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Towards biochemical fuel cells

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Abstract. A biochemical fuel cell is a device which converts chemical energy into electrical power. The catalysts used in this process can be either inorganic or organic type giving rise to 'inorganic fuel cells' or 'biochemical fuel cells', respectively. Biochemical fuel cells use either micro-organism or enzymes as active components to carry out electrochemical reactions. The efficiency of such a device theoretically can be as high as 90%. The difficulty in attaining these values arises due to sluggishness of electron transfer from active site to conducting electrode. This can be overcome by using mediators or by immobilizing active components on conducting electrode. We have immobilized FAD-glucose oxidase on a graphite electrode using a semiconducting chain as a bridge. At the present stage of development, such a device lacks high current densities, which is essential for commercial power generation but can be used in applications such as pacemakers and glucose sensors.

Keywords. Biochemical fuel cell; enzyme-coenzyme system; immobilization; modified electrode; cyclic voltametry.

1. Introduction

Human body can be considered as the oldest, yet the most refined fuel cell where food (fuel) is catalytically burned (oxidized) in an electrolyte (blood) to produce energy required for cellular functions. In this process, the chemical energy residing in the chemical bonds of fuel molecules is converted directly into other forms of energy without employing an intermediate thermal cycle. The process of conversion of chemical into electrical energy has received increasing attention during the last two decades, following the high demands for fossil fuels and the consequent depletion of the existing stocks of fossil fuels at an alarming rapid rate.

For several years, fuel cell devices which accomplish a direct conversion of chemical to electrical energy, remained a mere laboratory curiosity on account of problems of electrode development, short operational life and high costs. However, the space exploration programme gave an impetus for development and respectability to fuel cell as a practical device for energy transfer and energy storage.

In this article, we wish to review attempts at developing biochemical fuel cells, with some emphasis on our own work in this direction.

2. What is a fuel cell?

A fuel cell consists of two electrodes—an anode and a cathode separated by an electrolyte which transmits ions but not electrons. A fuel (generally hydrogen) is supplied to the anode and oxygen (from air) is supplied to the cathode. A catalyst

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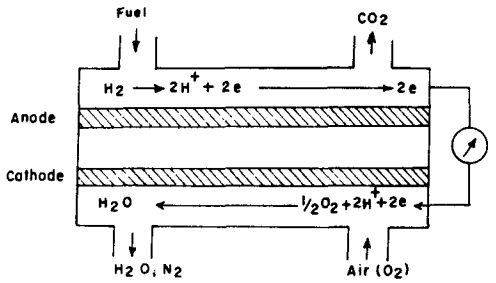

Figure 1. Prototype fuel cell.

situated on the anode causes hydrogen molecules to dissociate into hydrogen ions (H^+) and electrons. If the electrolyte is acidic as in the case of phosphoric acid fuel cells, the hydrogen ions migrate through it to the cathode, where they react with electrons (supplied through the external-circuit load) and oxygen to form water. The nature of migrating ions depends upon the type of electrolyte used. As shown in figure 1, an electric current can flow if the electrodes are connected by means of a conductor.

The maximum direct current developed by a fuel cell is a thermodynamic function of the fuel and the oxidant (Wingard *et al* 1982). Considering that the operation is isothermal and takes place at constant pressure without transference, the efficiency can be calculated. The theoretical amount of work that can be obtained, depends on the net release of Gibb's free energy ΔG during the course of the reaction.

$$\Delta G = nFE,$$

where n is the number of electrons transferred per mole, F is the Faraday constant and E is the net reversible potential. At constant temperature (T) and pressure (P) the free energy is related to the change in enthalpy ΔH and entropy ΔS

$$\Delta G = \Delta H - T\Delta S.$$

The efficiency of fuel cell is defined as

$$\eta = \Delta G/\Delta H = 1 - (T\Delta S/\Delta H).$$

For most systems $T\Delta S$ is 10% of ΔH. Therefore the theoretical efficiency can be as high as 90%. This is considerably higher than that for the systems which are governed by Carnot's cycle $\sim$ 40%. Experimentally efficiencies of the order of 50% have been achieved in fuel cells.

