INTRODUCTION

All organic natural products and most synthetic organics are susceptible to microbial degradation. In the case of hydrocarbons we have quite a detailed understanding of the biochemical mechanisms involved and these were discussed at a recent meeting of the Institute of Petroleum (Higgins & Gilbert, 1978). Microbial hydrocarbon degradation is largely an aerobic process and a group of enzymes, the oxygenases, play an important role in initiating many degradative sequences of biochemical reactions involved and in some other catabolic steps. These enzymes incorporate molecular oxygen into their substrates, thereby effecting highly specific, controlled partial oxidation to products which are in some cases commercially valuable. Many of the reactions involved are difficult, expensive or even impossible to effect by conventional chemical catalysis. The organic chemist has a general, seemingly insoluble problem in controlling oxidation reactions involving molecular oxygen. For example, the simplest commercially important hydrocarbon oxidation process, the industrial conversion of methane to methanol, involves oxidation of the hydrocarbon to carbon monoxide followed by hydrogenation over a catalyst at 270°C and 50 atmospheres pressure. Some recently discovered microbial enzyme systems discussed in this volume (Dalton, 1980) convert methane to methanol in a single step reaction at 30°C and 1 atmosphere pressure with a yield approaching 100%.

There is a wide range of oxygenase enzymes found in a variety of aerobic organisms including microorganisms, plants and animals. They fall into several different classes which between them catalyse a vast range of specific oxygen addition or insertion
### TABLE I. Common oxygenase enzymes which introduce molecular oxygen into hydrocarbons and related compounds

**Monooxygenases** (incorporate one atom of an O₂ molecule into the substrate)

<table>
<thead>
<tr>
<th>Type</th>
<th>Examples</th>
<th>Reactions catalysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome-P₄₅₀</td>
<td>Mammalian liver, Pseudomonas putida camphor hydroxylase, Corynebacterial and yeast alkane hydroxylases</td>
<td>Steroids, polycyclic hydrocarbons, drugs, pesticides + hydroxylated derivatives</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Camphor + 5-(exo)-hydroxycamphor</td>
</tr>
<tr>
<td>Flavoproteins</td>
<td>Methyloccoccus capsulatus (Bath) methane monooxygenase (iron sulphur protein also involved)</td>
<td>Variety of alkanes + alkan-1-ols</td>
</tr>
<tr>
<td></td>
<td>Cyclohexanone monooxygenase</td>
<td>Methane = methanol. Also hydroxylates extremely wide range of other compounds</td>
</tr>
<tr>
<td></td>
<td>p-Hydroxybenzoate hydroxylase</td>
<td>Cyclohexanone = caprolactone</td>
</tr>
<tr>
<td></td>
<td>Orcinol hydroxylase</td>
<td>p-Hydroxybenzoate = protocatechuate</td>
</tr>
<tr>
<td></td>
<td>Salicylate hydroxylase</td>
<td>Orcinol = trihydroxytoluene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salicylate = catechol</td>
</tr>
<tr>
<td>Copper enzyme</td>
<td>Methylosinus trichosporium methanemonooxygenase (possibly)</td>
<td>Methane = methanol. Also hydroxylates extremely wide range of other compounds</td>
</tr>
<tr>
<td>Non-haem iron enzyme</td>
<td>Pseudomonas oleovorans alkane hydroxylase (also contains flavoprotein component)</td>
<td>Alkanes + alkan-1-ols</td>
</tr>
</tbody>
</table>

