4
Biosensors and Biofuel Cells

W. J. ASTON AND A. P. F. TURNER

Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Beds., MK43 0AL, UK

Introduction

Revival of interest in the art of coupling biological systems with electrochemical techniques has resulted from a subtle blend of scientific advances and commercial requirement. The science of bioelectrochemistry has a distinguished academic lineage stemming from the classic work of Galvani on frog muscle, in the 18th century, and encompassing fundamental work on the redox potentials of biological materials fostered by Ehrlich's investigations into dye reduction by animal tissue, published in 1885. In retrospect, perhaps one of the most premonitory papers published on the subject of this review was a short work by Cohen (1931), in which he described a bacterial cell acting as an electrical half cell, using mediators such as ferricyanide and benzoquinone to shuttle electrons from the biological catalyst to an electrode. This principle forms the basis of some of the most commercially attractive bioelectrochemical systems demonstrated to date. Effective coupling of redox proteins to electronic systems by direct electron transfer has potential applications ranging from biological memories for computers to electrically driven biocatalysts for chemical synthesis, but probably the best short-term practical proposition is the development of biosensors for industrial and clinical monitoring. The technology required to produce efficient biocatalytic electrodes may also be exploited in biofuel cells, but as in the case of bio-organic synthesis, the advantages are more subtle and success may eventually depend on the vagaries of political and economic pressure.

The combination of a large number of studies revealing the elegance of biological catalysts, together with developments in the production and purification of enzymes, has led to their increasing use for analysis, therapy and industrial catalysis. Analytical applications of enzymes represent by far the largest market, the principal usage being in diagnostics and food analysis. It is difficult to estimate future trends in commercial biochemical analysis and authoritative opinions on the matter are generally coveted secrets. It is very obvious, however, that enzymatic methods of analysis have found increasing acceptance and are likely to continue to rise in popularity (*see also* Chapter 1). *Appendix A* to this chapter shows a survey of enzymes used routinely in either clinical or quality

Biotechnology and Genetic Engineering Reviews—Vol. 1, February 1984
0264-8725/84/01/89-32$10.00 + $0.00

control laboratories; all the enzymes listed are widely available and marketed either for automated procedures or as test kits. Although relatively pure enzymes with high specific activity may now be obtained at reasonable prices, significant economic advantages are offered by systems using immobilized enzymes and it is likely that these will occupy an increasing share of the market (*see* Chapter 5).

The ubiquity of the spectrophotometer in conventional assays (*Appendix A*) is meeting mounting opposition from electrochemical alternatives. Electrochemistry has made notable inroads into analytical instrumentation over the past two decades, matching a rapidly expanding range of applications with improved reproducibility and stability. Elegant, yet simple devices such as the ion-selective potentiometric electrode, the polarographic oxygen membrane electrode and coulometric electrochemical detectors, provide a tempting alternative to the complex instrumentation and/or methods traditionally associated with enzyme assays. The proposal, originally attributable to Clark and Lyons (1962), to combine the specificity of immobilized enzymes with such electrochemical devices to produce the enzyme electrode, has blossomed into a new multidisciplinary field. With a range of over 2000 enzymes now available, effecting dozens of readily measurable physical or chemical changes, the breadth of research activity proposed or under way is hardly surprising. While there is undoubtedly a shortage of novel sensors compatible with microprocessor control of a variety of processes and environments, it may be argued that the greatest immediate demand is for more efficient versions of the assays represented in *Appendix A*. A large number of sensors based on enzymes and whole organisms have already been proposed as alternatives to these routine assays, these generally relying on the electrochemical detection of enzyme products or substrates. It is worth reflecting, however, on a common feature of the assays shown in *Appendix A*: that all but the formino-glutamate assay involve an oxidoreductase. This key group of enzymes is widely distributed with commercially available members representing only a fraction of the redox catalysts available from nature. One premise of this contribution is that these electron transfer proteins may be more effectively coupled to a transducer by direct electrochemistry than by the widely propounded indirect methods.

In this short review it is not intended to give a complete guide to biosensors and biofuel cells, as many excellent works have already been written on the numerous possible configurations of these devices. It is hoped, however, to present a commercially aware picture of some of the most important principles and to draw attention to a few of the exciting recent developments in this rapidly expanding field (*see also* Chapter 7).

Indirect and direct systems

Bioelectrochemistry has principally involved the study of either indirect or direct interactions of biological material with an inert electrode. The practical advantages conferred on an electrochemical system by the biological element are the ability to operate at ambient temperatures and under mild chemical conditions, coupled with extraordinary catalytic specificity and high substrate affinity. While whole organisms, tissue slices and organelles have been exploited

for their stable wide-ranging activity, the ultimate goal must be to harness the specific (sometimes even stereospecific) nature of enzymes, membrane recognition sites and the manipulable properties of the antibody.

Conventional enzymatic analysis involves the determination of the concentration of either substrates or products of the catalysed reaction or of some coupled reaction (*Appendix A*). Two principal techniques have been used, depending either on dynamic or end-point measurements. In the former method, a physical or chemical change is followed continuously for a short period, giving a rapid result. The initial velocity of the reaction is dependent on enzyme concentration, activity, substrate affinity and the concentration of non-saturating substrates. In the second method relatively large amounts of enzyme are used and the reaction is allowed to reach equilibrium, rendering the techniques relatively insensitive to physical and chemical conditions affecting enzyme activity. Whereas the latter technique is favoured for substrate determination, the former may be applied to inhibitor and activator assays, although care must be taken to control other conditions that may affect the rate of reactions, especially pH and temperature. Immobilization of the biocatalyst introduces a further dimension; most importantly, such heterogeneous systems offer barriers to the free passage of molecules both to and from the enzyme, either by diffusion limitation or by some partitioning effect. Many of the advantages (and disadvantages) of the end-point assay may thus be conferred on the immobilized enzyme system by making substrate access limiting and measuring the rate of reaction. Enzyme electrodes generally rely on achieving an appropriate steady state for the required operational characteristics, by balancing diffusion of substrates and products against conversion rates (*Figure 1*). Some of the parameters that may be

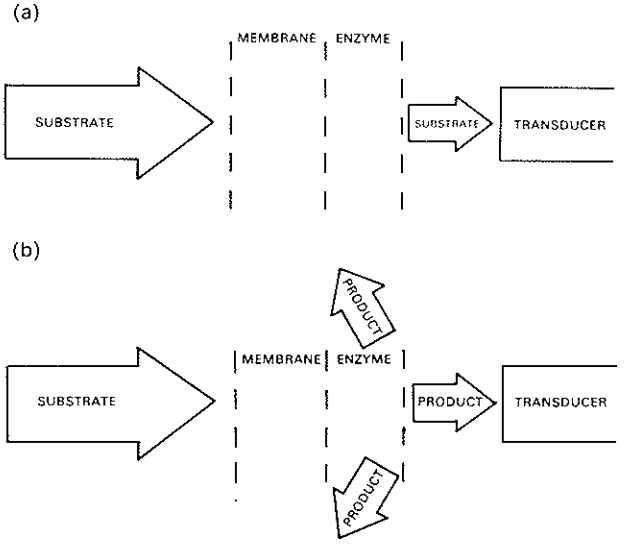


Figure 1. Two types of indirect diffusion-limited enzyme electrodes. (a) Reduction in substrate concentration determined, e.g. oxygen consumption, catalysed by an oxidase, monitored polarographically. (b) Product formation determined, e.g. ammonia liberation, catalysed by urease, monitored with an ion-selective electrode.

manipulated are discussed more fully by Carr and Bowers (1980); the principal advantage of diffusion-limited enzyme electrodes is stability, but this is achieved at the expense of increased response times.

The enzyme electrodes shown in *Figure 1* represent the vast majority of previously proposed systems, consisting of an enzyme immobilized on a membrane and placed in the vicinity of a transducer. These sensors may be considered to be indirect systems because the enzyme-catalysed reaction and the electrochemical sensor involve two quite discrete reactions. A wide variety of secondary transducers have been proposed for use in biosensors (*Table 1*)

Table 1. Some transducers used in indirect enzyme electrodes.

Transducer	Species detected
Amperometric electrodes	O_2, H_2O_2, NADH, I_2
Ion-selective electrodes	H^+, NH_4^+, NH_3, CO_2, I^-, CN^-
Field-effect transistors	H^+, H_2, NH_3
Photomultiplier (in conjunction with fibre optics)	Light emission or chemiluminescence
Photodiode (in conjunction with a light-emitting diode)	Light absorption
Thermistor	Heat of reaction
Piezoelectric crystal	Mass adsorbed

based on both physical and chemical measurements. The end-product of these devices is an electric current and yet in many cases the enzyme-catalysed reaction of interest involves electron transfer (*Appendix A*) and could theoretically be coupled directly to an electrode or semi-conductor device. In order to achieve such direct electron transfer from a redox protein to an electrode, the electron acceptor must replace one of the natural redox partners in the biological system. A variety of possibilities exist for achieving direct interactions (*Figure 2*), the most elegant and desirable solution being an electrode surface that resembles the natural partner. Practical electrodes based on this premise have been difficult to achieve. The demonstration of rapid and reversible electron transfer between cytochrome *c* and a gold electrode modified with 4,4′-bipyridyl (Albery *et al.*, 1981), however, provided the basis for a variety of bioelectrochemical systems, based on cytochrome *c* as a redox partner for oxidoreductases. Other mechanisms shown in *Figure 2* illustrate some practical alternatives to the ideal solution. The principle of using a solution mediator of low molecular weight to shuttle electrons from biological systems to electrodes has been known for many years, but has recently been shown to provide the basis for highly sensitive assays for methanol (Plotkin, Higgins and Hill, 1981; Aston *et al.*, 1983) and microbial activity (Turner, Ramsay and Higgins, 1983).