2.1 *Characteristics and disadvantages*

Ideally a fuel cell should possess the following characteristics: (i) the cell should have high conductivity *i.e.* low internal resistance; (ii) the electrodes and electrolyte should be stable *i.e.* there should not be any change due to corrosion or due to accumulation of products leading to a change in the cell reactivity with time; (iii) the cell should exhibit high reactivity; the fuel and oxidant should react rapidly, completely and electrochemically; (iv) It is desirable to have inexpensive materials and the operation should take place under conditions of moderate temperature and pressure.

The majority of inorganic fuel cells in use today, utilize hydrogen as fuel. The electrodes used in these cells are made from expensive materials like platinum, nickel

etc. Moreover, the electrolytes used (*e.g.* phosphoric acid, KOH, molten carbonate) are highly corrosive and the operating temperatures are high, 150–200°C in case of phosphoric acid fuel cell and 600–700°C for molten carbonate device). Some of these problems can be overcome by developing a biochemical fuel cell system.

3. Biochemical fuel cells

In a biochemical fuel cell (BFC), the oxidation of bio-organic materials is catalysed by specific bio-matter either in the form of bacteria (Austin 1967) or purified enzymes (Wingard 1978). There are certain advantages in using biochemical catalysts. Biocatalysts are known to be extremely efficient and selective. They normally operate at neutral pH, at or slightly above room temperature. The concentrations of reactants can be low.

The biochemical fuel cells can be either 'direct' type (figure 2a) or 'indirect' type (figure 2b) (Shaw 1963). In the direct biochemical fuel cell biological oxidation of a raw material (starch, carbohydrate or protein) is achieved with the help of bacteria which facilitate stepwise transfer of protons involving several classes of enzymes and coenzymes provided by the bacteria. The combustion rate in such cells is low since the reactions have to be carried out within narrow temperature and pH ranges. Further the electrodes may get contaminated by bacteria. Moreover, there is very little freedom of

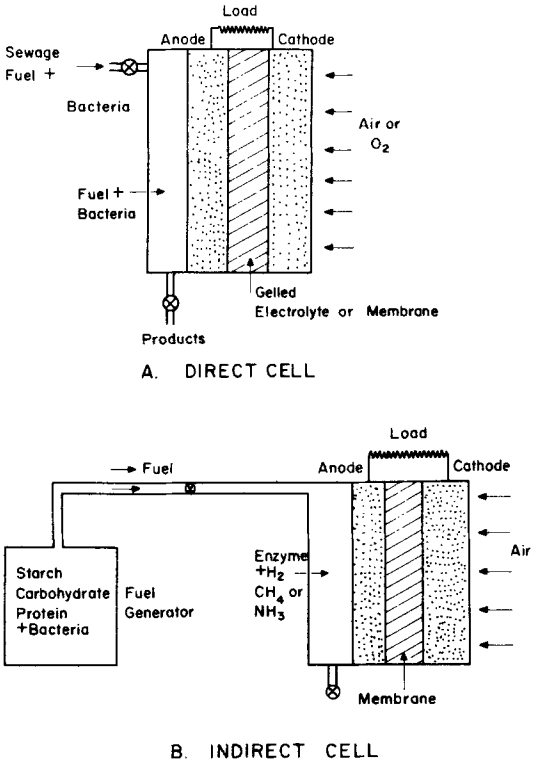

Figure 2. Biochemical fuel cell. (**a**) direct (**b**) indirect.

control or optimisation, as the conditions have to be those which are favourable for bacterial growth.

In indirect biochemical fuel cell, the processes of oxidation and power generation are separated out (figure 2b). The raw fuel is first converted into simpler biochemical end products like H_2, CH_4, CH_3OH, NH_3 etc (secondary fuel) with the help of suitable bacteria in fuel generator (figure 2b). The secondary fuel thus generated is transferred to a second compartment where the electrical power is generated. Such systems are more convenient and easier to optimise but bring in an additional engineering problem, namely, the transfer of secondary fuel.