**Dioxygenases** (incorporate both atoms of an O₂ molecule into the substrate)

<table>
<thead>
<tr>
<th>Type</th>
<th>Examples</th>
<th>Reactions catalysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-haem iron enzymes</td>
<td>Catechol-1,2-dioxygenase (pyrocatechase)</td>
<td>Catechol = cis,cis muconate</td>
</tr>
<tr>
<td></td>
<td>Catechol-1,3-dioxygenase (metapyrocatechase)</td>
<td>Catechol = α-hydroxy-muconic semialdehyde</td>
</tr>
<tr>
<td>Flavoprotein enzymes</td>
<td>Benzene dioxygenase</td>
<td>Benzeno + dihydroxycyclohexadiene</td>
</tr>
<tr>
<td></td>
<td>Toluene dioxygenase</td>
<td>Toluene = 2,3-dihydroxy-2,3-dihydroxytoluene</td>
</tr>
<tr>
<td></td>
<td>Benzoate dioxygenase</td>
<td>Benzoate = 2-hydro-1,2-dihydroxybenzoic acid</td>
</tr>
</tbody>
</table>
reactions. The more important oxygenases that act on hydrocarbons and closely related compounds are shown in Table I. For a detailed account of these enzymes see Hayashi (1974) and Gunsalus et al. (1975). Clearly, they are of great potential value to the chemical and pharmaceutical industries either as models for the development of new catalysts or for direct catalysis using microbial cultures, immobilised organisms or cell-free enzyme preparations. They have already made a major impact in steroid and antibiotic synthesis. A classical example of the importance of these enzymes in synthetic chemistry involves the production of cortisone which has been used in the treatment of rheumatoid arthritis since 1949. The drug was originally made from deoxycholeic acid by a process involving 37 steps giving a 0.16% yield. The discovery that the mould, Rhizopus oryzae, could specifically hydroxylate the readily available steroid hormone, progesterone, to 11α-hydroxyprogesterone (Peterson & Murray, 1952) made synthesis of cortisone much simpler and reduced its cost from $200/gm to 68c/gm. This reaction is catalysed by a monooxygenase and it is this type of oxygenase which offers the most potential for hydrocarbon biotransformation.

**ELECTROENZYMOLOGY**

Monooxygenases (mixed function oxidases) incorporate one atom of an oxygen molecule into the substrate whilst the other is reduced to water. There are two major classes of monooxygenase depending upon the source of reducing power. Internal monooxygenases catalyse the reaction:-

\[
SH_2 + O_2 \rightarrow S - O + H_2O.
\]

External monooxygenases require a reduced cofactor (DH₂), usually a pyridine nucleotide (NADH or NADPH), and catalyse the reaction:-

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

Most monooxygenases are of the latter type, requiring reduced pyridine nucleotide coenzymes and this has constituted a major impediment to their exploitation using cell-free preparations. The reduced coenzymes are expensive and unstable but, although the reaction consumes stoichiometric amounts of reducing agents, they can be regenerated in cyclic fashion by coupling to an enzymic reaction requiring oxidised coenzyme. A particularly suitable enzyme is formate dehydrogenase since formate is relatively inexpensive, the product is CO₂ and quite stable immobilised preparations of this enzyme have been obtained. The two enzymes operate thus:-

![Diagram of monooxygenase and formate dehydrogenase](image-url)
However, this introduces a further complexity into the system, the initial cofactor charge remains expensive and there may still be a fairly rapid loss of cofactor with time, necessitating replenishment. It might prove possible to regenerate the reduced cofactor electrochemically, e.g., the electrochemical reduction of NAD to NADH has recently been demonstrated (Aizawa et al., 1976). Indeed, electrochemical regeneration of NADPH has now been used to drive a cytochrome P₄₅₀ monooxygenase from rabbit liver (Scheller et al., 1977) although the enzyme showed only 30% of normal activity. Of course, this does not solve the problem of the inherent instability of the reduced coenzyme.

An alternative potential solution is to reduce the prosthetic groups of monooxygenases (and indeed other enzymes) directly using electrochemical techniques (Higgins and Hill, 1978; 1979). For this approach to be of value, systems which effect rapid, efficient electron transfer between enzyme active centres and electrodes are required. There are two main approaches to this problem. Either a readily diffusible mediator which shuttles electrons between enzyme and electrode may be employed or in some cases it is proving possible to effect direct electron transfer between enzyme and electrode, if necessary employing a chemical promoter which binds to the electrode in such a way as to facilitate electron transfer to the active site.

Until very recently attempts to reduce proteins directly at an electrode have either failed or the reduction has proved irreversible. It has been assumed that the peripheral protein structures hinder facile electron transfer between electrode and protein redox centre. However, techniques which allow rapid reversible electron transfer from electrodes to proteins are now being developed in several laboratories. Generally, these techniques involve the use of a three-electrode cell comprising a cathode (working electrode), anode (secondary electrode), and a reference electrode; potential is then controlled by a potentiostat. Electron transfer to proteins in such systems has been facilitated in two main ways:–

i) By using a lower molecular weight intermediary electron carrier in solution. Such mediators include ascorbic acid, methylene blue or a viologen dye.

ii) By using an electrode modified in some way by permanent or transient binding of a conjugated molecule such as 4,4'-bipyridyl which 'promotes' (lowers the overpotential for) electron transfer.

