basis of previous work (*6,7*) *Proteus vulgaris* (NCTC 10020) was the preferred biocatalyst. It has an extremely small endogenous store of carbohydrate, so that the current response arising from utilization of exogenous (i.e., added) carbohydrate "fuel" is easily separated from the endogenous contribution. The organisms, obtained from the culture collection of King's College London (Microbiology Department), were maintained in nutrient broth (lab-m, No. 2). They were cultured aerobically in nutrient broth with no extra glucose at 37°C for 18 h on a rotary shaker (200 rpm), washed, and resuspended in potassium phosphate buffer (50 m*M*, pH 7). The dry wt was estimated photometrically, and 10 μmol of glucose for every 20 mg of dry wt were added to the stock suspension for use in fuel cells. From previous work (*7,10*) it is established that, under the conditions of operation of the fuel cells, the viability of bacterial cells (and more importantly, their biological reducing activity) falls only slowly, and any consequent losses are likely to be of minor importance for periods of operation used in the present study.

Preparation of Immobilized Bacterial Electrodes

Bacteria were immobilized onto the electrode surface by the formation of an amide link between oxidized carbon atoms on the electrode surface and amino groups in the bacterial cell wall. The amide link was created using a well-known carbodiimide technique (*12*). Felt electrodes were

activated by soaking in 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluene sulfonate solution (Aldrich, Gillingham, UK, 0.1 *M* in 0.1 *M* sodium acetate buffer, pH 4.5) with constant agitation at 4°C for 5 h. They were rinsed in water and potassium phosphate buffer (50 m*M*, pH 7), and were then exposed to a suspension of *Proteus vulgaris* (40 mL, 20 mg dry wt, 50 m*M* phosphate, pH 7) with constant agitation at 4°C for 12 h. Excess *Proteus vulgaris* was then washed off with cold buffer, and the electrodes were used immediately. Bacteria were also grown onto the felt surface by immersing an electrode in nutrient broth that was innoculated with *Proteus vulgaris* and incubated for 24 h at 37°C. The amount of bacteria immobilized was similar to that obtained by the covalent bonding method.

After a fuel-cell experiment, the immobilized bioanodes were removed from the cell compartment, washed in potassium phosphate buffer (200 mL, 50 m*M*, pH 7), and then stored at 4°C in phosphate buffer (100 mL, 50 m*M*, pH 7), with the addition of glucose (100 μmol).

Bacterial Content of Immobilized Electrodes

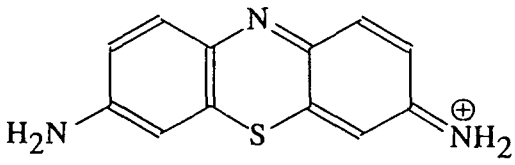
The amount of bacteria immobilized in the felt was determined by a modified Lowry method (*13*). The felt was soaked in alkaline solution (NaOH, 2 *M*) to digest the bacteria, and the protein content of the resultant liquor was determined by comparison with that of a "free" bacterial suspension of known concentration. It was assumed that the size of a bacterium was 1×3 μm and the dry mass such that 1 g contained 5×10^{12} organisms. The results were consistent with an immobilization process that produced a firmly fixed monolayer of bacteria on the electrode surface. A small amount of loosely trapped bacteria could be removed by breaking up the electrode and washing it further.

Mediators

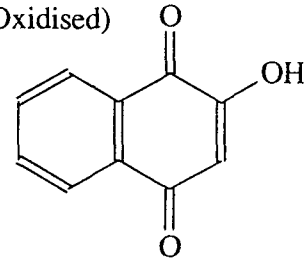
An ideal mediator would form a reversible redox couple at the electrode with a highly negative E^0 value, in order to maximize the open-circuit cell voltage. It would also be stable in both the oxidized and reduced forms, and would not decompose during long-term redox cycling. The polarity of the mediator should be such that it is soluble in aqueous systems (near pH 7) and is able to pass through the bacterial cell membrane reversibly. In order to maximize the coulombic efficiency of the cell, the mediator should accept electrons from as far up the electron transport chain as possible or from more than one point along its length. Previous work on mediator/organism combinations (*7*) showed that mediators with the most negative redox potentials tend to be unstable and/or are not completely reduced by the bacteria. Thionine is reduced rapidly and gives a relatively high cell voltage and coulombic efficiency (40% for glucose), but it is not very soluble or stable. It also becomes adsorbed onto the electrode surface, reducing the long-term performance of the cell. In