Efforts have been directed to develop both the direct and indirect fuel cells (Videta and Arvia 1975; Karube *et al* 1977; Ahn *et al* 1976). Almost any waste or organic material can serve as a fuel. The electrical current produced is proportional to the rate of bacterial metabolism. Fifty percent of the bio-fuel gets consumed by the micro-organisms as their nutrient. To date the electrical power produced in such devices is in the milliwatt range (50–60 % efficiency). The inability to attain higher power outputs is probably due to a lack of knowledge about conditions or micro-organisms which can yield higher metabolic rates and/or due to lack of knowledge about the electrode kinetics. Moreover in actual cells when current flows, the potential is not available to produce work as a result of polarization (overpotentials) effects. The main contributions to polarization, arise from activation, concentration and ohmic factors. Activation over-potentials result from the slowness of one of the intermediate steps in the chemical or electron transfer processes. The polarization is due to slow migration of substrate, charge transfer agents or products towards or away from the electrodes. The ohmic over-potentials are on account of slow transport of the ionic species, through the electrolyte medium.

The major difficulty in using micro-organisms is apparently due to the difficulty involved in mild conditions and the process of transferring the electrons across cell membranes. This can be overcome by making fuel matter to undergo electrochemical oxidation with the help of purified enzymes. If the reduced form of an enzyme can be oxidized electrochemically and its oxidized form is allowed to react with a substrate, the enzyme can cycle back and forth, getting oxidized at the electrode surface and reduced by chemical reaction in solution. The net result would be the apparent oxidation of substrate at the electrode.

$$E + R \rightleftharpoons ER$$

$$ER + M \rightleftharpoons M[ER]$$

$$M[ER] \rightleftharpoons M + E + P + H^+ + e^-$$

where E is the enzyme, R is the reactant, M is the electrode surface and P is the product. Several enzyme systems have been examined for their electrochemical activity.

If the enzyme works in conjuction with a coenzyme the whole system can be incorporated into anode or cathode compartment which can act as catalyst for the redox reactions. In the case of enzymes which catalyse redox reactions, the associated cofactors serve as the actual electron and proton transfer agents.

The major problem in such fuel cells is the inefficient transfer of electrons from substrate to electrodes. This can be catalysed by (i) diffusion of cofactor from enzyme active site to the electrode surface; (ii) using electroactive mediators; (iii) immobilization

of enzyme-cofactor system on the electrode using a conducting matrix and; (iv) making the cofactor as an integral part of electrode surface.

Of the schemes described above, immobilization techniques hold great promise and are described below in some detail.

3.2 *FAD based bioanodes*

Though the flavins (flavin mononucleotide FMN and flavin adinine dinucleotide FAD) and nicotinamide (NAD^+, $NADP^+$) coenzymes are primary acceptors of electrons from various substrates and cellular metabolites, the tricyclic flavins are more versatile catalysts as they can undergo facile two-electron as well as one-electron chemistry (Walch 1980). Reduced flavin can readily undertake reductive oxygen metabolism. The isoalloxazine ring system offers several positions at which the coenzyme can be immobilized on the electrodes. Wingard (1980) utilized the 8 methyl position to covalently link riboflavin to carbon electrodes through a conjugated double bond system and report that the immobilized species retains its electroactivity. Other workers (Bourdillon *et al* 1980; Ianniello *et al* 1982) have reported immobilization of glucose oxidase, L-amino acid oxidase and xanthine oxidase on modified carbon electrodes. The attachments in these reports are through ε–NH_2 groups of amino acid residues of the protein part, which may or may not be on the active site of the enzyme.

In our approach to the FAD-glucose oxidase system we have first studied the electron fluxes produced at different sites of the flavin ring system of FAD during its redox reaction. A site for modification has been identified, and used for immobilization of the coenzyme on the electrode. The details are given below.

The oxidation-reduction cycle of FAD involves two protons and two electrons. Schematically the complete cycle is represented in figure 3. When a substrate SH_2 is oxidised in the presence of a suitable enzyme (E) it liberates two protons and two electrons. These can be taken up by the oxidised form of FAD (I) to give $FADH_2$ (III), sequentially giving rise to an intermediate species—protonated form II. Similarly, formation of oxidized form of FAD may involve sequential release of two protons (IV)

