

# Electricity generation by microorganisms

H.P. Bennetto

Microbial electrochemistry provides many opportunities for promoting an interdisciplinary approach in science teaching, perhaps in an integrated science course. Simple experiments to demonstrate microbial generation of electricity are described which can be carried out with relatively unsophisticated equipment, and a guide to more advanced project work is presented.

## Introduction

The electrical activity of microorganisms is a fascinating and instructive area of science. Although much work remains to be done at the research level to elucidate the chemistry and biochemistry of such phenomena, recent studies of microbial fuel cells have greatly advanced our understanding of microbial electricity generation (Bennetto, 1984, 1987, 1990).

Demonstrations of microbial electricity devices never fail to capture the imagination of young audiences, and the intention of this paper is to provide a guide to some relatively simple experiments which can be performed in a college or high school laboratory. In some countries, notably the UK, recent policies in secondary education have failed to deter a traditional tendency for students specializing in biological science to veer away from chemistry and physics (and *vice-versa*); there remains a need to de-segregate these subjects. Experience has shown that experiments such as those described below not only stimulate the curiosity of students in biotechnology and its relation to basic sciences, but encourage a more general flexibility of scientific thinking in their interpretation.

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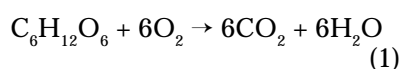
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In the account which follows, some over-simplification of the basic biochemical and microbiological principles is perhaps inevitable, and where necessary reference should be made to standard texts and the papers cited.

## Background and general principles of the method

### *Microbial metabolism and electricity generation*

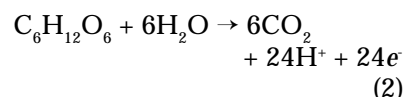
When living creatures metabolize food to provide them with energy, they are tapping the energy of oxidation of energy-rich (electron-rich) substances liberated, from carbohydrates for example, in reactions such as:



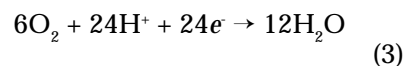
In the living cell (or in single-celled micro-organism, on which we focus our attention) this process is of course a complex one involving many enzyme-catalysed reactions. It progresses through a series of intermediates (e.g., *NADH*, *quinones*) involving successive oxidation-reduction changes, and in this respect resembles an electrochemical process; it is an example of “cold combustion”. The energetics of metabolism are of fundamental importance in areas such as nutrition, and have inspired evocative labels such as the “fire of life”, but that “fire” is one that proceeds at body temperature.

In “normal” microbial catabolism, a substrate such as a carbohydrate is oxidized initially without participation of oxygen when its electrons are released by enzymatic reactions. The electrons are stored as intermediates which become reduced, and in this state

they are used to fuel the reactions which provide the living cell with energy for maintenance and growth via bio-synthetic reactions. The ultimate “electron sink” is molecular oxygen (dioxygen). To an electrochemist, a simplified representation of the charge separation involved in the oxidation of glucose by a whole bacterial cell would be as follows:



The large harvest of electrons is stored as reduced intermediates, but the eventual terminus in the respiratory chain is oxygen:



Inspection shows that equations (2) and (3) combine to give the same result as equation (1).

### *Intervention of mediators*

In the absence of oxygen, electrons may be diverted from the respiratory chain by an oxidation-reduction (“redox”) mediator, which enters the outer cell membrane, becomes reduced, and leaves again in the reduced state. The reduced mediator then shuttles the “stolen” electrons to an electrode (which becomes the electro-generic, negatively charged, electrode) and they appear as an electric current driven around an external circuit. To complete this circuit a second (positive, oxidizing) electrode is required which is again the electron sink, but is now external to the biological system. The oxidizing material can be oxygen gas, but it is more convenient to use a simple soluble solid oxidizing reagent such as potassium ferricyanide (potassium

hexacyano-ferrate III). The main features of the cell are shown schematically in Fig. 1.