The main problems associated with the use of mediators are retention of these small molecules at the electrode surface and their potential reactivity giving rise to a source of interference. Immobilization of the mediator, either on the electrode surface or on the enzyme, solves retention problems (*Figure 2*). Reactivity of the mediator, especially with oxygen, remains a more serious problem, however, necessitating anaerobic conditions for efficient electron transfer from most mediators to electrodes. Exceptions to this rule are various derivatives of the organometallic compound, ferrocene, which form the basis of a recently

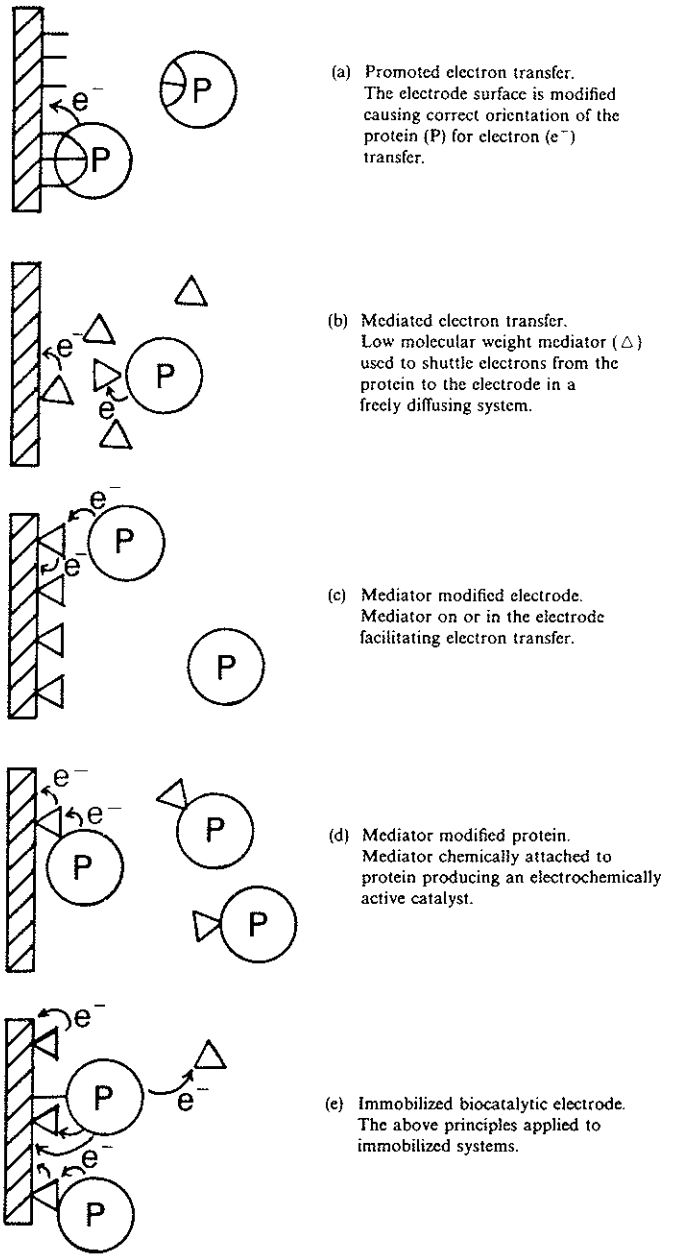


Figure 2. Some possible configurations facilitating direct electron transfer from a redox protein to an electrode.

patented series of oxygen-insensitive devices (Higgins, Hill and Plotkin, 1983; Aston *et al.*, 1983).

The advantages of the tightly coupled direct enzyme-based sensor lie in its simplicity, cheapness, reliability and accuracy. Interferences are restricted to those electrochemically active substances that reach the electrode surface and can be minimized by the use of membranes and low working potentials. The production of an effective biocatalytic electrode, however, finds wider application than sensors: biofuel cells may also be considered in the two classes of indirect and direct. The most highly developed indirect biofuel cell reported to date relies on the microbial production of hydrogen and its subsequent oxidation in a conventional hydrogen/oxygen fuel cell (Suzuki, Karube and Matsunaga, 1979). Cells based on direct electrochemistry potentially offer advantages in simplicity and cost, but limitations imposed by the need to achieve high current densities may necessitate the use of soluble mediators (Davis *et al.*, 1983). The limitation on current is imposed by the large size of protein molecules (and even larger size of micro-organisms) which have to interact with the surface of the electrode. Similar considerations are intrinsic to the successful application of direct electrochemistry for chemical synthesis. Biotransformations of particular relevance to the future of the chemical industry are a variety of alkane, aromatic and steroid hydroxylations catalysed by external mono-oxygenases (Higgins, Best and Hammond, 1980). A major problem in the exploitation of these enzymes, however, is their requirement for reduced cofactors, usually NADH. One proposed solution involves the direct electrochemical reduction of the enzyme prosthetic group (Higgins and Hill, 1978; 1979). For both chemical and energy production, however, the overriding factor will be the relative economics of the proposed processes, with the balance undoubtedly proving more subtle than the clear case for biosensors.

Indirect biofuel cells

The present state of development of biofuel cells has been excellently discussed by Wingard, Shaw and Castner (1982). Indirect biofuel cells typically involve the microbial production of an electroactive species such as hydrogen by anaerobic fermentation and the subsequent oxidation of the gaseous fuel at the anode of a conventional hydrogen/oxygen fuel cell (Suzuki *et al.*, 1980a, b):

at the anode

$$H_2 \rightarrow 2H^+ + 2e^- \qquad E^0 = 0\,V$$

at the cathode

$$2H^+ + 2e^- + 1/2O_2 \rightarrow H_2O \qquad E^0 = 1{\cdot}23\,V$$

overall

$$H_2 + 1/2O_2 \rightarrow H_2O \qquad E^0 = 1{\cdot}23\,V$$

Whereas the electrochemical conversion may be highly efficient, there are inevitable losses associated with the microbial transformation of the fuel to

hydrogen. The microbial component of the system, however, effectively broadens the range of fuels available to the fuel cell and is particularly suited to the utilization of dilute aqueous fuels or wastes. Despite recent efforts to achieve a commercially viable indirect biofuel cell, however, continuous net power production from such a device remains to be reported. The principal hurdle to be overcome is the high energy input in the form of stirring, gassing and temperature control required for the continuous efficient microbial production of hydrogen. Biochemical processes may also be adapted to sustain the cathodic reaction; photosynthetic systems from plants and algae may be used to produce oxygen for a conventional fuel cell (Suzuki *et al.*, 1980a). Alternatively, chloroplasts may be coupled directly to an electrode via a mediator to produce electricity (Pan, Bhardwaj and Gross, 1983).

The concept of a 'hydrogen economy' has been widely debated (McAuliffe, 1980; McGown and Bockris, 1980; Beghi, 1981). Such an approach is dependent upon the economic availability of large quantities of the gas and a suitable means of handling and transporting it. Hydrogen gas, even when compressed, contains relatively little energy per unit volume; the ideal storage medium would be a liquid which would readily release the gas when required. Two compounds meeting these requirements are methanol and formate, both of which provide substrates for microbial production of hydrogen and can be produced cheaply either from fossil fuels or from vegetable matter (Egorov, Recharshky and Berezin, 1981). Hydrogen may be burnt, providing pollution-free power to generate electricity, but the efficiency of this process is limited according to the Second Law of Thermodynamics. The advantage of direct energy conversion was recognized as early as 1894 (Oswald), with the proposal that fuel oxidation could be performed at an electrode, effecting the direct conversion of chemical energy to electric current without heat losses. The hydrogen/oxygen fuel cell is now relatively well developed, being used in certain situations for domestic power generation (Daggitt, 1982). Despite intense research effort, however, suitable inorganic catalytic electrodes for the oxidation of other fuels remain elusive. One obvious goal is the efficient direct electrochemical oxidation of methanol, avoiding hydrogen as an intermediate.

Experiments performed in the early part of this century first showed that microbial cultures could develop electrode potentials directly (Potter, 1911). Two half cells containing glucose were connected by a salt bridge. Addition of yeast to one side resulted in an observable current. Yudkin (1935) studied the redox potentials of washed suspensions of a facultative anaerobic, a strict anaerobic and a strict aerobic bacterium. Although these studies did not involve electron transfer to an external circuit to derive useful energy, they did show a good correlation between redox potentials measured at an electrode and those determined using redox dyes. The potentials developed, however, were attributed to electromotively active molecules capable of passing through a collodion sac and not to any direct interaction between the bacteria and the electrode. Interest in the subject of biofuel cells was revised in the 1960s by NASA in the United States. Work was primarily concerned with microbial cells and fermentation broths within the anodic compartment (Austin, 1967; Cenek, 1968), but performance was poor when compared with that of inorganic fuel cells (Rourback,

Scott and Canfield, 1962). A number of patent applications were granted for biofuel cells both in England and America during the 1960s, including alcoholic fermentation by yeast (Hunger and Perry, 1966), hydrocarbon oxidation by bacteria (Young, 1965), hydrocarbon oxidation by actinomycetes (Davis and Yarborough, 1967) and methane oxidation by *Pseudomonas methanica* (Van Hees, 1965). The latter type of cell was capable of developing a maximum power density of 2·8 W/cm^2 at 0·34 volts.

Explanations of the mechanism by which current is generated in this group of bioelectrochemical fuel cells fall into two categories: one possibility is that the bioelectrode reaction is achieved through the discharge of organic substrates irrespective of the presence of bacteria; the other requires active participation of the biological system in the bioelectrode reaction. It has been shown (Disalvo, Videla and Arvia, 1979) that the kinetics of the bioelectrode system are due to two consecutive reactions which occur at the electrode surface. The early work of Yudkin (1935) has been related to these more recent systems by Videla and Arvia (1975) who showed that current was still produced in these biofuel cells when the biologically active material was isolated from the electrode, although the current was reduced due to ohmic and diffusional effects. While it is not clear exactly what electrochemical reactions were responsible for the currents observed, it is likely that the majority of previously reported glucose-powered (Wingard, Shaw and Castner, 1982) and hydrocarbon-powered (Higgins *et al.*, 1980) biofuel cells did not operate by direct electron transfer from the catalyst to the electrode. It is more likely that changes in dissolved oxygen concentration (where appropriate) and formation of electroactive products such as hydrogen and ammonia were responsible for the currents observed.

Direct biofuel cells

An alternative to electrochemical oxidation of microbial products as a basis for a biofuel cell is the development of promoted or mediated systems. The attraction of fuel cells lies in their thermodynamic efficiency with their energy output being dependent merely on the difference in Gibbs free energy (ΔG) between the reactants and the products, with small losses due to entropy effects. The net release of Gibbs free energy is related to the reversible potential difference (E) between the two half cells and the number of electrons transferred per mole of fuel (n) according to:

$$-\Delta G = n\,\mathrm{FE}$$

where F = the Faraday Constant.

The use of either mediators or redox proteins as intermediates in the electron transfer from substrate to electrode effectively reduces the potential difference between the two half cells, since the redox potential seen by the electrode is that of the last component of the chain. The efficiency of electron transfer (coulomic efficiency) using intermediates, however, can be high (Davis *et al.*, 1983; Roller *et al.*, 1983) and they facilitate reasonable current densities otherwise

hampered by the large size of biological catalysts (especially whole organisms). The theoretical output of a fuel cell is also proportional to the number of electrons (n) transferred per mole of substrate. Complete biological oxidation of complex substrates, such as dairy wastes or sewage, involves numerous enzymatic steps and is therefore more efficiently carried out by whole organisms containing an effective package of the correct enzymes. The complete oxidation of methanol, however, is catalysed by just two enzymes, methanol dehydrogenase (MDH) and formate dehydrogenase (FDH):

$$\text{Methanol} \xrightarrow{\text{MDH}} \underset{+\,2H^+ + 2e^-}{\text{Formaldehyde}} \xrightarrow[H_2O]{\text{MDH}} \underset{+\,2H^+ + 2e^-}{\text{Formate}} \xrightarrow{\text{FDH}} \underset{2H^+ + 2e^-}{CO_2}$$

and is probably the most commercially interesting enzyme-based fuel cell investigated to date.