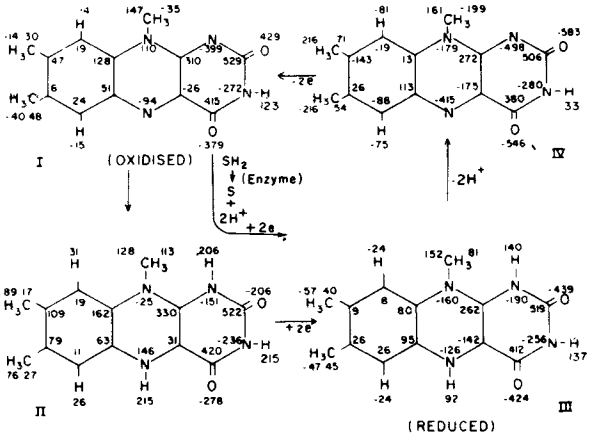

Figure 3. Charge densities obtained from INDO calculations of various redox states of flavin ring of FAD. Charge densities are in units of 10^{-3} atomic units.

and two protons and two electrons from the substrate (I → II → III) and releases them (III → IV → I) on oxidation. The two protons released can be taken up easily by the aqueous medium and the two electrons can be made to pass through an external circuit thereby giving rise to a current in external circuit. The major difficulty arises in affecting efficient electron transfer. One possible way to overcome this difficulty is by immobilising the coenzyme-enzyme system onto an electrode at a site which undergoes large change in electron density during the step IV → I. The charge distribution in FAD molecule was calculated making use of semiempirical quantum chemical technique of INDO. One can notice from figure 3 that in accordance with general expectations, the two carbonyl groups in the isoalloxazine ring and four nitrogens in the two heterocyclic rings possess such characteristics. Of these one of the four nitrogens is substituted by ribose and two are involved in proton transfer reactions, while the fourth nitrogen is known to be involved in coenzyme-enzyme linkage. The two carbonyl sites therefore appear to be potential sites for hooking up the semiconducting side chains.

FAD has been immobilized on carbon electrode employing a series of chemical steps which are schematically shown in figure 4. The steps involve creating suitable active centres on the electrode surface which are then made to react with the carbonyl groups of FAD to form conjugated double bond linkages between FAD molecules and graphite. The linkage is quite stable and FAD is not leached out from the electrode on repeated washing or use in electrochemical cells. This step had been carried out in dark to prevent the photoreaction of FAD.

Glucose oxidase is immobilized by incubating the FAD modified electrode with its apoenzyme. The electrodes were washed till the washings showed no absorbance at 280 nm and stored refrigerated in dark when not in use. Immobilized enzyme activity was assayed by using the modified method of Bergmeyer (1974). The immobilized glucose oxidase is found to be enzymatically active.

The electrochemical activity of modified electrodes has been assessed using cyclic voltametry. A conventional three-electrode system has been used in a cell where platinum mesh is used as counter electrode and saturated calomel electrode as reference. Supporting electrolyte in all cases was 1 M KCl pH 7. The potential was

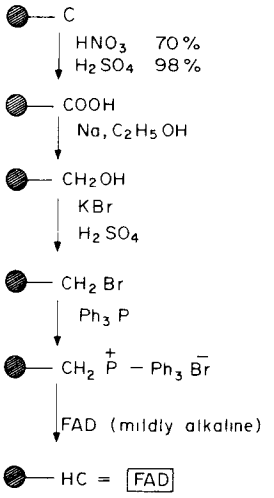

Figure 4. Reaction scheme for immobilization of FAD on graphite electrode.

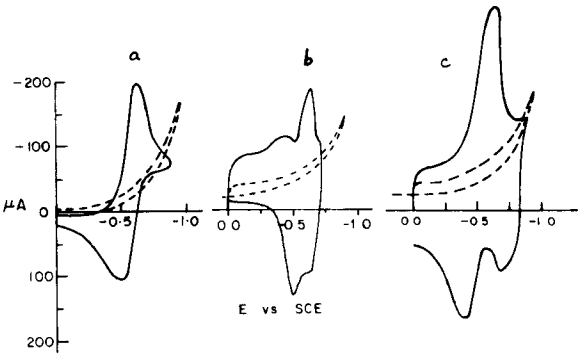

Figure 5. Cyclic voltamograms (**a**) FAD (1 mg/ml) in 1 M KCl pH 7; (**b**) FAD immobilized on carbon black; and (**c**) glucose oxidase immobilized on FAD modified carbon black; Broken lines—unmodified carbon black in 1 M KCl pH 7.

swept at the rate of 150 mV/sec. Analar grade nitrogen was bubbled through the electrolyte for 30–60 min prior to the experiments which were performed in nitrogen atmosphere.