The current generated may be detected with a microammeter (or multimeter), while the transformation of energy and power ("bugpower") from the microbial oxidation into mechanical energy (power) may be demonstrated by including a load in the external circuit in the form of a small motor. Alternatively, the current may produce light and heat from a small bulb (light-emitting diode (LED)). In the absence of any regeneration of the substrate the system will eventually run out of oxidizable fuel, and the flow of electrical current will cease. In this mode the system is operating as a simple galvanic cell or *battery*, which, like a torch battery after Prolonged use, will run out of "juice": but it is also demonstrably rechargeable, because the current can be restored by further addition of substrate. When a continuous supply of fuel is maintained regeneratively, the device becomes a *fuel cell* which can support the generation of electricity for long periods.

### Elementary demonstration experiments

Various forms of two-compartment electrochemical cell may be devised to demonstrate microbial electricity generation, and those described here are only two of many possibilities. The first example given is sufficiently simple for rapid assembly and operation in a teaching environment affording modest facilities. The use of a more sophisticated cell is described for which the construction and investigation is suitable for more advanced students, especially where time is available for an extended project.

#### *A simple demonstration cell*

One of the simplest forms of cell which we have used for demonstrations is represented in Fig.2. The components are conveniently contained in a large glass boiling tube (the "cell": any type of pot or simple container could be used, but the use of a transparent vessel allows

observation of colour changes, as noted later).

The electrodes are made from carbon rods — large if possible — which can be scavenged from old "dry" batteries. Although inert metals can be used, most common ones (copper, iron, zinc, aluminium) should be avoided, as they may give rise to spurious generation of current from electrochemical dissolution of the metal. Ordinary copper wire may be used for connections, wound tightly around the electrodes with pliers, but care should be taken to avoid these connections making contact with the cell solutions.

The oxidizing solution (catholyte) is a solution of potassium or sodium ferricyanide, 0.01–0.10 M, in potassium phosphate buffer (anywhere in the region of pH 7). One electrode is immersed in this solution to form the oxidizing half-cell. The separation of the anode and cathode solutions is achieved with the aid of a length of tubular dialysis membrane, made into a "bag" by tying a knot in one end of the wetted tube. The bag and contents, forming the reducing half-cell, are assembled before placing in the catholyte contained in the main cell container to complete the assembly of the cell. (The membrane is of course permeable to ions, including the oxidant; the separation of the two halves is therefore only temporary, but is adequate for a demonstration which can be completed in 30 minutes or so.)

The bag contains the second electrode immersed in pH 7 buffer containing the micro-organisms, the mediator, and a small amount of glucose. *E. coli* is a very suitable organism, and its use is permitted by school safety regulations in most countries. (*Proteus vulgaris* is excellent, but may not be permitted, as some strains are pathogenic.) Ordinary yeast may be used, but is sluggish (*i.e.*, less active, giving less reducing power) and being heavier gradually settles out. The amount of micro-organism required is upwards of 50 mg (dry wt.) per mL — sufficient to give a turbid (watery milk-white) suspension. A rubber

band wound several times tightly around the top of the bag seals off anolyte solution before the entire bag and contents is placed in the cell vessel, leaving the top of the bag and the exposed electrode well above the liquid level to avoid contamination by the oxidant ferricyanide solution and the possibility of a short-circuit. Further down in the solution, close contact of the negative electrode with the inside of the bag and pressing together of the two electrodes should be avoided.

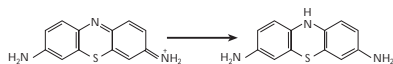
Suitable redox mediators are thionine or methylene blue (readily obtainable from suppliers, *e.g.*, Eastman, Fluka). These substances resemble dyestuffs, and although water-soluble, they are strongly adsorbed at surfaces; hence contact of the solids or concentrated solutions with skin or clothing should be avoided. Mediators are best prepared as a concentration solution (c. 5–10 mM in water), and then added to the anolyte suspension of micro-organisms from a syringe to give the required final concentration.