To date, most work has been concentrated on two proteins, cytochrome-c (Edwards and Hill, 1977; Yeh and Ramana, 1977) and ferredoxin (Landrum et al., 1977). In the case of cytochrome-c, the use either of a gold working electrode and 4,4'-bipyridyl as promoter or of an indium oxide electrode yield rapid electron transfer, the rates being limited only by chemical diffusion. In addition, however, a number of other proteins have proved amenable to electrochemical reduction using a variety of electrochemical
TABLE II. Proteins for which direct electrochemical oxidation and/or reduction of the prosthetic group have been demonstrated

<table>
<thead>
<tr>
<th>Type of prosthetic group/metal ion used</th>
<th>Working electrodes</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haem</td>
<td>Modified gold</td>
<td>Variety of cytochromes-c</td>
</tr>
<tr>
<td>Indium oxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome-P_450</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camphor hydroxylase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>Modified gold</td>
<td>Azurin</td>
</tr>
<tr>
<td>Gold</td>
<td></td>
<td>Laccase</td>
</tr>
<tr>
<td>Flavin or haem/copper</td>
<td>Gold</td>
<td>Methylophilus trichosporium</td>
</tr>
<tr>
<td>Methane monooxygenase</td>
<td>Gold</td>
<td>Methane monooxygenase</td>
</tr>
<tr>
<td>V. fischeri</td>
<td></td>
<td>Pilimio fisheri luciferase</td>
</tr>
<tr>
<td>Non-haem iron</td>
<td>Mercury</td>
<td>Variety of ferredoxins</td>
</tr>
</tbody>
</table>

Figure 1. Experimental arrangement for electroenzymology (schematic)

Techniques (Table II). Of particular relevance to this discussion are the methane monooxygenase and camphor cytochrome-P_450 enzymes. In both cases a gold working electrode, platinum anode and calomel reference electrode were used and products were generated in the presence of substrate (Figure 1). In the former case, methane was oxidised to methanol and ethylbenzene to phenylethyl alcohol and p-hydroxyethylbenzene at about 10% of the NADH-dependent rate and with about 15% electrochemical efficiency. Hydroxycamphor (5-exo) was generated from camphor in the latter case. It is expected that rates and efficiencies will be improved by developments in electrode and cell design. The methane oxygenase is of particular significance as it has become clear in recent years that this
enzyme and those from closely related species have extremely wide substrate specificities (see article by Dalton in this volume). A wide variety of products could therefore be generated from hydrocarbons using this enzyme and these techniques.

The only other clear demonstration of product generated by electro-enzymology to date is light formation using Vibrio fischeri luciferase (Table II). However, product formation from benzphetamine, aminopurine and p-nitroanisole as a result of the demethylase activity of rabbit liver microsomal cytochrome-P450 has been reported in which a related electrochemical procedure was used to generate hydrogen peroxide electrochemically, thereby replacing the reduced coenzyme (Scheller et al., 1976, 1977; Mohr et al., 1978). Hydrogen peroxide destroys cytochrome-P450 activity but its electrochemical generation may avoid this highly active compound accumulating to damaging concentrations. The cytochrome-P450 class of monoxygenases constitutes an important group of enzymes widely distributed in nature and capable of oxidising hydrocarbons, steroids, drugs and a variety of natural products. A number of them have now been purified and immobilised (Mohr et al., 1978). This, together with the demonstration of electrochemical reduction techniques, bodes well for their future industrial exploitation. The various methods of supplying reducing power electrochemically to cytochrome-P450 enzymes are summarized in Fig. 2.