Cyclic voltametric behaviour of FAD in solution is shown in figure 5a. A well-defined peak centred at −0·62 V is observed corresponding to the reduction of FAD. In the return sweep, a peak is seen at −0·50 V corresponding to its oxidation. Cyclic voltammogram of FAD immobilized on CB (carbon black) in supporting electrolyte is shown in figure 5b. Predominant peaks at 0·62 V and −0·50 V due to reduction and oxidation are observed indicating definite coupling of FAD to the electrode surface. Absorption of FAD on heterogenous electrode surface renders it a bit more difficult to reduction, which occurs at a potential higher than that of the coenzyme in solution (Gorton and Johansson 1980; Ianniello *et al* 1982). As this is not so in the modified electrodes in our case, it is reasonable to overrule the possibility of adsorption being the mode of attachment. Moreover these peaks have been found to be much sharper than those observed when carbon black electrodes dipped in FAD solution (*i.e.* adsorbed FAD) are used. Figure 5c depicts the results when glucose oxidase has been immobilised on FAD modified electrodes. It may be observed that while the reduction potential remained unaltered, the peak potential for oxidation has been shifted by 0·11 V more anodic. The redox peaks are sharper than those when free enzyme was used. In short covalent attachment of FAD on carbon electrode through carbonyl group of the flavin ring structure seems to favour the exchange of electrons between the FAD molecule and the electrode. Immobilization of glucose oxidase on electrode so modified, apparently orients the enzyme molecule with respect to the electrode and leads to direct electron swapping between enzyme active site and electrode.

3.3 *Biocathodes*

The biocathode system in wide use is an oxygen cathode where simultaneous uptake of electrons and reduction of oxygen to water takes place. This reaction is sluggish and mostly results in lower overall efficiency of the fuel cell. The equilibrium potential of the oxidation reduction pair O_2/H_2 of 1·23 V is obtainable only on specially treated platinum (Watanabe and Devartrathan 1964; Bogdanovskaya *et al* 1972). Exchange

currents on platinum are as low as 10^{-11} A/cm^2. This can be overcome by supplying oxygen, to the electrode through photosynthesis of algae (Lewis 1964). In this way the source of oxygen can be put directly on the electrode. But the disadvantage arises due to dependence of the cell on light energy. Alternatively, enzymes which can actively reduce oxygen to water can be immobilized on cathode. Cytochrome C oxidase, ceruloplasmin, ascorbatoxidase and laccase are known to reduce oxygen to water *via* a four-electron mechanism without hydrogen peroxide as an intermediate. Yaropolov *et al* (1976) and Berezin *et al* (1977) have described systems where peroxidase and cytochrome C oxidase have been used as catalysts for oxygen reduction. The electrons get transferred from the electrode to active centres by mediators. The electrode potentials of 0·6–0·8 V have been obtained which is dependent on the ratio of the reduced and oxidized forms of mediators. Berezin *et al* (1977) have described another system where *Poluporos versicolor* laccase has been used as catalyst, laccase had been directly absorbed on the electrode. The maximal potential value of 1·207 V near equilibrium has been obtained on carbon black electrodes that had been kept in 10^{-5} M laccase solution for 24 hr.

4. Scope and future projections

Efficient bridging between the redox proteins and electrodes of the type discussed above can eventually give rise to three types of devices (i) biochemical fuel cells working at low temperatures (ii) sensors based on direct electrode interactions rather than conventional ones which detect products or substrates of biological reactions (iii) bioelectrodes for electrochemical synthesis.

Inspite of the many advantages projected for biochemical fuel cells, work on the preparation of bioelectrodes is still in its infancy. The major constraint, of course, is the poor electron transfer from enzyme active site to electrode. Although, at present, biochemical fuel cells are far from being used as commercial power generators, their use in sensors provides excellent potential. A couple of examples in this regard are the alcohol sensors, the glucose and urea sensors. The glucose sensor for instance can find wide applications in estimating an monitoring glucose in food and fermentation industries and clinical laboratories. One other use of biochemical fuel cells is in running devices which require low power outputs. For example, a micro glucose-electrode conveniently implanted in the body, can serve as an excellent power source for pacemaker, generating low-power pulses from the plasma glucose. However, one can foresee the most potential application of indirect type of biofuel cell as power generators in conjunction with sewage, biomass and agricultural waste fermentation.

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