The first clear example of a direct biofuel cell may be attributed to Cohen (1931) who demonstrated that a current of 2 mA at a potential of 35 V could be produced by connecting several bioelectrochemical cells in series. Interest during the 1960s in the United States was focused on the biofuel cell as a method of efficient energy production for silent military generators and vehicle power supplies (Huff and Orth, 1960) and as an implantable power source for cardiac pacemakers (Drake, 1968). Methylene blue was shown to act as a mediator for glucose oxidase in a glucose-powered biofuel cell (Davis and Yarbrough, 1962; Scott and Cohn, 1962) and hydrogenase activity was coupled to an anode using either methylene blue or methyl viologen (Mizuguchi, Suzuki and Takahashi, 1966). More recently high coulomic efficiencies in the region of 90% have been reported using dichloroindophenol with glucose oxidase (Weibel and Dodge, 1975). The methanol biofuel cell has been developed and studied in our laboratory (Plotkin, Higgins and Hill, 1981; Turner, Higgins and Hill, 1982; Aston *et al.*, 1983; Davis *et al.*, 1983) using the quinoprotein methanol dehydrogenase (MDH) principally with phenazine etho-sulphate (PES) or *N*, *N*, *N'*, *N'*-tetramethyl-*p*-phenylenediamine (TMPD) as mediator. While PES gave the largest currents, TMPD-based cells were more stable, yielding a steady current output decreasing by less than 10% over a 24-hour period of continuous operation. The basic scheme of the cell is shown in *Figure 3*. The maximum current density achieved at a platinum gauze anode was 0·2 A/m^2 geometric area; power density was 20 mW/m^2 representing 14 kW/mol catalyst. Various designs have been tested and cells requiring no power input in the form of gassing or stirring have been constructed (Turner, Higgins and Hill, 1982; Aston, unpublished work). Roller *et al.* (1983) reported a biofuel cell based on whole organisms for utilization of lactose wastes, using thionine to mediate electron transfer from *Escherichia coli* to the anode and the reduction of ferricyanide at the cathode. The 20 ml cell yielded approximately 0·4 mW for over two weeks with continuous additions of lactate. While an equivalent-sized MDH fuel cell might be expected to yield 10 mW, whole organisms offer potential advantages of wide substrate range and stability. A major disadvantage shared by all these mediated fuel cells is the reactivity of the mediators with oxygen. Substantial power losses occur if the anode compartment is not kept strictly anaerobic,

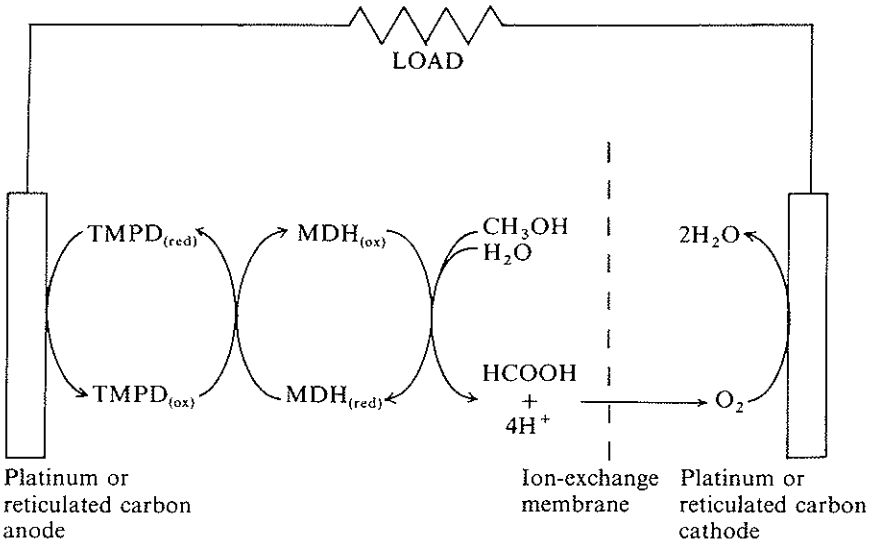
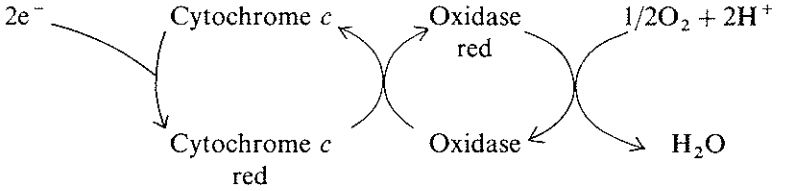


Figure 3. Methanol dehydrogenase-based biofuel cell.

because electrons are passed to oxygen as well as to the electrode (Turner, Higgins and Hill, 1982). Realistic configurations for power generation without these losses are difficult to conceive, especially as the cathodic reaction usually proposed is the reduction of oxygen to water, and proton-permeable membranes required to separate the anodic and cathodic compartments also allow the diffusion of oxygen (Turner, Higgins and Hill, 1982).

Although ferrocene and its derivatives previously were largely ignored, presumably because of their apparent insolubility and unexciting optical properties, they provide an alternative to conventional electron acceptors (Higgins, Hill and Plotkin, 1983; A. E. G. Cass *et al.*, unpublished work). Preliminary experiments have shown that these compounds will rapidly accept electrons from several oxidases and NAD-independent dehydrogenases and undergo reoxidation at carbon electrodes. Moreover, they remain stable in the reduced form allowing enzyme half cells to be constructed which show no variation in current output on changing from oxygen-free nitrogen to pure oxygen saturation. Ferrocene and its insoluble derivatives such as dimethyl ferrocene may be used to produce modified electrodes for use in immobilized systems, whereas more soluble ferrocenes, e.g. carboxyferrocene, are of interest in homogeneous configurations.

The cathodic reaction of the fuel cell has also received some attention. Reduction of molecular oxygen is catalysed by cytochrome oxidase at a gold/bipyridyl electrode (Hill, Walton and Higgins, 1981):



Laccase also catalysed the reduction of oxygen at an electrode, an effect that was enhanced by hydroquinone (Mizuguchi, Suzuki and Takahashi, 1966). The replacement of laccase with an analogous non-biological system, however, such as ammonium chloride and copper sulphate, was found to be as effective as the enzyme.

Although the power densities of biofuel cells demonstrated to date are extremely low compared with their inorganic counterparts, some practical applications may be envisaged. The potential of biofuel cells to utilize waste products such as urine, carbon dioxide and faecal material led to the proposal that they may be valuable for space programmes, producing electricity, oxygen and food while removing waste materials. Systems were devised on the basis of previous work (Sisler, 1962; McNeil, 1969) using both bacteria and algae. Bioelectrochemical fuel cells may be of significance for electricity generation in the Third World where various plant and animal wastes could be converted directly to small quantities of electricity. Specialized military needs may be met by, for example, a noiseless battery recharger operating at ambient temperatures using readily available diesel or methanol/water anti-freeze mixtures as fuels. The direct conversion of industrial wastes to electricity as part of a detoxification process may also find some application, one particularly interesting example being the carbon monoxide biofuel cell (Turner, Higgins and Hill, 1982). It is unlikely that biological fuel cells will offer realistic alternatives for general power transduction, but they can operate under peculiarly mild and dilute chemical conditions.

Indirect biosensors

The obvious appeal of straightforward and inexpensive measurement of industrial and clinical biochemicals has led to a rapid expansion of the basic principle of combining immobilized biological material with a secondary detector (*Figure 4*). Surprisingly, the bacterial electrode was suggested as late as 1975 (Diviès), but its development has since been intensely pursued, particularly in Japan (Suzuki, Satoh and Karube, 1982). Equally, the range and form of secondary detectors has expanded, aided particularly by concurrent interest in ion-selective electrodes and semi-conductor devices. Recent reviews on biosensors (Carr and Bowers, 1980; Kobos, 1980; Wingard, Katzir and Goldstein, 1981; Guilbault, 1980, 1982; Suzuki, Satoh and Karube, 1982; Lowe, Goldfinch and Lias, 1983; Mosbach, 1983) detail the numerous configurations of biological catalysts coupled with either potentiometric, amperometric, calorimetric or photometric transducers that have been reported in the literature. *Appendix B* of this chapter shows some examples of these.

Clark and Lyons (1962) coined the term 'enzyme electrode' when they proposed that glucose oxidase could be held between two cuprophane membranes at a polarographic oxygen electrode and glucose concentration determined by measuring the oxygen consumption. Updike and Hicks (1967) reported a more practical system where glucose oxidase was immobilized in a polyacrylamide gel over an oxygen electrode and a second electrode containing heat-inactivated enzymes was introduced to correct for fluctuations in oxygen tension

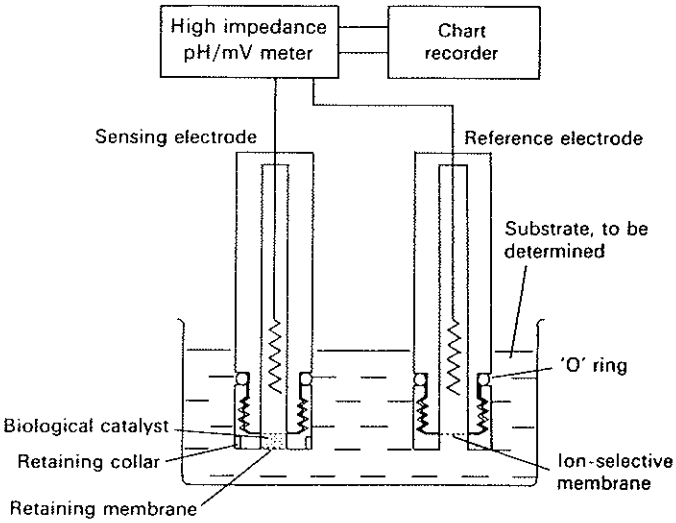


Figure 4. Schematic diagram of a typical ion-selective electrode biosensor.

and interfering substances. When the enzyme was placed in contact with a biological solution or tissue, glucose and oxygen diffused into the gel layer of immobilized enzyme. Instability of the Clark electrode, however, was caused by hydrostatic pressure variations, through loosening of the membranes (Severinghaus, 1968) and fluctuations in oxygen tension, although the latter may be overcome by supplying sufficient oxygen. (Rossette, Froment and Thomas, 1979). Oxygen-based sensors have been used industrially to monitor a variety of oxidase substrates (Karube *et al.*, 1977; Okada, Karube and Suzuki, 1981; Karube, Okada and Suzuki, 1982).