view of these disadvantages, the mediator preferred for fuel-cell runs longer than 24 h was 2-hydroxy-1,4-naphthoquinone (HNQ), which is soluble, stable, and much less prone to adsorption than thionine. Whereas thionine is known to interact at many positions in the electron transport chain, accounting in some way for the relatively high coulombic efficiencies achieved (*7*), HNQ, which is reduced more slowly than thionine, gives a lower coulombic efficiency (20% for glucose). This appears to be a result of more limited access of this mediator to points on the electron transport chain. It is of interest to note that, in the absence of cellular enzymes, HNQ is not reduced by free NADH (*14*). The molecular structures of these mediators are shown below:

Thionine (Oxidised)



HNQ (Oxidised)



Fuel-Cell Assembly

Experiments were carried out in stacks of "Perspex" (methyl methacrylate) cells built up from molecular units of simple rectangular design (*1,7*). Each cell unit had anode and cathode compartments (internal dimensions 58×52×6 mm) separated by a central divider (*see* Fig. 2). The separating membrane (BDH, cationic-exchange membrane) was held between the cell units by silicone rubber gaskets (1.5 mm, ESCO). The stack was held in a frame that was lightly bolted together, applying enough pressure to seal the cells. Each cell compartment had inlets at the top for the addition of solutions and at the bottom for the passage of nitrogen (BOC, O_2 free). The stack temperature was maintained at 30.0 (±0.1)°C by immersion in a water bath.

Procedure for Discharge of Cells

Cells were initially set up containing only potassium phosphate buffer (50 m*M*, pH 7) in the anode compartment. In making up the anode buffer, allowance was made for water remaining in the electrode after the initial wetting procedure. The cathode was set up with either potassium ferricyanide (50 m*M*, in pH 7 buffer) for the RVC electrodes or with potassium phosphate buffer (500 m*M*, pH 7) for the PCP electrodes. The voltages of the cells were measured simultaneously with a voltmeter (Solartron Datastore, 7066) linked to a multichannel scanner (Solartron Minate, 7010) and recorded digitally on a computer (Research Machines,

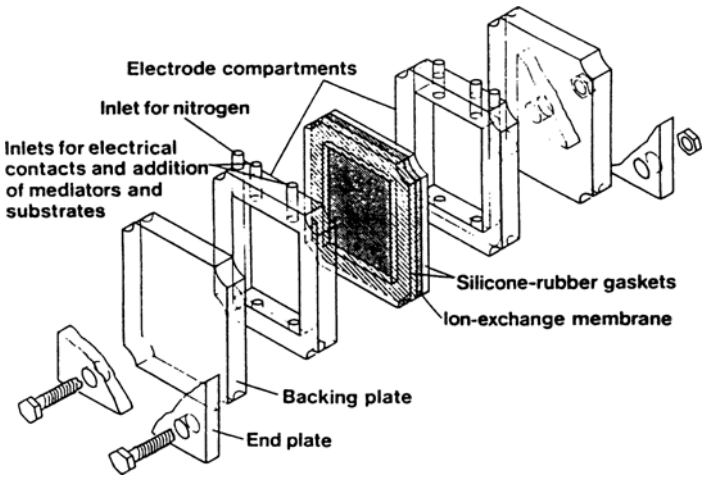


Fig. 2. Expanded view of the bio-fuel cell.