Approaches involving direct electrochemical reduction of enzyme prosthetic groups offer a unique advantage over other

\[ \text{CATHODE} \]

\[ \text{NAD(P)}^+ \]

\[ 2e^- \rightarrow \]

\[ \text{NAD(P)H} \]

\[ \text{NAD(P)H-REDOXIN} \]

\[ \text{REDUCTASE} \]

\[ \text{REDOXIN} \]

\[ \text{O}_2 \]

\[ \text{SUBSTRATE} + \text{O}_2 \]

\[ \text{PRODUCT} + \text{H}_2\text{O} \]

Figure 2. Possible routes by which reducing power may be supplied electrochemically to drive a cytochrome P-450 monoxygenase.
methods in that by the accurate control of potential it should prove possible to select a particular enzyme in a mixture. There is, therefore, the opportunity for high efficiency without high levels of enzyme purity; indeed, in some cases, crude extracts may be suitable. Any practical applications of protein electrochemistry for synthesis of chemical products will depend upon the development of suitable high surface area electrodes perhaps with adsorbed enzyme and the design of suitable electroenzymology reactors. The use of precious metal working electrodes, if they prove most suitable, is not in itself prohibitive. Extremely high surface areas can be obtained by application of ultra-thin layers to suitable supports. The source of electrons for electroenzymology need not be mains electricity. Hydrogen or water may also be used and both these configurations have been achieved using horse heart cytochrome-c as acceptor.

**BIOFUEL CELLS**

The successful development of electroenzymology as a technique for biotransformation of hydrocarbons depends upon the development of rapid, efficient electron transfer between electrode and enzyme and of high surface area electrodes. This is also true if there is to be a future for biofuel cells. The conventional hydrogen-oxygen fuel cell is now beginning to make a major contribution in the power industry in the United States as well as having a variety of specialist uses. It seems likely that the fuel cell will play increasingly important roles in the energy industry in coming years. Its great asset is that it converts the free energy of a chemical reaction into electricity with high efficiency. Since the reactions involved are ones of charge transfer and not heat transfer, they are not subject to the limitations of the Carnot cycle and practical efficiencies of 50-70% are common. The simple hydrogen-
oxygen cell is shown in Fig. 3. Oxidation occurs at the anode and reduction at the cathode, the two electrode compartments being separated by a membrane. As the hydrogen gas passes over the anode surface it is electrochemically oxidised to hydrogen ions which enter the electrolyte and migrate towards the cathode, where oxygen is reduced to water. The electrons flow through the external circuit from anode to cathode, thus providing useful work. (For detailed discussion of fuel cells see Bockris and Reddy, 1970; Bockris and Nagy, 1974; Briefer, 1969.)

At present, the hydrogen-oxygen cell is the only one with an immediate commercial future; this is because of lack of effective, stable catalysis of electron transfer from other fuels. For example, although low temperature methanol fuel cells have been developed, they are not particularly stable. Cells employing hydrocarbons including methane require very high operating temperatures as a result of the low electroactivity of these fuels. Such cells suffer corrosion problems and are inefficient.

Many biofuel cells have been demonstrated over the last seventy years in which various microorganisms have been used to supply electrons to the anode using a wide variety of substrates including waste carbohydrate, plant material, water (in the presence of light), hydrocarbons and alcohols (Lewis, 1966; Sisler, 1970; Williams, 1966). Bacteria-containing biofuel cells have been marketed commercially, one producing 40 mA at 6v., fuelled by powdered rice husks (Williams, 1966). Of particular relevance are biocells using methane (Van Hees, 1965) or higher hydrocarbons (Young, 1965) as fuels.

There are three major types of biofuel cells, i.e. product cells, depolarizer cells and regeneration cells.

Product Cell

In this type of cell, organisms or enzymes derived therefrom are used to convert compounds, which are not electrochemically active, into electroactive species. The most well known example (Table III) is the production of hydrogen from e.g. glucose.

\[
\text{Glucose} \rightarrow H_2
\]

\[
H_2 \rightarrow 2H^+ + 2e \quad \text{(Anode)}
\]

\[
2H^+ + \frac{1}{2}O_2 + 2e \rightarrow H_2O \quad \text{(Cathode)}
\]

Some other examples are given in Table III and clearly many possibilities exist for the conversion of inexpensive surplus materials into electroactive species. Whether or not these processes can be competitive with other methods of hydrogen production is speculative at this stage but in some situations they may prove attractive.