Hydrogen peroxide, a product of the enzymatic oxidation of glucose, is itself electrochemically active and may be measured in as low as picomolar concentrations at an electrode (Sittampalam and Wilson, 1982). Enzyme electrodes based on this system have been developed for both *in vitro* (Chua and Tan, 1978) and *in vivo* blood glucose analysis (Shichiri *et al.*, 1982). Glucose oxidase from *Aspergillus niger* has been shown to contain a glycoprotein structure (Pazur, Kleppe and Cepure, 1965) conferring resistance to inactivation by sodium dodecyl sulphate, urea and heating (Nakamura, Hayashi and Koya, 1976), but the enzyme is rapidly inactivated by hydrogen peroxide (Greenfield, Krittrell and Laurence, 1975) which must be removed from an enzyme electrode. The intrinsic dependence of the reaction on oxygen also presents a problem for process control and *in vivo* applications, where oxygen tensions may fluctuate. The production of oxygen by electrolysis has been used to produce an oxygen-stabilized enzyme electrode for glucose analysis (Enfors, 1981). Electrochemical interference from substances such as ascorbic acid and drugs can occur (Farrance and Aldons, 1981; Lidh *et al.*, 1982), but much of this may be eliminated by electrode modification such as the incorporation of ascorbate oxidase (Nagy, Rice and Adams, 1982) used for *in vivo* electrochemical analysis of catecholamines, or the use of membranes (Newman, 1976; Lobel and Rishpon, 1981).

Acetyl cellulose membranes, for example, enhance peroxide diffusion under alkaline conditions and may effectively be used between the enzyme and the electrode to reduce ascorbate interference. Alternatively, ascorbate may be assayed concurrently using an enzyme-based ascorbate sensor (Schenic, Miller and Adams, 1982). Hydrogen peroxide production may be linked to iodide oxidation (Nanjo, von Strop and Guilbault, 1973), catalysed by molybdate ions (Malmstadt and Pardue, 1961; Pardue, 1963) or a peroxidase enzyme, allowing the use of an iodine-selective electrode (Llenado and Rechnitz, 1973; Nagy, Rice and Adams, 1982).

The use of ion-selective electrodes has increased enormously over the last few years (Fricke, 1980; Freiser, 1980); their mode of operation and basic principles are well documented (Koryta, 1975; Moody and Thomas, 1975; Gammann, 1977). They have been applied successfully to physiological monitoring of blood sodium, potassium, calcium and lithium (Berman, 1974; Meir *et al.*, 1980; Zhukov *et al.*, 1981). Modification of ion-selective membrane materials (Rechnitz, 1967) has led to the use of liquid membrane electrodes for *in vivo* analysis of chlorpheniramine (Fukamachi and Ishibashi, 1978), novocaine (Negoiu, Ionescu and Cosofret, 1981), sulpha drugs (Ionescu *et al.*, 1981), naproxinate (Hogue and Landgraf, 1981), codeine and morphine (Goiha, Hobai and Rozenberg, 1978). Miniaturization of ion-selective electrodes has enabled research to be directed towards microscale enzyme electrodes (Brown and Flaming, 1974) which will provide a valuable tool in medicine, biology and physiological research (Gammann, 1977). With the exception of the Corning liquid membrane acetylcholine electrode (Baum and Ward, 1971), all biosensors based on ion-selective electrodes function by the indirect determination of ion formation or utilization. An example (*see Appendix B* for further examples) is the urea sensor incorporating urease (Katz and Rechnitz, 1963; Guilbault and Montalvo, 1970; Guilbault and Nagy, 1973a; Herman and Rechnitz, 1975). Two products are formed, both of which may be assayed using an ion-selective electrode.

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$$

Ammonium ions may be measured using an ammonium-selective liquid membrane electrode (Montalvo and Guilbault, 1969) and carbonate ions determined using a carbonate liquid membrane electrode. These two methods have the disadvantage that they are susceptible to interference caused by other ions present in biological samples (Katz and Cowans, 1965). This interference may be overcome by the use of gas-sensitive electrodes and air gap electrodes, which have been the subject of excellent reviews serving to describe the study, design and use of gas-sensing electrodes (Bailey and Riley, 1975; Fricke, 1980). Air gap electrodes introduced in the urease assay were later refined for measurements in both serum and whole blood (Hansen and Růzuičha, 1974), measuring the ammonia evolved upon exposure to strong alkali. A more rapid and reliable one-step assay was developed (Guilbault and Tarp, 1974) using immobilized urease. The other product under acidic conditions, carbon dioxide, may also be measured using a gas-sensitive electrode (Guilbault and Shu, 1972). These methods are not affected by ions present in the measuring solution and exhibit

superb selectivity, although hydrogen ions may affect the equilibrium (Ross, Riseman and Krueger, 1974).

The enzyme electrode generally offers superior specificity to that given by sensors based on whole organisms and tissue. Workers in Japan, however, have shown that a variety of practical devices may be based on immobilized whole organisms at an oxygen electrode (Suzuki, Satoh and Karube, 1982). Judicious selection of organisms and membrane configurations has yielded devices that are sufficiently selective for use in a variety of industrial processes, particularly for fermentation control. Whole organisms also provide the opportunity to exploit enzymes that become unstable on purification, for example methane mono-oxygenase (Higgins, Best and Hammond, 1980).

While the amperometric oxygen electrode and potentiometric ion-selective electrode have attracted the most attention as secondary transducers for biosensors, considerable effort has been expended on other systems. The technique of microcalorimetry has been studied in relation to automated analysis for clinical and process control situations resulting in the development of the enzyme thermistor (Mosbach, 1983). The ion-selective field effect transistor (ISFET) has been used in place of traditional probes to detect product formation in enzyme-based sensors (Janata and Huber, 1980; Danielsson *et al.*, 1983) and miniaturized optical systems have been devised using light-emitting diodes combined with photodiodes to detect colorimetric reactions in transparent films (Lowe, Goldfinch and Lias, 1983). These developments are indicative of a trend towards the fusion of biological systems and electronics in an attempt to achieve low-cost miniaturized sensors. It is questionable, however, whether these relatively complex indirect systems will provide the key to computer-compatible microbiosensors.

Direct biosensors

The advantages in simplicity, cost and accuracy of achieving a direct interaction between biological recognition sites and electronic systems has focused attention on this area. As in the case of earlier sensors, effort has polarized into potentiometric and amperometric approaches, the former involving charge and capacitance effects with the latter exploiting direct electron transfer.

Yamamoto and Hiroshi (1981) have described immunosensors based on immobilized antibodies at a platinum electrode. Small potential changes were observed when the antigen was bound. This type of interaction may be more sensitively monitored using chemically sensitive field effect transistors (CHEMFET) with a polarized interface to measure the interfacial charge density resulting from the immunochemical reaction (Janata and Huber, 1980). Immunochemically sensitive field effect transistors (IMFET) consist of either antibodies or antigens covalently attached to an inert hydrophobic layer at the gate of an FET. The insulated gate FET measures the change in charge resulting from the formation of the antibody/antigen complex. Considerable effort is being expended to achieve reliable commercial devices based on this principle, with some attention also being paid to measuring charge differences associated with the formation of enzyme/substrate complexes.

While direct amperometric sensors may be made immunosensitive by protein binding interfering either with an electrochemical or a coupled enzymatic reaction, the most obvious application of this technique is for the assay of whole organisms, oxidoreductases and their substrates. The output of direct biofuel cells, both enzyme-based (Turner, Higgins and Hill, 1982) and those based on whole organisms (Turner, Ramsay and Higgins, 1983), is proportional to the concentration of the biological catalyst and this can be used as an assay technique. In place of the fuel cell configuration, a potential may be applied to the biological half cell by means of a defined power source or a potentiostat. Using a standard reference such as a calomel or silver/silver chloride electrode and a mediator to facilitate electron transfer to the working electrode, a sensitive bioactivity monitor may be constructed (Turner, Ramsay and Higgins, 1983). Such a technique may be suitable for assessing microbial populations, especially when rapid methods are required and the sample is coloured or opaque, for example, fermentation monitoring and microbial contamination of milk or cutting oils. Of wider application, however, is the use of bioelectrochemical systems to detect either substrates, inhibitors or activators of the catalyst. Recent work on the use of the MDH fuel cell to detect methanol contamination in drinking water (Aston *et al.*, 1983) confirmed the remarkable sensitivity of this procedure. Using very simple equipment final concentrations of less than 10^{-3} ppm methanol were detectable, well below the limits of routine gas–liquid chromatography and mass spectrometry.

The performance of the direct enzyme-based sensor may be attributed to the tight coupling of the redox reaction allowing expression of the enzymes' high substrate affinity. Current density is far less critical in this application since it may be calculated that even a monolayer of enzyme may be expected to yield tens of microamperes per square centimetre of electrode, which is well within the sensitivity of very simple electronic metering. In addition, there is plenty of scope for miniaturization of such electrodes. More important to the commercial success of a biosensor is the development of a stable immobilized configuration, which is free from interference. Previous enzyme sensors using redox compounds of low molecular weight, such as ferricyanide, have suffered from mediator leakage and oxygen sensitivity, the latter caused by the reaction of the mediator with molecular oxygen.

There are many suitable substrates for an oxidoreductase-based sensor, some of which are detailed in *Table 1*, and others include formaldehyde, explosives, carbon monoxide, methane, NADH and NADPH. Probably the most widely beneficial and consequently highly marketable enzyme sensor, however, is for glucose. Diabetes is one of the commonest chronic diseases and affects about 6% of the adult population of the Western World (WHO, 1980). Associated complications include heart disease, strokes, amputations and blindness; these should be reduced by improved control (Albisser and Spencer, 1982). Several devices may be envisaged that would improve general diabetes management: cheaper and more reliable instruments for clinical analysis; convenient subcutaneous sensors activating a hypoglycaemia alarm and giving a continuous read-out; ultimately, a continuous sensor activating a portable insulin pump as required. Each of these applications possesses specialized problems: longevity

is paramount for *in vivo* sensors, but not essential for disposable personalized testers, whereas a fast response time is critical for sample analysis, yet less important in continuous sensors. Despite considerable research effort in the area there is, as yet, no glucose sensor which has proved to be suitable for *in vivo* use. It is hoped, however, that the use of dimethyl ferrocene modified carbon electrodes will rectify this situation (Aston *et al.*, 1983; Higgins, Hill and Plotkin, 1983). These electrodes accept electrons from immobilized glucose oxidase or NAD-independent glucose dehydrogenase, but do not react with oxygen. The electrode may be operated at low potentials (100 mV–160 mV) thus reducing electrochemical interference from blood components such as ascorbate. Prototype electrodes based on plastic strips with a coat of carbon produce a linear range of 0·1–35 mM and have a response time of less than 30 s (to 95% of maximum current).