380Z). The measuring system also allowed the continuous calculation of coulombic outputs. After adjusting flow of nitrogen to the anode to give gentle agitation, the open-circuit cell voltages were allowed to stabilize. Bacteria (typically 30–50 mg, prefed with glucose) were added to the anode compartment from a stock suspension. When the voltage had stabilized, mediator solution (50 m*M*, HNQ) was added to give the required final concentration. When the voltages had reached their plateau values following the bacterial reduction of the mediator, the cells were connected to their load resistances (R_{load}), and the resultant current (I) was measured as $I = V/R_{load}$, where V is the voltage drop across R_{load}.

Cell Polarization

To test the current-producing capabilities of the cells, they were allowed to discharge through a range of different known resistances (10 kΩ–1Ω) for 60 s. The cell voltage was allowed to recover to a plateau value between each discharge. The voltage and current were measured instantaneously and after 60 s. A plot of instantaneous current against voltage drop on closing the circuit had a linear slope that gave an estimate of the internal resistance of the cell. The internal resistance was also measured using a conductance bridge (Wayne Kerr, B221) that gave roughly comparable values. A plot of current against voltage at 60 s gave a measure of the concentration polarization taking place, and for small resistances, the mass-transport limited current (50 mA with 10 m*M* HNQ in the 20-mL cell).

Computer Control of Fuel Cells

The arrangement for the microcomputer control of the small-volume system has been previously documented (*11*). Voltages across R_{load} was measured by the analog-to-digital converter of a microcomputer (BBC,

Model B) and transferred to digital form. The computer was interfaced through the I/O user-port to the remote control of a pump (Pharmacia, P-1), and information could be transferred using BASIC and Assembly language to read from the A/D converter and to write to the interface. A supply of carbohydrate (glucose, 50 m*M* in pH 7 buffer) was injected from a reservoir into the anode compartment in response to changes in the electrical output signals, so that under suitable conditions, the power could be sustained at a constant level through regeneration or maintained according to some other prescribed mode. The reactor was programmed to operate either as a constant current/voltage source that could be optimized for power generation or as a device for maximizing the (coulombic) efficiency of oxidation of the substrate. The precalibrated rate of addition of fuel could be controlled by the length of time the pump was switched on. The self-adjusting mode of operation was achieved by feeding the cell output voltage signal to the computer, which, according to some programmable input instructions, turned the pump on or off. Net coulombic yields were used to compute the coulombic efficiency, which could also be used as a criterion for pump operation.

RESULTS

Comparative Studies of Fuel-Cell Anodes Containing Immobilized and Free Bacteria

A fuel-cell stack containing four cells was set up. In two of these, the anodes contained immobilized bacteria, whereas the other two had anodes that contained the same dry mass of freely suspended bacteria. The cathodes were of RVC in potassium ferricyanide solution (50 m*M*). One cell of each type was fed with glucose, and one was not to act as a "blank." Both types of cell gave similar current vs time profiles on initial discharge through a load resistance of 1000 Ω. The unfed free bacteria cell produced more charge than its immobilized equivalent. This is attributed to the unavoidable exposure of the "immobilized" electrode to oxygen during its preparation, with consequent oxidation of endogenous reducing equivalents stored in the bacteria. The stack was stored at 20°C for 10 d on open circuit and then discharged to the background current level before adding glucose (20 μmol) to each cell under load. The cell currents rose, reached a plateau, and then decayed back to the background value (Fig. 3). Glucose added to the bacteria is assimilated in seconds and immediately begins to reduce mediator. As the ratio of reduced-to-oxidized mediator increases, the cell voltage increases in accordance with the Nernst equation. As the cell is discharged through a load resistance, the reduced mediator becomes oxidized at the electrode surface. Eventually, as the concentration of oxidized mediator drops, the rates of bacterial reduction and reoxidation at the electrode become equal, and the current stabilizes