The equipment required for electrical measurements needed can be quite simple and inexpensive. A small digital multimeter is ideal (preferably reading to millivolts and to microamps). For current readings, a school-type moving-coil needle microammeter will suffice if an electronic microammeter is not available, but large home-use needle ammeters or multimeters are generally not suitable.

#### *A preliminary experiment: microbial reduction of a mediator*

A useful introduction to the subject is provided by a simple mediator reduction test which may be carried out in a small sample tube. A suspension of micro-organisms in buffer solution (a few mL) is placed in the tube to almost fill it. A drop or two of concentrated mediator solution is added—enough to give on mixing a just medium blue colour (like watery ink), and the tube is then stoppered and inverted to mix the contents. The reducing action of "bugs" is

observed as they turn the oxidized (blue) mediator to its reduced form (colourless). For thionine, the reaction is as follows:



If the “bugs” are active the colourless condition will be maintained almost indefinitely. The reaction may take some time, and will depend on the ambient temperature, so that assistance from hand-warming may be in order. It will be noticed that at the top of the tube, where a little air is trapped in the tube, the decolorization may be incomplete; an indication that oxygen is successfully competing with the mediator for electrons, or is rapidly re-oxidizing the reduced mediator.

A little experimentation will show that the rate of decolourisation depends on the concentrations of organisms and mediators (Bennetto *et al.*, 1981), and if the biological reducing activity is sufficiently high the whole of the contents will soon become completely exhausted of oxygen. If the stopper is removed, allowing more aerial oxygen to enter, the reaction will be seen to be reversed as the blue colour of oxidized mediator is re-established, and when the stopper is replaced the continued reducing activity will again be demonstrated. Successive reductions and re-oxidations may be carried out almost indefinitely, because the drain on the supply of electrons is relatively small, while the organisms (in most cases) have large endogenous supplies of oxidizable food store which may readily be converted to carbohydrate. The decolourisation test confirms that the “bugs” are actively producing electricity which can be stored as reduced mediator, and represents the first phase of generation of an electric current in the microbial fuel cell. The second stage, transfer of the electrons to the electrode and thence to the outside circuit, is best demonstrated in the complete cell.

### Procedure for operation of the cell

Having established that a batch of organisms has reducing activity in decolorization tests, the anode should be assembled for use in the cell, but at first in a “blank” experiment without any mediator added to the solution. The leads from the electrodes should be connected to a voltmeter. (If a multimeter is used, make sure it is initially set to ‘voltage’.) It is instructive to first observe what happens in the absence of a mediator, and to then see the effects of adding a mediator according to the following procedure. On immersing the anode “bag”, a residual low voltage (generally about 0.2–0.3 V) is generated. If the meter is then switched to ‘amps’, only a very low current is produced, which is not sustained. It rapidly falls as the system becomes polarized, an indication that there is no effective coupling of the source of the electrons with the electrode.

The effect of adding mediator solution should now be studied. The addition is conveniently made by carefully locating a syringe needle in the top of the bag (held in position inside the bag by the rubber band; this may be done during assembly). With the electrode leads connected to the voltmeter (to measure voltage, not current), about 1–2 mL of mediator solution is injected rapidly from a syringe into the anolyte suspension. As the blue colour of oxidized mediator gradually fades, the electrical effect is observed: the voltage increases as the electrode in the bag becomes more negative, generating 0.4–0.7 V maximum when the mediator is completely reduced.

When the voltage has reached a maximum, the cell is charged up and “ready to go”: the reduced mediator is ready to deliver electricity to the electrode. The meter should now be switched to measure current, and the cell will be found to easily produce 50–100  $\mu\text{A}$  for a limited period. (A more constant lower current can be sustained if a resistor is included in series with the meter in the current-reading circuit.) The

greatly increased current effected by the mediator demonstrates the principles outlined above. Evidence that transfer of electricity from the reduced mediator to the electrode has occurred may be seen in the region of the electrode, where the blue colour of oxidized mediator becomes visible.