Exploitation of the range of oxidoreductases that may be coupled to modified electrodes offers an important industrial opportunity. Not only may the substrates for these enzymes be detected, but inhibiting and activating reactions may also be monitored. The demands of micro-miniaturization are stimulating investigation of direct electron transfer from enzymes and whole organisms to materials compatible with silicon-based devices; such enzyme-effected transistors are likely to play a major part in future monitoring and control systems.

Acknowledgements

The authors are indebted to Professor I. J. Higgins (Cranfield), Dr H. A. O. Hill (Oxford University), Mr G. Davis (Oxford University) and our colleagues at Cranfield for numerous invaluable discussions and ideas. The generous sponsorship of the joint Cranfield/Oxford sensor programme by Genetics International Inc., Boston, is greatly appreciated. A. P. F. Turner is a Senior Research Fellow of the British Diabetic Association and W. J. Aston's studentship was provided by the SERC.

Appendix A. Enzymes commonly used in analysis.

Recommended name of enzyme [Systematic name in square brackets]	Used to determine	Reaction(s)	Principle
1. Acetyl-CoA synthetase [Acetate: CoA ligase (AMP-forming)] EC 6.2.1.1	Acetic acid in foodstuffs.	Acetate + CoASH + ATP $\xrightarrow{1}$ acetyl-CoA + AMP + PP_i. Malate + NAD^+ $\xrightleftharpoons{24}$ oxaloacetate + NADH + H^+ Acetyl-CoA + oxaloacetate + H_2O $\xrightarrow{10}$ citrate + CoASH + H^+	Malate dehydrogenase reaction[31] used as an indicator. Formation of NADH determined spectrophotometrically.
2. Alcohol dehydrogenase [Alcohol: NAD^+ oxidoreductase] EC 1.1.1.1	Ethyl alcohol in foodstuffs, blood, serum or plasma.	Ethanol + NAD^+ $\xrightarrow{2}$ acetaldehyde + NADH + H^+	Formation of NADH determined spectrophotometrically.
3. Alkaline phosphatase [Orthophosphoric-monoester phosphohydrolase (alkaline optimum)] EC 3.1.3.1	Lecithin in foodstuffs	Lecithin + H_2O $\xrightarrow{36}$ 1,2-diglyceride + phosphorylcholine Phosphorylcholine + H_2O $\xrightarrow{3}$ choline + P_i Choline + ATP $\xrightarrow{8}$ phosphorylcholine + ADP ADP + phospho(enol) pyruvate $\xrightarrow{37}$ pyruvate + ATP Pyruvate + NADH + H^+ $\xrightarrow{29}$ Lactate + NAD^+ + H_2O	Lecithin converted to a kinase substrate (36 & 3). ADP produced by choline kinase (8) measured using pyruvate kinase and lactate dehydrogenase (37 & 29). Oxidation of NADH determined spectrophotometrically.
4. Amyloglucosidase (Exo-1,4-α-D-glucosidase) [1,4-α-D-Glucan glucohydrolase] EC 3.2.1.3	Starch in foodstuffs	Starch + $n H_2O$ $\xrightarrow{4}$ n glucose Glucose + ATP $\xrightarrow{27}$ glucose-6-phosphate + ADP Glucose-6-phosphate + NADP $\xrightarrow{20}$ 6-phosphogluconate + NADPH	Starch converted to a dehydrogenase substrate (4 & 27). NADPH formed by glucose-6-phosphate dehydrogenase (20) determined spectrophotometrically.
5. Ascorbate oxidase [L-Ascorbate:oxygen oxidoreductase] EC 1.10.3.3	L-Ascorbic acid in foodstuffs.	Ascorbate + $\frac{1}{2}O_2$ + chromogen $\xrightarrow{5}$ dehydro-ascorbic acid + reduced chromogen	Reduced dye determined spectrophotometrically.

Appendix A (continued)

Recommended name of enzyme [Systematic name in square brackets]	Used to determine	Reaction(s)	Principle
6. Cholesterol esterase [Sterol-ester acylhydrolase] EC 3.1.1.13	Total cholesterol in serum or plasma	Cholesterol esters $\xrightarrow{6}$ cholesterol + fatty acids	Cholesterol esters converted to cholesterol (6)
		Cholesterol + O_2 $\xrightarrow{7}$ cholest-4-en-3-one + H_2O_2	Peroxide produced by cholesterol oxidase (7) reacted to produce dye.
		$2H_2O_2$ + 4-amino antipyrine + phenol $\xrightarrow{32}$ quinoeimine dye + $4H_2O$	
7. Cholesterol oxidase [Cholesterol: oxygen oxidoreductase] EC 1.1.3.6	Cholesterol in foodstuffs, serum or plasma	As above (6)	As above (6).
8. Choline kinase [ATP: choline phosphotransferase] EC 2.7.1.32	Lecithin in foodstuffs	As above (3)	As above (3)
9. Citrate (*pro*-3*S*)-lyase [Citrate oxaloacetate-lyase (*pro*-3*S*-$CH_2COO \rightarrow$ acetate)] EC 4.1.3.6	Citric acid in foodstuffs	Citrate $\xrightarrow{9}$ acetate + oxaloacetate	Citrate converted to dehydrogenase substrates (9). NADH oxidation (29) determined spectrophotometrically.
		Oxaloacetate + NADH + H^+ $\xrightarrow{31}$ malate + NAD^+	
		(Oxaloacetate $\xrightarrow{\text{decarboxylase?}}$ pyruvate + CO_2)	
		Pyruvate + $NADH^+$ $\xrightarrow{29}$ lactate + NAD^+	
10. Citrate (*si*)-synthase [Citrate oxaloacetate-lyase (*pro*-3*S*-$CH_2COO- \rightarrow$ acetyl-CoA)] EC 4.1.3.7	Acetic acid in foodstuffs	As above (1)	As above (1)
11. Diaphorase [NADH: lipoamide oxidoreductase] EC 1.6.4.3	L-Glutamic acid in foodstuffs and lactate dehydrogenase (LDH) in serum or plasma	Glutamate + NAD^+ + H_2O $\xrightarrow{22}$ NH_4^+ + 2-oxoglutarate + NADH *or*	NADH produced by a dehydrogenase determined by coupling oxidation of NADH to reduction of a dye (11).

		Lactate + $NAD^+ \xrightarrow{LDH}$ pyruvate + NADH + H^+ *THEN* NADH + H^+ + chromogen $\xrightarrow{11}$ NAD^+ + reduced chromogen	
12. 2,3-Diphosphoglyceric acid phosphatase (phosphoglyceromutase) [2,3-Bisphospho-D-glycerate : 2-phospho-D-glycerate phosphotransferase] EC 2.7.5.3	2,3-Diphosphoglyceric acid in blood	2,3-Diphosphoglycerate $\xrightarrow{12}$ 3-phosphoglycerate + P_i 3-phosphoglycerate + ATP $\xrightarrow{33}$ 1,3-diphosphoglycerate + ADP 1,3-diphosphoglycerate + NADH $\xrightarrow{25}$ glyceraldehyde-3-phosphate + NAD^+	2,3-Diphosphoglycerate converted to a dehydrogenase substrate (12 & 33). NADH oxidation (25) determined spectrophotometrically.
13. Formimino-L-glutamic acid transferase (glutamate formiminotransferase) [5-Formiminotetrahydrofolate: L-glutamate *N*-forminotransferase] EC 2.1.2.5.	Formimino-L-glutamic acid in urine	Formimino-L-glutamate + tetrahydrofolic acid $\xrightarrow{13}$ formimino-tetrahydrofolic acid + glutamate	Formation of 5,10-methenyl-tetrahydrofolic acid determined spectrophotometrically.
14. Formiminotetrahydrofolate cyclodeaminase [5-Formiminotetrahydrofolate ammonia-lyase (cyclizing)] EC 4.3.1.4	As above (13)	Formimino-tetrahydrofolic acid $\xrightarrow{14}$ 5,10-methenyl-tetrahydrofolic acid + NH_4 As above (13)	As above (13)
15. β-D-Fructofuranosidase [β-D-Fructofuranoside fructohydrolase] EC 3.2.1.26	Sucrose and glucose in foodstuffs	Sucrose + H_2O $\xrightarrow{15}$ β-D-fructose + D-glucose D-glucose + ATP $\xrightarrow{27}$ glucose-6-phosphate + ADP Glucose-6-phosphate + $NADP^+$ $\xrightarrow{20}$ gluconate-6-phosphate + NADPH + H^+	Sucrose converted to dehydrogenase substrate (15 & 27). NADP reduction (20) determined spectrophotometrically.

Appendix A (continued)

Recommended name of enzyme [Systematic name in square brackets]	Used to determine	Reaction(s)	Principle
16. α-D-Galactosidase [α-D-Galactosidase galactohydrolase] EC 3.2.1.22	Raffinose in foodstuffs	Raffinose + $H_2O \xrightarrow{16}$ Sucrose + D-galactose D-galactose + $NAD^+ \xrightarrow{18}$ galactono-γ-lactone + NADH + H^+	Raffinose converted to a dehydrogenase substrate (16). NAD reduction (18) determined spectrophotometrically.
17. β-D-Galactosidase [β-D-Galactosidase galactohydrolase] EC 3.2.1.23	Lactose and galactose in foodstuffs	Lactose + $H_2O \xrightarrow{17}$ galactose + glucose Galactose + $NAD^+ \xrightarrow{18}$ galactono-γ-lactone + NADH + H^+	Lactose converted to a dehydrogenase substrate (17). NAD reduction (18) determined spectrophotometrically.
18. Galactose dehydrogenase [D-Galactose: NAD^+ 1-oxidoreductase] EC 1.1.1.48	Raffinose (16) or lactose and galactose (17) in foodstuffs	As above (16 + 17)	Formation of NADH determined spectrophotometrically
19. Gluconokinase [ATP: D-gluconate 6-phosphotransferase] EC 2.7.1.12	D-Gluconic acid and D-glucono-δ-lactone in foodstuffs	D-Gluconate + $ATP \xrightarrow{19}$ D-gluconate-6-phosphate + ADP D-Gluconate-6-phosphate + $NADP^+ \xrightarrow{34}$ D-ribulose-5-phosphate + NADPH + CO_2 + H^+	Gluconate converted to a dehydrogenase substrate (18). NADP reduction (34) determined spectrophotometrically.
20. Glucose-6-phosphate dehydrogenase [D-Glucose-6-phosphate: $NADP^+$ 1-oxidoreductase] EC 1.1.1.49	Glucose in serum or plasma and glucose, starch (4), sucrose (15) or fructose (35) in foodstuffs. Kinase enzymes.	Glucose + $ATP \xrightarrow{27}$ glucose-6-phosphate + ADP Glucose-6-phosphate + $NADP^+ \xrightarrow{20}$ gluconate-6-phosphate + NADPH + H^+	Glucose or ATP determined by conversion to a dehydrogenase substrate. NADPH determined spectrophotometrically.
21. Glucose oxidase [β-D-Glucose: oxygen 1-oxidoreductase] EC 1.1.3.4	Glucose in blood, serum, plasma or urine	Glucose + $O_2 \xrightarrow{21}$ δ-gluconolactone + H_2O_2 H_2O_2 + chromogen $\xrightarrow{32}$ H_2O + oxidized chromogen	Oxidase used to produce peroxide (21) which reacts to give coloured product (32) determined spectrophotometrically.