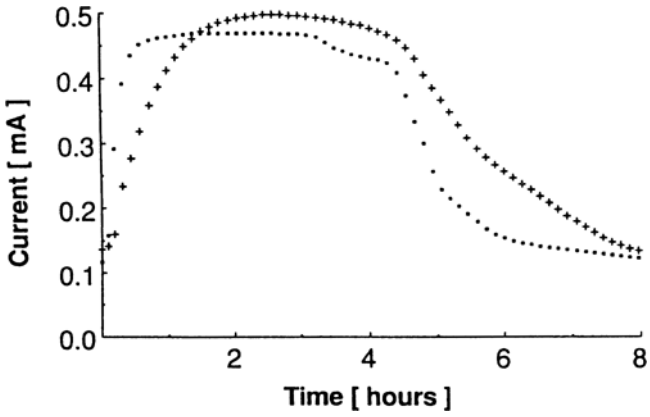


Fig. 3. Current production by fuel cells containing "free" (■) and immobilized (+) *Proteus vulgaris* (45 mg) after storage at 20°C for 10 d. At time = 0 h, glucose (20 μmol) was added. The load resistance was 1000 Ω.

at the plateau value. When the bacteria run out of charge from the glucose, the reduction rate drops, and the current decays.

The current produced by the "free" bacterial cell rose more rapidly than the "immobilized" cell, reaching the plateau value earlier. However, the plateau for the "immobilized" cell was 7% higher than that of the "free" case. These results can be interpreted by considering the location of the bacteria relative to the electrode surface in the two different configurations. The reduction of mediator by immobilized bacteria can occur only at the electrode surface, whereas in the case of freely suspended organisms, it may occur throughout the anode electrolyte. On addition to glucose to bioanodes containing immobilized bacteria, mediator must travel to the electrode surface before it can be reduced. Because of the constraints of mass transport, immobilized bacteria take longer to reduce all the mediator in the anode than an equivalent amount of freely suspended bacteria. However, once the plateau is reached and the mediator is predominantly in the reduced form, under mass-transport limited conditions, the average distance that the mediator must travel in order to give up its electrons is shorter for the immobilized organisms, and the current yield per organism is consequently larger for immobilized bacteria than for those in suspension. The coulombic efficiencies of the immobilized and "free" electrode systems were the same, however, at 15% for the 20 μmol of glucose added for the examples shown in Fig. 3.

In a further example of the characteristic behavior of the fuel cells over extended periods, two similar fuel cells were set up with *Proteus vulgaris* (45 mg, fed with glucose) in potassium phosphate buffer (40 mL, 50 m*M*, pH 7). One cell contained "free" bacteria, and the other immobilized bacteria. An air cathode in concentrated potassium phosphate

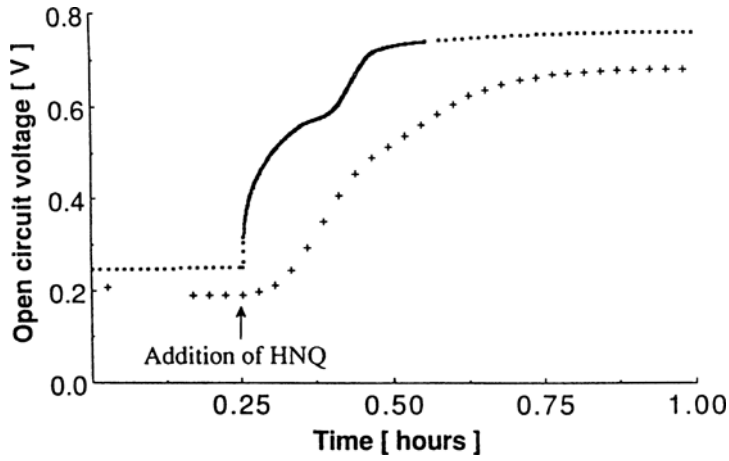


Fig. 4. Increase in open-circuit fuel-cell voltage owing to bacterial reduction of mediator (HNQ) by "free" (■) and immobilized (+) *Proteus vulgaris* (45 mg). At time = 0.25 h, sufficient oxidized HNQ was added to make the anode concentration 0.5 m*M*.