Depolarizer Cell

In this type of cell, organisms or enzymes act as depolarizers or catalysts of simple electrochemical reactions such as $H_2$ oxidat-
### TABLE III. Product type biofuel cells

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Organisms</th>
<th>Electroactive product</th>
<th>Remarks</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Clostridium Welchii</td>
<td>H₂</td>
<td>3.5V</td>
<td>1</td>
</tr>
<tr>
<td>Maltose</td>
<td>?</td>
<td>C₂H₅OH</td>
<td>?</td>
<td>2</td>
</tr>
<tr>
<td>Glucose</td>
<td>Yeast</td>
<td>C₂H₅OH</td>
<td>V&lt;sub&gt;open&lt;/sub&gt;=0.17-0.8V</td>
<td>3</td>
</tr>
<tr>
<td>Hydrocarbon</td>
<td>Nocardia salmonicolor</td>
<td>H₂</td>
<td>V&lt;sub&gt;open&lt;/sub&gt;=0.115V (without H acceptor)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+0.245V (with H acceptor)</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>Clostridium butyricum</td>
<td>H₂</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>H₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacillus pasteurii</td>
<td>NH₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urease</td>
<td>NH₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L-amino acid oxidase</td>
<td>NH₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aeromonas formalis</td>
<td>HCOOH</td>
<td>40mA/cm²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>HCOOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H₂S forming bacteria</td>
<td>H₂S</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>Micrococcus cerifaciens strain</td>
<td>H₂</td>
<td>V&lt;sub&gt;open&lt;/sub&gt;=0.2V</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>max 3.5mA/cm²</td>
<td></td>
</tr>
</tbody>
</table>

See p. 193 for references

ion or O₂ reduction. Hydrogenase, as a hydrogen oxidation reaction catalyst is the most obvious example (Berezin et al., 1975; Kimura et al., 1972).

**Hydrogenase**

\[
\text{H}_2 + 2\text{H}^+ + 2\text{e} \rightarrow \text{H}_2\text{O} \quad \text{(Anode)}
\]

\[
2\text{H}^+ + \frac{1}{2}\text{O}_2 + 2\text{e} \rightarrow \text{H}_2\text{O} \quad \text{(Cathode)}
\]

This system has been used as an assay method for hydrogenase activity (Kimura et al., 1972) since the activity is proportional to the short circuit current of the cell. It will be important to isolate enzymes with high turnover numbers that are thermally stable if they are to be employed in fuel cells. Whether they can be immobilized to provide enzyme modified electrodes remains to be demonstrated.

**Regenerative Cell**

In such devices, organisms, disrupted cells or enzymes are used to regenerate the redox compounds which in turn carry out the electrochemical reaction. In one example (Takahashi et al., 1970) the following reactions are involved:
TABLE IV. Regenerative biofuel cell

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Organisms</th>
<th>Redox System</th>
<th>Remarks</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolic acid or Glucose</td>
<td>-</td>
<td>Mn(ll), Mn(lll)</td>
<td>0.2mA/cm²</td>
<td>8(a)</td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
<td>Fe(III)/Fe(II)</td>
<td>0.5mA/cm²</td>
<td>8(b)</td>
</tr>
<tr>
<td>Glucose</td>
<td>E. coli</td>
<td>Fe(III)/Fe(II)</td>
<td>40mA/cm²</td>
<td>9</td>
</tr>
<tr>
<td>HON</td>
<td>Acetobacter</td>
<td>Fe(III)/Fe(II)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See p. 193 for references

Glucose-6-P + NADP $\xrightarrow{G-6-P$ dehydrogenase} D-Glucono-6-lactone-6-P + NADPH

NADPH $\rightarrow$ NADP$^+$ + H$^+$ + 2e (Anode)

$2H^+ + \frac{1}{2}O_2 + 2e \rightarrow H_2O$ (Cathode)

Other examples are given in Table IV.

In addition, there have been attempts to convert light into electricity by means of biofuel cells or rather photoassisted biofuel cells. For example, light is used to initiate the bacterial photoreaction,

$$CO_2 + 2H_2R \xrightarrow{hv} (CH_2O) + H_2O + 2R$$

Then (CH$_2$O) may be used as the fuel for a biofuel cell (Helmuth, 1967; Sisler, 1971).