22. Glutamate dehydrogenase ($NAD(P)^+$) [L-Glutamate: $NAD(P)^+$ oxidoreductase (deaminating)] EC 1.4.1.3	Urea and ammonia in serum, plasma or urine. Ammonia in foodstuffs and urine. Glutamate in foodstuffs (11).	$Urea + H_2O \xrightarrow{38} 2NH_4 + CO_2$ $NH_4 + \alpha\text{-Ketoglutarate} + NADH \xrightarrow{22} glutamate + NAD^+$	NH_4 required for reductive deamination of α-ketoglutarate. NADH oxidation determined spectrophotometrically.
23. Glutamate–oxaloacetate transaminase (aspartate aminotransferase) [L-Aspartate: 2-oxoglutarate aminotransferase] EC 2.6.1.1	L-Malic acid in foodstuffs	$Malate + NAD^+ \overset{31}{\rightleftarrows} oxaloacetate + NADH + H^+$ $Oxaloacetate + glutamate \xrightarrow{23} \alpha\text{-oxoglutarate} + aspartate$	Formation of NADH determined spectrophotometrically
24. Glutamate-pyruvate transaminase (alanine aminotransferase) [L-Alanine: 2-oxoglutarate aminotransferase] EC 2.6.1.2	Lactic acid in foodstuffs	$L\text{-Lactate} + NAD^+ \overset{28}{\rightleftarrows} pyruvate + NADH + H^+$ $Pyruvate + glutamate \xrightarrow{24} \alpha\text{-oxoglutarate} + alanine$	Formation of NADH determined spectrophotometrically.
25. Glyceraldehyde-phosphate dehydrogenase [D-Glyceraldehyde-3-phosphate: NAD^+ oxidoreductase (phosphorylating)] EC 1.2.1.12	2,3-Diphosphoglyceric acid in blood	As above (12)	NADH oxidation determined spectrophotometrically
26. Glycerol kinase [ATP: glycerol 3-phosphotransferase] EC 2.7.1.30	Triglycerides in serum or blood and glycerol in foodstuffs	$Triglycerides \xrightarrow{39} glycerol + fatty\ acids$ $Glycerol + ATP \xrightarrow{26} glycerol\text{-}1\text{-}phosphate + ADP$ $ADP + phospho(enol)\ pyruvate \xrightarrow{37} pyruvate + ATP$ $Pyruvate + NADH + H^+ \xrightarrow{29} lactate + NAD^+ + H_2O$	Triglycerides converted to a dehydrogenase substrate (30, 26 & 37). NADH oxidation determined spectrophotometrically.
27. Hexokinase [ATP: D-hexose-6-phosphotransferase] EC 2.7.1.1	Glucose in serum or plasma and glucose, starch (4), sucrose (15) or fructose (35) in foodstuffs. Creatine phosphokinase and kinase in serum or plasma.	$ATP + glucose \xrightarrow{27} ADP + glucose\text{-}6\text{-}phosphate$ $Glucose\text{-}6\text{-}phosphate + NADP^+ \xrightarrow{20} gluconate\text{-}6\text{-}phosphate + NADPH + H^+$	ATP or glucose determined by conversion to a dehydrogenase substrate. NADPH determined spectrophotometrically.

Appendix A (continued)

Recommended name of enzyme [Systematic name in square brackets]	Used to determine	Reaction(s)	Principle
28. Isocitrate dehydrogenase ($NADP^+$) [*threo*-D_S-Isocitrate: $NADP^+$ oxidoreductase (decarboxylating)] EC 1.1.1.42	D-Isocitric acid in foodstuffs	Isocitrate + $NADP^+$ $\overset{28}{\rightarrow}$ 2-ketoglutarate + CO_2 + NADPH + H^+	NADPH determined spectrophotometrically
29. Lactate dehydrogenase [L-Lactate: NAD^+ oxidoreductase] EC 1.1.1.27	Glycerol (26) or L-lactate in foodstuffs. Triglycerides (26) or lactate in blood, serum or plasma	L-Lactate + NAD^+ $\overset{29}{\rightarrow}$ pyruvate + NADH + H^+	NADH determined spectrophotometrically
30. Lipase (triacylglycerol lipase) [Triacylglycerol acylhydrolase] EC 3.1.1.3	Triglycerides in serum or plasma (26)	As above (26)	As above (26)
31. Malate dehydrogenase [L-Malate: NAD^+ oxidoreductase] EC 1.1.1.37	Malate, citrate (9) or acetate (1) in foodstuffs and aspartate aminotransferase in serum or plasma	Malate + NAD^+ $\overset{31}{\rightleftarrows}$ oxaloacetate + NADH + H^+	Reaction used as an indicator. NAD(H) followed spectrophotometrically
32. Peroxidase [Donor: hydrogen-peroxide oxidoreductase] EC 1.11.1.7	Cholesterol (6) or glucose (21) in food, serum or plasma	As above (6, 21)	Dye-linked determination of peroxide
33. Phosphoglycerate kinase [ATP: 3-phospho-D-glycerate 1-phosphotransferase] EC 2.7.2.3	2,3-Diphosphoglyceric acid in blood (12)	As above (12)	As above (12)
34. Phosphogluconate dehydrogenase (decarboxylating) [6-Phospho-D-gluconate: $NADP^+$ 2-oxidoreductase (decarboxylating)] EC 1.1.1.44	D-Gluconic acid and D-glucono-δ-lactone in foodstuffs (19)	As above (19)	As above (19)

35. Phosphoglucose isomerase (glucosephosphate isomerase) [D-Glucose-6-phosphate ketol-isomerase] EC 5.3.1.9	Fructose and glucose in foodstuffs (27)	Fructose + ATP $\xrightarrow{27}$ fructose-6-phosphate + ADP Fructose-6-phosphate $\xrightarrow{35}$ glucose-6-phosphate Glucose-6-phosphate + $NADP^+$ $\xrightarrow{20}$ gluconate-6-phosphate + NADPH + H^+	Fructose converted to a dehydrogenase substrate (27 & 35). NADPH determined spectrophotometrically.
36. Phospholipase C [Phosphatidylcholine cholinephosphohydrolase] EC 3.1.4.3	Lecithin in foodstuffs (3)	As above (3)	As above (3)
37. Pyruvate kinase [ATP: pyruvate 2-*O*-phosphotransferase] EC 2.7.1.40	Glycerol (26) or lecithin (3) in foodstuffs and triglycerides (26) in serum or blood	As above (3 & 26)	Substrate (pyruvate) produced for a dehydrogenase
38. Urease [Urea amidohydrolase] EC 3.5.1.5	Urea and ammonia in foodstuffs and urine (22)	As above (22)	As above (22)
39. Uricase (urate oxidase) [Urate: oxygen oxidoreductase] EC 1.7.3.3	Uric acid in serum or urine	Urate + O_2 + $2H_2O$ $\xrightarrow{39}$ allantoin + H_2O_2 + CO_2	Allantoin determined spectrophotometrically

Appendix B. Some typical indirect biosensors.

a) Enzyme-based sensors

Type	Enzyme	Sensor	Immobilization	Stability	Response	Linear range	Reference
Alcohol	Alcohol oxidase	O_2	Glutaraldehyde	>2 weeks	1–2 min	1–25 mg/ℓ	Verduyn, Van Dijken and Scheffers (1983)
Acetyl choline	Acetyl cholinesterase	pH	Gelatin	40 days	Several weeks	1×10^{-5}–1×10^{-4} M	Durland, David and Thomas (1978)
Ascorbic acid	Ascorbate oxidase	O_2	Collagen paste, glutaraldehyde	3 weeks	1–5 min	5×10^{-5}–5×10^{-4} M	Matsumoto, Yamata and Osajima (1981)
L-Amino acids	L-Amino acid oxidase	O_2	Glutaraldehyde	4 months	2 min	1×10^{-6} – 6×10^{-5} M	Nanjo and Guilbault (1974)
L-Asparagine	Asparaginase	NH_3	Polyacrylamide	1 month		10^{-4}–10^{-3} M	Guilbault and Hrabenkova (1971)
L-Amino acids	L-Amino acid oxidase	NH_3	Polyacrylamide	1 month	–	10^{-4}–10^{-3} M	Guilbault and Hrabenkova (1970)
Choline	Choline oxidase	H_2O_2	Glutaraldehyde	>20 days	7 seconds	0–1×10^{-4} M	Matsumoto *et al.* (1980)
β-Glucose	β-Glucose oxidase	O_2	Chemical	3 weeks	–	0·5–4% weight	Chotani and Constantinides (1982)
Glutamate	Glutamate decarboxylase	CO_2	Glutaraldehyde	>7 days	10 min	$4{\cdot}4 \times 10^{-4}$– $4{\cdot}7 \times 10^{-3}$ M	Kuriyama and Rechnitz (1981)
Glutamic acid	Glutamine decarboxylase	CO_2	Glutaraldehyde	–	18 min	0·4–>10 mg%	Ahn, Wolfson and Yao (1975)
L-Lysine	L-Lysine carboxylase	CO_2	Glutaraldehyde	500 assays	3 min	1×10^{-2} – 1·0 g/ℓ	Tran, Romette and Thomas (1983)
Oxalate	Oxalate decarboxylase	CO_2	Glutaraldehyde	1 month	8–10 min	2×10^{-4}–1×10^{-2} M	Kobos and Ramsay (1980)
Penicillin	Penicillinase	pH	Polyacrylamide	>2 weeks	15–30 seconds	10^{-4}–10^{-2} M	Papariello, Mukherji and Shearer (1973)
L-Phenyl alanine	L-Phenyl alanine oxidase and peroxidase	I^-	Chemical	>3 weeks	60–180 sec steady state 30 seconds slope	3×10^{-5}–1×10^{-4} M	Guilbault and Nagy (1973b)
Proteins	L-Amino acid oxidase	NH_3	Glutaraldehyde	>30 days	7 mins	0·1–100 μg/ml	Mascini and Giardini (1980)