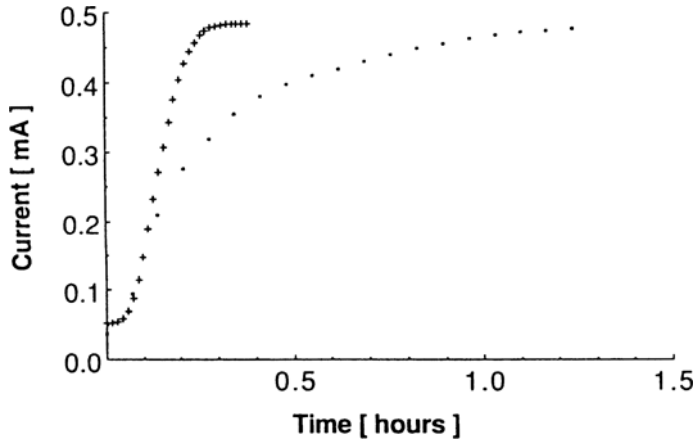


Fig. 5. Current production by fuel cells containing "free" (■) and immobilized (+) *Proteus vulgaris* (45 mg). The cells had previously been discharged and contained oxidized HNQ (0.5 m*M*) when glucose was added at $t = 0$ h. The load resistance was 1000 Ω.

buffer (0.5*M*, pH 7) provided a relatively stable cathode potential. When HNQ was added to give an anode concentration of 0.5 m*M*, the open-circuit voltages of both cells rose to plateau values of ca. 0.7 V (Fig. 4). The cells were then discharged through 1000 Ω until constant background currents were obtained. With the load resistance still applied, glucose (5 μmol) was added, causing the currents to rise to ca. 0.5 mA (Fig. 5). The response of immobilized cells to the addition of HNQ and the resultant rise in voltage occurred more slowly than for the "free" cells. However,

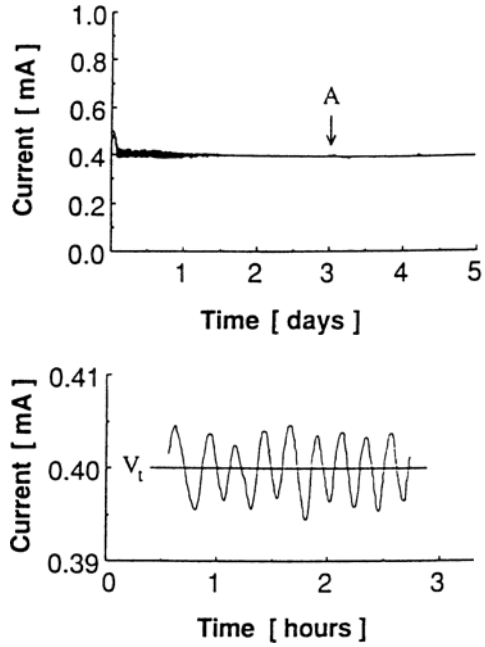


Fig. 6. Current–time traces for a computer-controlled fuel cell containing *Proteus vulgaris* (435 mg dry wt) immobilized in graphite felt, with HNQ as a mediator. The upper trace shows the stable electrical output over 5 d. The lower trace shows the fine detail of the current output at point 'A''. Glucose was automatically added when the current was <0.4 mA.

on addition of glucose after discharging the cells, the ''immobilizied'' cell reached its plateau current first. This difference may be explained by the argument that, when HNQ is first added, approach of HNQ to bacteria immobilized at the electrode surface is hindered by comparison with the approach to freely suspended bacteria. However, once the HNQ is at the surface, the transport distance required for mediator cycling becomes less than before, and consequently, the current rises more rapidly.