Practical applications of biofuel cells include implantable cells to supply power for cardiac pacemakers (Coltan and Orahe, 1969; Foutenier et al., 1975; Ahu et al., 1976) and use in space vehicles (McNeil, 1969). The use of immobilised bacteria has also been applied to biofuel cells. For example, gel-entrapped Clostridium butyricum organisms have been used to convert glucose to hydrogen which is used as a fuel, 0.6 mA and 0.4 V being achieved by using 0.4 g wet weight of bacteria (Karube et al., 1977).

All examples given so far have been concerned with the anodic reactions. However, the main inadequacy in the electrochemistry of the hydrogen-oxygen fuel cell concerns the higher overvoltage, i.e. slow reaction of oxygen reduction at the cathode. Since we have demonstrated the reversible electrochemical reduction of cytochrome-c at a 4,4'-bipyridyl modified gold electrode (Eddowes and Hill, 1977, 1979), we have applied this system to a depolarizer cell for oxygen reduction, using cytochrome oxidase, thus:

$$4\text{Cyt-c} + 4e \rightarrow 4\text{Cyt-c (RED)} \quad \text{(Cathode)}$$

$$4\text{Cyt-c (RED)} + \text{Cyt-ox} \rightarrow 4\text{Cyt-c} + \text{Cyt-ox (RED)}$$

$$\text{Cyt-ox (RED)} + O_2 + 4H^+ \rightarrow \text{Cyt-ox} + 2H_2O$$

$$2H_2 \rightarrow 4H^+ + 4e \quad \text{(Anode)}$$
OVERALL REACTION: \[ 2\text{CH}_4 + 2\text{O}_2 \rightarrow \text{CH}_3\text{OH} + \text{HCOOH} + \text{H}_2\text{O} \]

\[ 4\text{e}^- + 2\text{O}_2 \rightarrow 4\text{H}^+ \]

\[ \text{ELECTRICAL ENERGY} \]

**Figure 4. Hypothetical enzyme fuel cell (C is a hydrogen carrier)**

In this way we have achieved an open circuit potential of 600mV, although the current obtained is still low. We have also applied the electrochemical reduction of cytochrome-c to a photoassisted biofuel cell. Electrons were derived from water using illuminated titanium oxide:

\[ 2\text{H}_2\text{O} \xrightarrow{TiO_2, \text{hv}} 4\text{H}^+ + \text{O}_2 + 4\text{e}^- \text{ (Anode)} \]

\[ 4\text{Cyt-c} + 4\text{e}^- \rightarrow 4\text{Cyt-c (RED)} \text{ (Cathode)} \]

\[ 4\text{Cyt-c (RED)} + \text{Cyt-ox} \rightarrow 4\text{Cyt-c + Cyt-ox (RED)} \]

\[ \text{Cyt-ox (RED)} + \text{O}_2 + 4\text{H}^+ \rightarrow \text{Cyt-ox + 2H}_2\text{O} \]

This is, of course, an enzyme photocell rather than a biofuel cell. An open circuit potential of 300mV was achieved and the current was proportional to the light intensity.

The development of low temperature cells using enzymes to catalyse electron transfer between electrodes and fuels with low electroactivity, especially hydrocarbons and particularly methane which can be generated from waste material by fermentation, would be an exciting project. It may even prove possible to combine electricity generation with biotransformation as depicted in Fig.4. Here methanol dehydrogenase (an enzyme widely distributed in methylo trophic bacteria) at the anode would oxidise methanol via formaldehyde to formate, yielding four electrons for the circuit (possibly via a carrier) and four protons which would diffuse to a cathode incorporating methane monooxygenase. Oxygen would be reduced to water and methane would be oxidised to methanol. The overall process would generate two useful products and
electricity. Although these ideas are attractive, the only really viable biofuel that can be constructed with existing technology is a conventional hydrogen-oxygen cell, the fuel being produced microbiologically from waste material or via biophotolysis.

Future developments in this field and in electroenzymology will depend upon advances in electrode technology and an understanding of the processes involved in electron transfer between enzymes and electrodes.

REFERENCES


Young, T. G. (1965), British Patent No. 981,803.