Sucrose	Invertase, glucose oxidase and mutarotase	O_2	Glutaraldehyde	10 days	3–7 min	0–10 mM	Satoh, Karube and Suzuki (1976)
Sulphate	Arylsulphatase	4-Nitrocatechol	Chemical	25–50 min	5 min	10^{-6}–10^{-4} M	Cserfalvi and Guilbault (1976)
Urea	Urease	NH_4^+	Polyacrylamide	>19 days	20–40 seconds	5×10^{-4}–1×10^{-1} M	Montalvo and Guilbault (1969)

b) Whole-organism-based sensors

Type	Organism	Sensor	Immobilization	Stability	Response time	Range	Reference
Ammonia	*Nitrosomonas europea*	O_2	Physically entrapped	>2 weeks	8 min	$0{\cdot}5 \times 10^{-2}$–$1{\cdot}3$ mg/ℓ	Hikuma *et al.* (1980)
Arginine	*Streptococcus faecium*	NH_3	Physically entrapped	>20 days	20 min	5×10^{-5}–1×10^{3} M	Rechnitz *et al.* (1977)
Aspartate	*Bacterium cadaveris*	NH_3	Physically entrapped	2 days	5 min	3×10^{-4}–7×10^{-3} M	Kobos and Rechnitz (1977)
Cholesterol	*Nocardia erythropolis*	O_2	Physically entrapped	4 weeks	35–70 sec	15×10^{-6}–130×10^{-6} M	Wollenberg, Scheller and Atrat (1980)
Cysteine	*Proteus morganii*	H_2S gas-sensing electrode	Physical entrapment	6 days	5–8 min	5×10^{-5}–9×10^{-4} M	Jensen and Rechnitz (1978)
Formic acid	*Clostridium butyricum*	Fuel cell using H_2 produced	Adsorbed	>20 days	20 min	10–1000 mg/ℓ	Matsunaga, Karube and Suzuki (1980)
Glutamine	*Sarcina flava*	NH_3	Physically entrapped	2 weeks	5 min	10^{-4}–10^{-2} M	Kobos and Rechnitz (1977)
Methane	*Methylomonas flagellata*	O_2	Physical entrapment	720 days	1 min	$1{\cdot}3 \times 10^{-6}$–$6{\cdot}6 \times 10^{-3}$ M	Okada, Karube and Suzuki (1981)
Nitrate	*Azotobacter vinelandii*	NH_3	Physical entrapment	2 weeks	7–8 min	1×10^{-5}–8×10^{-4} M	Kobos, Rice and Flourney (1979)
Nitroacetic acid	*Pseudomonas* sp.	NH_3	Physical	1 month	5 min	1×10^{-4}–1×10^{-3} M	Kobos and Pyon (1981)
L-Serine	*Clostridium acidiurici*	NH_3	Physical	>3 days	3–5 min	$1{\cdot}6 \times 10^{-2}$–$1{\cdot}8 \times 10^{-4}$ M	Di Paolanionio, Arnold and Rechnitz (1981)

c) Organelle-based sensors

Substrate	Tissue or organelle	Sensor	Immobilization	Stability	Response time	Linear range	Reference
Adenosine	Mouse small intestine containing adenosine deaminase	NH_3	Glutaraldehyde	–	11 min	$3{\cdot}1 \times 10^{-4}$–1×10^{-3} M	Arnold and Rechnitz (1981)
Sulphite	Microsomes	O_2	Cellulose acetate	2 days	10 min	–	Karube *et al.* (1983)

(d) Hybrid sensors

Substrate	Biological components	Sensor	Immobilization	Stability	Response time	Linear range	Reference
Nicotinamide adenine dinucleotide	NADase and *Escherichia coli*	NH_3	Dialysis	1 week	5–10 min	$2{\cdot}5 \times 10^{-1}$–$2{\cdot}5 \times 10^{-3}$ M	Riechel and Rechnitz (1978)
Phenylalanine	Lactate oxidase and *Leuconostoc mesenteroides*	O_2	–	–	5 min (total test time 6 h)	$0{\cdot}75$–6×10^{-7} g/ml	Karube *et al.* (1980)

References

AHN, B.K., WOLFSON, S.K. AND YAO, S.J. (1975). An enzyme electrode for the determination of L-glutamic acid. *Bioelectrochemistry and Bioenergetics* **2,** 142–153.

ALBERY, W.J., EDDOWES, M.J., HILL, H.A.O. AND HILLMAN, A.R. (1981). Mechanism of the reduction and oxidation of cytochrome *c* at a modified gold electrode. *Journal of the American Chemical Society* **103,** 3904–3910.

ALBISSER, A.M. AND SPENCER, W.J. (1982). Electronics and the diabetic. *IEEE Transactions on Biomedical Engineering* **29,** 239–247.

ARNOLD, M.A. AND RECHNITZ, G.A. (1981). Selectivity enhancement of a tissue-based adenosine sensing membrane electrode. *Analytical Chemistry* **53,** 575–578.

ASTON, W.J., SCOTT, L.D., HIGGINS, I.J., TURNER, A.P.F. AND HILL, H.A.O. (1983). Enzyme based methanol sensor. In *Proceedings, Bioelectrochemistry and Bioenergetics Symposium, Nottingham, 4–9 September, 1983* (in press).

AUSTIN, L.G. (1967). *Fuel Cells, a Review of Government-Sponsored Research, 1950–1964.* NASA SP 120.

BAILEY, D.L. AND RILEY, M. (1975). Performance characteristics of gas-sensing membrane probes. *Analyst* **100,** 145–156.

BAUM, G. AND WARD, F.B. (1971). General enzyme studies with a substrate selective electrode. *Analytical Biochemistry* **42,** 487–493.

BEGHI, G., ED. (1981). *Hydrogen: Energy Vector of the Future.* Graham and Trotman Ltd, London.

BERMAN, H.J. (1974). Ion selective microelectrodes: Their potential in the study of living matter. In *Ion Selective Electrodes* (H.J. Berman and N.C. Herbert, Eds), pp. 3–11. Plenum Press, New York.

BROWN, K.T. AND FLAMING, D.G. (1974). Beveling of fine micropipette electrodes by rapid precision method. *Science* **185,** 693–695.

CARR, P.W. AND BOWERS, L.D. (1980). *Immobilised Enzymes in Analytical and Clinical Chemistry.* John Wiley, New York.

CENEK, M. (1968). Biochemical fuel cells. *US Air Force AD* 694072. *August* 1969: (translated from *Chemiche Listy* **62,** 927–974).

CHOTANI, G. AND CONSTANTINIDES, A. (1982). On line glucose analyser for fermentation studies. *Biotechnology and Bioengineering* **24,** 2743–2745.

CHUA, K.S. AND TAN, I.K. (1978). Plasma glucose measurements with the Yellow Springs Glucose Analyser. *Clinical Chemistry* **24,** 150–152.

CLARK, L.C. AND LYONS, C. (1962). Electrode systems for continuous monitoring in cardiovascular surgery. *Annals of the New York Academy of Sciences* **102,** 29–45.

COHEN, B. (1931). The bacterial cell as an electrical half cell. *Journal of Bacteriology* **21,** 18–19.

CSERFALVI, T. AND GUILBAULT, G.G. (1976). An enzyme electrode based on immobilised arylsulfatase for the selective assay of sulphate ion. *Analytica chimica acta* **84,** 259–270.

DAGGITT, G.E.G. (1982). *Fuel Cells.* Science and Engineering Research Council, Didcot, Oxon.

DANIELSSON, B., WINQUIST, F., MOSBACH, K. AND LUNDSTROM, I. (1983). Enzyme transistors. In *Biotech 83: Proceedings of the International Conference on the Commercial Applications and Implications of Biotechnology,* pp. 629–688. Online Publications, Northwood, London.

DAVIS, G., HILL, H.A.O., ASTON, W.J., TURNER, A.P.F. AND HIGGINS, I.J. (1983). Bioelectrochemical fuel cell and sensor based on a quinoprotein. *Enzyme and Microbial Technology* **5,** 383–388.

DAVIS, J.B. AND YARBOROUGH, H.F. (1962). Preliminary experiments on a microbial fuel cell. *Science* **137,** 615–616.

DAVIS, J.B. AND YARBOROUGH, H.F. (1967). US Patent 3331, 848.

DI PAOLANIONO, C.L., ARNOLD, M.A. AND RECHNITZ, G.A. (1981). Serine-selective

membrane probe based on immobilised anaerobic bacteria and a potentiometric ammonia gas sensor. *Analytica chimica acta* **128,** 121–127.

DISALVO, E.A., VIDELA, H.A. AND ARVIA, A.J. (1979). Kinetic model of a depolarisation-type bioelectrochemical fuel cell. *Bioelectrochemistry and Bioenergetics* **6,** 493–508.

DIVIÈS, C. (1975). Remarques sur l'oxidation de l'ethanol par une 'electrode microbienne' de *Acinetobacter xylinium*. *Annals of Microbiology* **126A,** 175–186.

DRAKE, R.F. (1968). *Implantable Fuel Cell for an Artificial Heart*. US Government PB 177695.

DURLAND, P., DAVID, A. AND THOMAS, D. (1978). An enzyme electrode for acetylcholine. *Biochimica et biophysica acta* **527,** 277–281.

EGOROV, A.M., RECHARSHKY, M.V. AND BEREZIN, I.V. (1981) Coal-derived liquid fuels provide hydrogen for fuel cell power vehicle propulsion. *Chemical Institute* **5,** 20–26.

ENFORS, S.O. (1981). Oxygen stabilised enzyme electrode for D-glucose analysis in fermentation broths. *Enzyme and Microbial Technology* **3,** 29–32.

FARRANCE, I. AND ALDONS, J. (1981). Paracetamol interference with YS1 glucose analyser. *Clinical Chemistry* **27,** 782–783.

FREISER, H., ED (1980). *Ion Selective Electrodes in Analytical Chemistry*, volumes 1 and 2. Plenum Press, New York.

FRICKE, G.M. (1980). Ion selective electrodes. *Analytical Chemistry* **52,** 259R–275R.

FUKAMACHI, K. AND ISHIBASHI, N. (1978). Chlorpheniramine sensitive electrode. *Bunseki Kagaku* **27,** 152–155.

GAMMAN, K. (1977). Biosensors based on ion-selective electrodes. *Analytical Chemistry* **287,** 1–9.

GOIHA, T., HOBAI, S. AND ROZENBERG, L. (1978). Liquid membrane ion selective electrodes for some alkaloids. *Farmaco (Bucharest)* **26,** 141–147.