Computer Control of Fuel Cells Containing "Free" and Immobilized Bacteria

The performance of fuel cells containing immobilized and ''free'' bacteria under computer control (*11*) was compared. By adding a known quantity of glucose to the cells when the voltage dropped below a threshold value (V_t), the voltage and current could be maintained for periods of at least a week. Typical data for immobilized bacterial electrodes are shown in Fig. 6 (upper trace), where V_t had been set to 0.4 V. The load resistance was 1000 Ω, and a current of 0.4 mA was sustained. The cathode did not consume all of the acid produced in the anode reaction, and consequently, the pH dropped slowly, causing the cathode potential

to become more positive. In order to maintain a constant cell voltage, the computer feedback system automatically compensated for this by making the anode potential more positive, resulting in a decrease in the ratio of reduced-to-oxidized mediator. The pH was measured every 24 h, and the cathode buffer was replaced when it fell below pH 6 in order to protect the bacteria from overacidification. The "free" and "immobilized" systems both oxidized glucose with a coulombic efficiency of 33% for 48 h, which subsequently stabilized at around 25% after a week. These efficiencies are double those achieved by large, single additions of glucose, a difference that can be attributed to the fact that under computer control, the excess of glucose is much smaller than that resulting from a single addition. The presence of an excess of glucose is likely to activate metabolic pathways in the bacteria, which lead to incomplete oxidation of the glucose (*11*). It should be noted, however, that much higher yields are to be expected with mediators other than HNQ, which was selected primarily for its long-term stability and high solubility.

The typical fine structure of the computer-controlled cell voltage is shown in Fig. 6 (lower trace, corresponding to a short interval after 3 d as indicated by A on the upper trace). The voltage drops as the cell begins to run out of glucose, but when the voltage falls below V_t, the computer-controlled feed system adds a pulse of glucose (0.01 μmol) every 6 s, causing the current to increase. Glucose addition ceases when V_t is exceeded, and eventually, the voltage drops once more to produce the "saw tooth" variation in voltage shown in Fig. 5. The period of current oscillation was 22 min for the "free" bacteria, compared with 12 min for the immobilized bacteria, and was virtually independent of mediator concentration. The amplitude of current oscillation was 26 mV for the freely suspended organisms and 19 mV for the immobilized organisms with an HNQ concentration of 0.5 m*M*. At a higher HNQ concentration of 2 m*M*, the difference in amplitude between the two types of cells was greater ("free," 13 mV, immobilized, 1.4 mV). The faster response time of the "immobilized" bacterial electrode is attributed to the shorter mass-transport distance. These results were consistent with a simple simulation model of the computer-controlled system based on the finite difference method (*15*), which showed that when the bacterial reduction was concentrated at the electrode surface, the period of current oscillation was decreased.

CONCLUSIONS

Immobilization of the bacteria apparently improves the kinetics of mediator mass transport between the electrode and the bacteria to produce a stable, integral bioanode capable of generating a constant output

of electrical power over a period of at least several days. Another preliminary conclusion is that the functional lifetimes of the organisms are increased by immobilization, although further long-term studies are needed. In the computer-controlled experiments, it was confirmed that the relatively high coulombic yields, obtained previously with suspended organisms under substrate-limited conditions, are similarly achieved with immobilized organisms. The present results also indicate the scope for further future optimization of the bioanode. For example, improvements could be made in the redox mediation and the pH stability, whereas further development of anode materials will enable higher concentrations of organisms to be immobilized within the electrode structure. The role of reagents that facilitate permeation of bacterial cell membranes by mediators (*16*) also warrants further investigation. Such improvements and innovations are currently under investigation using cells of the type described in this article, whereas it will also be of interest in future work to examine possibilities for the utilization of alternative substrates of biotechnological interest, such as xylose or other pentoses. With the present cells, currents attainable are limited to much less than 1.0 A, mainly as a consequence of design factors and the electrode and internal resistances. Currents outputs extending into the ampere range are currently under investigation in a scaled-up cell of 4-L capacity, which has a much decreased internal resistance, and these results will later be presented elsewhere.

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