### Microbial fuel cells for advanced experiments

The maximum currents produced in the cell described above are too small to drive all but the most minute motors or to light a bulb. Some improvement is achieved if the two half cells are kept separate (*e.g.*, two separate tall beakers) and are connected by a salt bridge. However, to produce much greater currents, and for more rigorous, quantitative studies, cells such as that illustrated in Fig. 3 may be easily made from plastic and other simple materials where construction facilities are available. This design, used extensively for research purposes (Bennetto, 1984; Delaney *et al.*, 1984), is fitted with ports for addition of solution components and for passing a stream of nitrogen. This enables rapid flushing of oxygen from the solutions, and helps to keep the organisms in suspension. The ports are not essential, however, for many simple experiments. The two main cell compartments are separated by a cation exchange membrane (obtainable from BDH or other suppliers) held in place by rubber gaskets. Flat plates of carbon, graphite, or “glassy” carbon can be used for electrodes (not shown in Fig. 3) having connecting (platinum) wires attached with conducting epoxy cement. A cheaper and equally effective material for electrodes is carbon cloth or felt, when this can be obtained (see Appendix).

Experiments with the simple cell outlined above can all be performed with the larger cell, but obviously with greater control. If possible, two meters should be used to monitor current and voltage simultaneously. A single cell can generate a maximum of 3–50 mA current, depending on the concentrations. But on maximum

current the electrodes rapidly become polarized (the voltage falls, and takes some time to recover) and to avoid excessive drain on the cell the leads should not be connected directly to an ammeter for more than a few seconds.

#### *Continuous production of electrical power*

It is preferable to allow the current to discharge through resistor (200–1000  $\Omega$ ), which will allow a constant or slowly-decreasing current to be sustained. Since a single cell is capable of driving a small motor (see Appendix), it is easy to devise demonstrations, e.g., by attaching a small lightweight propeller to the motor shaft. Much interesting and instructive information can be derived from discharge experiments. For example, current readings can be taken at intervals and plotted against time, or, more conveniently, monitored with a chart recorder. (Currents may be measured by allowing the cell to discharge through a resistance, say 500–100  $\Omega$ , connected in parallel with a simple recorder on a “volts” scale.) Since the amount of electricity delivered is current ( $i$ ) x time ( $t$ ), the *coulombic* yield may be found from the area under the  $i$  vs.  $t$  curve (see Bennetto (1987)). By comparison, the yield from a “blank” run in the absence of mediator is very small.

One suggested experiment of fundamental importance is to attempt the measurement of the electrical yield from a given quantity of glucose (Roller *et al.*, 1984; Delaney *et al.*, 1984). According to equation (2) one molecule of glucose delivers a maximum of 24 electrons (1 mol  $\rightarrow$  1 Faraday, 96,500 C). In practice, it is easy to get yields of 40% or more (e.g., from *E. coli*), but the importance of doing “blank” experiments and the merits of doing differential experiments cannot be overemphasized. Thus the substantial yield from a “blank” cell containing mediator but no added glucose (which results from oxidation of endogenous “fuel” which the organisms keep as an energy reserve) needs to be

subtracted from the yield obtained in an “identical” cell with added glucose.

It is a fascinating exercise to run down a cell almost to zero, thus exhausting the microbes, and to see the effect of adding further “feed”. With active organisms, the recovery of current is observed within minutes, and the “feed”–discharge cycle can be repeated numerous times. This cannot be demonstrated, however, with yeast, which has relatively low reducing power, and “long legs”, i.e., *large endogenous energy reserves*. For project work, numerous experiments are possible investigating the longevity of the (current delivery) system under a variety of experimental conditions (concentrations, different mediators, increased temperature).

#### **Discussion**

The many areas of science brought into focus in the study of “bugpower” will be obvious to most, and it is hoped that the following brief list of topics, rhetorical questions and suggestions for discussion will stimulate interest in both the experimental and theoretical aspects of the subject.

#### *Microbiology and microbial physiology*

Substrate specificity of different organisms. Why does ordinary *E. coli* not respond to sucrose in a fuel cell? (Bennetto *et al.*, 1986). A great variety of species can be used under enormously different conditions: e.g., *in alkaline solution* (Akiba *et al.*, 1985), *with photosynthetic organisms* (Bennetto *et al.*, 1983), *in acid conditions, used for extraction of gold from ores* (Bennetto *et al.*, 1989b). Why does yeast not respond addition of substrate? What oxidation products are likely to be found where oxidation is incomplete? (Thurston *et al.*, 1985). Could the electrical response of micro-organisms be used to construct analytical biosensors? (Bennetto *et al.*, 1987a).

*General chemistry, electrochemistry; elementary physics*

Proper understanding of the electrical phenomena depends on some familiarity with basic definitions and units of current, voltage, energy, power, etc., and Faraday’s laws. (Hibbert and James (1984) give a useful comprehensive guide.) How many molecules of redox mediator are being taken up and reduced per microbe in 1 second, and how many electrons are transferred? What e.m.f. (voltage) might be expected using an efficient oxygen electrode, and what improvements to the power and energy yields might be affected? Diversions into thermodynamics and mechanical engineering may be entertained for advanced students.

#### *Possible applications in biotechnology, biomass conversion and environmental science*

Simple “scale-up” calculations can be made to see what power might be obtainable from a reactor the size of a tennis court (cubed—to give the volume to an order of magnitude). How much biocatalyst would be needed, and how could it be grown? How much carbohydrate would need to be supplied per hour to maintain a constant output? Could we use waste products or crude biomass such as lactose or sucrose molasses (Roller *et al.*, 1983; Bennetto *et al.*, 1986). What weight of glucose might be required to power the 1 mW motor (in short pulses) of a digital watch for one year, if the electricity could be tapped at maximum efficiency” (Bennetto *et al.*, 1987b). What factors might be involved in reversing the process, and feeding electricity *into* microbes? If a working bio-motor could be developed, how might the energy efficiency compare with that of the internal combustion engine?

#### **Acknowledgements**

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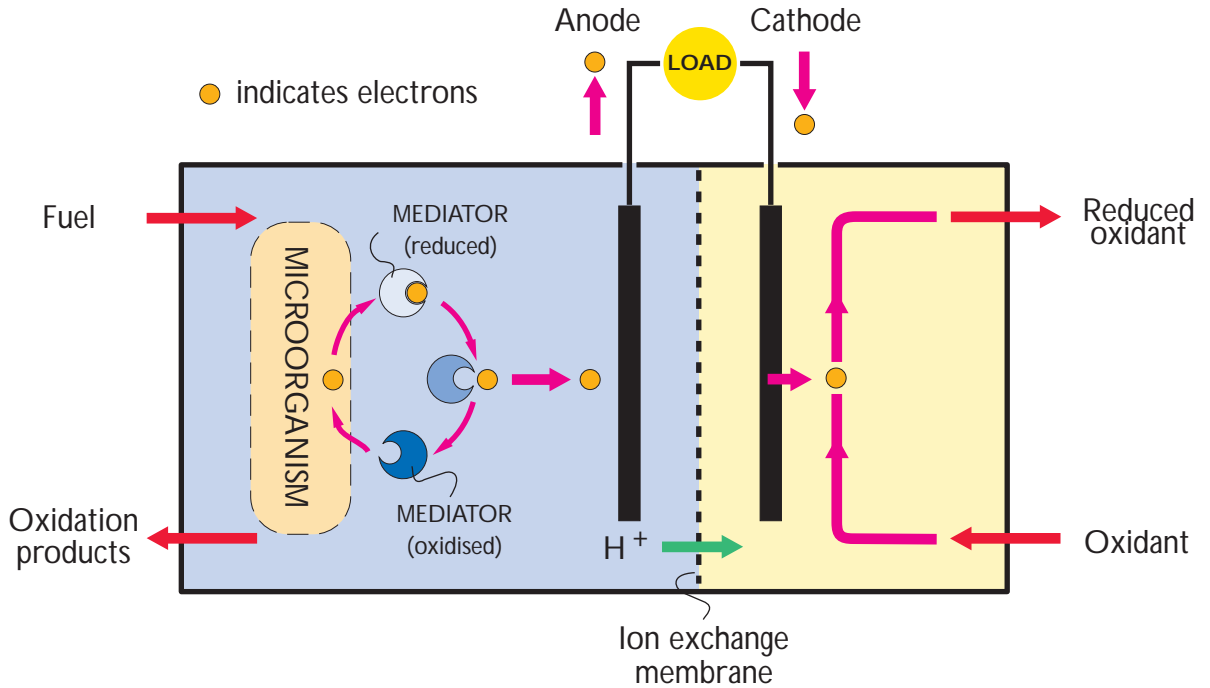
## Appendix

A simplified cell based on the design described here and costing less than £50 is available from the National Centre for Biotechnology Education, The University of Reading, who can also advise on availability of electrode materials and other components. Enquiries should be sent to NCBE@reading.ac.uk. A useful range of motors which can be driven is available from Portescap UK Ltd (Wimborne, Dorset). In the author's experience, the model 22.Cl 1.2051 is perhaps the most suitable, as the fairly high resistance (200  $\Omega$ ) prevents over-rapid discharge, and allows continuous slow rotation at outputs as low as 1.5 mA, 0.2 V, for very many hours. The 16.C11.210.30 model has a low resistance (8  $\Omega$ ) and gives high speeds, but more rapid drain. (Most motors used for model cars, aeroplanes, etc. have too high a power requirement; very low power ones are rare, although some have been found in stores selling odd electronic components.) The power output from one cell is insufficient to light even small bulbs; several cells in series may be needed to light an LED bulb, which requires more current (generally > 15 mA) at higher voltage (> 1.5 V). Electronic devices containing flashing LEDs (which require only short bursts of charging current) can be constructed, but generally 2–3 cells are needed to give over 1.2 V. For the information of secondary school instructors in the UK, a demonstration of the microbe-driven motor of the type described may be seen on a BBC TV educational series (“Science in Action”) that was screened from 1990–1993.

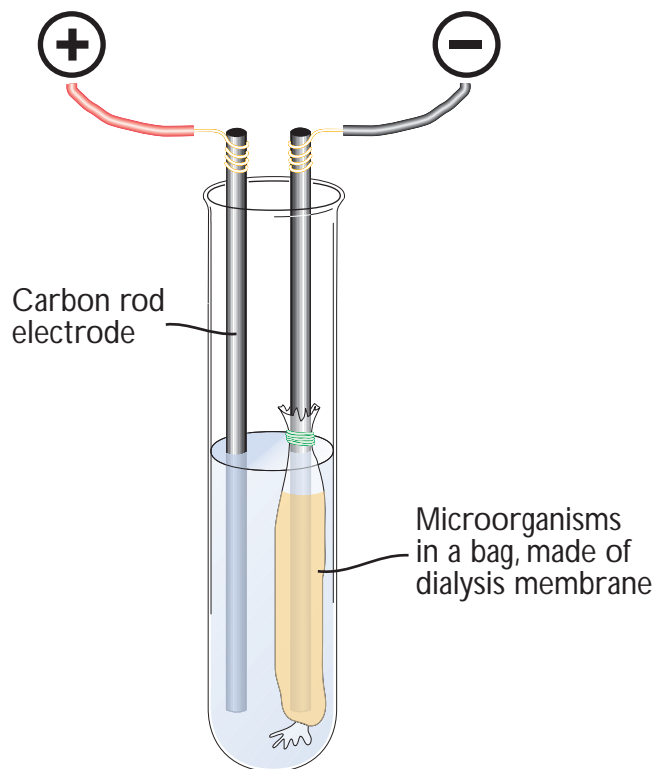
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**Figure 1**  
Schematic representation of a  
microbial fuel cell



**Figure 2**  
Simple demonstration cell.  
The microorganisms are  
contained in a dialysis  
membrane 'bag' surrounding  
the negative electrode.



**Figure 3**  
Construction of a microbial fuel cell for school experiments