GREENFIELD, P.F., KRITTRELL, J.R. AND LAURENCE, R.L. (1975). Inactivation of immobilised glucose oxidase by hydrogen peroxide. *Analytical Chemistry* **1,** 109–124.

GUILBAULT, G.G. (1980). Enzyme electrode probes. *Enzyme and Microbial Technology* **2,** 258–264.

GUILBAULT, G.G. (1982). Ion selective electrodes applied to enzyme systems. *Ion Selective Electrode Reviews* **4,** 187–231.

GUILBAULT, G.G. AND HRABENKOVA, E. (1970). An electrode for the determination of amino acids. *Analytical Chemistry* **42,** 1779–1783.

GUILBAULT, G.G. AND HRABENKOVA, E. (1971). New enzyme electrode probes for D-amino acids and asparagine. *Analytica chimica acta* **56,** 285–290.

GUILBAULT, G.G. AND MONTALVO, J.G. (1970). An enzyme electrode of the substrate urea. *Journal of the American Chemical Society* **92,** 2533–2538.

GUILBAULT, G.G. AND NAGY, G. (1973a). Improved urea electrode. *Analytical Chemistry* **45,** 417–419.

GUILBAULT, G.G. AND NAGY, G. (1973b). Enzyme electrodes for the determination of L-phenylalanine. *Analytical Letters* **6,** 301–312.

GUILBAULT, G.G. AND SHU, F.R. (1972). Enzyme electrodes based on the use of a carbon dioxide sensor, urea and L-tyrosine electrodes. *Analytical Chemistry* **44,** 2161–2166.

GUILBAULT, G.G. AND TARP, M. (1974). A specific enzyme electrode for urea. *Analytica chimica acta* **73,** 355–365.

HANSEN, E.H. AND RŮZUIČHA, J. (1974). Enzymatic analysis by means of the air gap electrode, determination of urea in blood. *Analytica chimica acta* **72,** 353–364.

HERMAN, H.B. AND RECHNITZ, G.A. (1975). Preparation and properties of a carbonate ion selective membrane electrode. *Analytica chimica acta* **76,** 155–164.

HIGGINS, I.J. AND HILL, H.A.O. (1978). British Patent 33388/78.

HIGGINS, I.J. AND HILL, H.A.O. (1979). Microbial generation and interconversions of energy sources. In *Proceedings, Society for General Microbiology Symposium on Microbial Technology* (A.T. Bull, D.C. Ellwood and C. Ratledge, Eds), volume 29, pp. 359–377. Society for General Microbiology, UK.

HIGGINS, I.J., BEST, D.J. AND HAMMOND R.C. (1980). New findings in methane-utilising

bacteria highlighting their importance in the biosphere and commercial potential. *Nature* **286,** 561–564.

HIGGINS, I.J., HILL, H.A.O. AND PLOTKIN, E.V. (1983). Sensor for components of a liquid mixture. European Patent Application 0078636.

HIGGINS, I.J., HAMMOND, R.C., PLOTKIN, E.V., HILL, H.A.O., UOSAKI, K., EDDOWES, M.J. AND CASS, A.E.G. (1980). Electroenzymology and biofuel cells. In *Hydrocarbons in Biotechnology* (D.E.F. Harrison, I.J. Higgins and R. Watkinson, Eds), pp. 181–193. Heyden, London.

HIKUMA, M., KUBO, T., YASUDA, T., KARUBE, I. AND SUZUKI, S. (1980). Ammonia electrode with immobilised nitrifying bacteria. *Analytical Chemistry* **52,** 1020–1024.

HILL, H.A.O., WALTON, W.J. AND HIGGINS, I.J. (1981). Electrochemical reduction of dioxygen using a terminal oxidase. *FEBS Letters* **126,** 282–284.

HOGUE, E.R. AND LANDGRAF, W.C. (1981). Naproxinate ion specific electrode: development and use. *Analytical Letters* **14,** 1757–1766.

HUFF, J.R. AND ORTH, J.C. (1960). The USACOM-MERC fuel cell. Electric power generation Program. *Advances in Chemistry Series*, volume 90, pp. 315–327.

HUNGER, H.F. AND PERRY, J. (1966). US Patent 3,284,239.

IONESCU, M., CILIANU, S., BUNACIN, A.A. AND COSSOFRET, V.V. (1981). Determination of sulpha drugs with ion selective membrane electrodes. *Talanta* **28,** 383–387.

JANATA, J. AND HUBER, R.J. (1980). Chemically sensitive field effect transistors: In *Ion Selective Electrodes in Analytical Chemistry* (H. Freiser, Ed.), pp. 107–174. Plenum, New York.

JENSEN, M.A. AND RECHNITZ, G.A. (1978). Bacterial membrane electrode for L-cysteine. *Analytica chimica acta* **101,** 125–130.

KARUBE, I., OKADA, T. AND SUZUKI, S. (1982). A methane gas sensor based on methane oxidising bacteria. *Analytica chimica acta*, **135,** 61–67.

KARUBE, I., MATSUNAGA, T., MITSUDA, S. AND SUZUKI, S. (1977). Microbial Electrode BOD Sensor. *Biotechnology and Bioengineering* **19**, 1535–1547.

KARUBE, I., MATSUNAGA, T., TERAOKA, N. AND SUZUKI, S. (1980). Microbioassay of phenylalanine in blood sera with a lactate electrode. *Analytica chimica acta* **119,** 271–276.

KARUBE, I., SOGABE, S., MATSUNAGA, T. AND SUZUKI, S. (1983). Sulfite ion sensor with use of immobilised organelle. *European Journal of Applied Biotechnology* **17,** 216–220.

KATZ, S.A. AND COWANS, J.A. (1965). Direct potentiometric study of the urea, urease system. *Biochimica et biophysica acta* **107,** 605–608.

KATZ, S.A. AND RECHNITZ, G.A. (1963). Direct potentiometric determination of urea after urease hydrolysis. *Analytische Chemie* **196,** 248–257.

KOBOS, R.K. (1980). Potentiometric enzyme methods. In *Ion-Selective Electrodes in Analytical Chemistry* (H. Freiser, Ed.), pp. 1–84. Plenum, New York.

KOBOS, R.K. AND PYON, H.Y. (1981). Application of microbial cells as multistep catalysts in potentiometric biosensing electrodes. *Biotechnology and Bioengineering* **23,** 627–633.

KOBOS, R.K. AND RAMSAY, T.A. (1980). Enzyme electrode system for oxalate determination utilising oxalate decarboxylase immobilised on a carbon dioxide sensor. *Analytica chimica acta* **121,** 111–118.

KOBOS, R.K. AND RECHNITZ, G.A. (1977). Regenerable bacterial membrane electrode for L-aspartate. *Analytical Letters* **10**, 757–758.

KOBOS, R.K., RICE, D.J. AND FLOURNEY, D.S. (1979). Bacterial membrane electrode for the determination of nitrate. *Analytical Chemistry* **57,** 1122–1125.

KORYTA, J. (1975). *Ion Selective Electrodes.* Cambridge University Press.

KURIYAMA, S. AND RECHNITZ, G.A. (1981). Plant tissue based bioselective membrane electrode for glutamate. *Analytica chimica acta* **131,** 91–96.

LIDH, M., LINDGREN, K., CARLSTROM, A. AND MOUSSON, P. (1982). Electrochemical interferences with the YSI glucose analyser. *Clinical Chemistry* **28,** 726.

LLENADO, R.A. AND RECHNITZ, G.A. (1973). Ion electrode based autoanalysis system for enzymes. *Analytical Chemistry* **45,** 2165.

LOBEL, E. AND RISHPON, J. (1981). Enzyme electrode for the determination of glucose. *Analytical Chemistry* **53,** 51–53.

LOWE, C.R., GOLDFINCH, M.J. AND LIAS, R.T. (1983). Some novel biomedical biosensors. In *Biotech 83: Proceedings of the International Conference on the Commercial Applications of Biotechnology*, pp. 633–641. Online Publications, Northwood, London.

MCAULIFFE, C.A. (1980). *Hydrogen and Energy*. Macmillan Press Ltd, London.

MCGOWN, L.B. AND BOCKRIS, J.O.M. (1980). *How to Obtain Abundant Clean Energy*. Plenum Press, New York.

MCNEIL, R.J. (1969). The development of a bioelectrochemical fuel cell for utilisation in space flight. *Proceedings of South Dakota Academy of Science* **48,** 174–187.

MALMSTADT, H.V. AND PARDUE, H.L. (1961). Quantitative analysis by an automatic potentiometric reaction rate method. Specific enzymatic determination of glucose. *Analytical Chemistry* **33,** 1040–1047.

MASCINI, M. AND GIARDINI, R. (1980). Potentiometric determination of proteins with an ammonia sensor. *Analytica chimica acta* **114,** 329–334.

MATSUMOTO, K., YAMATA, K. AND OSAJIMA, Y. (1981). Ascorbate electrode for determination of L-ascorbic acid in food. *Analytical Chemistry* **53,** 1974–1979.

MATSUMOTO, K., SEIJO, H., KARUBE, I. AND SUZUKI, S. (1980). Amperometric determination of choline with use of immobilised choline oxidase. *Biotechnology and Bioengineering* **22,** 1071–1086.

MATSUNAGA, T., KARUBE, I. AND SUZUKI, S. (1980). A specific microbial sensor for formic acid. *European Journal of Applied Microbiology and Biotechnology* **10,** 235–243.

MEIR, P.L., AMMANN, D., MORT, W.E. AND SIMON, W. (1980). Liquid membrane ion selective electrodes and their biomedical applications. In *Medical and Biological Applications of Electrochemical Devices* (J. Koryter, Ed.), pp. 13–91. John Wiley and Sons, New York.

MIZUGUCHI, J., SUZUKI, S. AND TAKAHASHI, F. (1966). Biochemical reaction cell: *Bulletin of the Tokyo Institute of Technology* **78,** 27–33.

MONTALVO, J.G. AND GUILBAULT, G.G. (1969). Sensitised carbon selective electrode. *Analytical Chemistry* **41,** 1897–1899.

MOODY, J. AND THOMAS, J.D.R. (1975). The analytical role of ion selective and gas sensing electrodes in enzymology. *Analyst* **100,** 609–619.

MOSBACH, K. (1983). New biosensor devices. In *Biotech 83: Proceedings of the International Conference on the Commercial Applications and Implications of Biotechnology*, pp. 665–678. Online Publications. Northwood, London.

NAGY, G., RICE, M.E. AND ADAMS, R.N. (1982). A new type of enzyme electrode: the ascorbic acid eliminator electrode. *Life Sciences* **31,** 2611–2616.

NAKAMURA, S., HAYASHI, S. AND KOYA, K. (1976). Effect of periodic oxidation on the structure and properties of glucose oxidase. *Biochimica et biophysica acta* **445,** 294–308.

NANJO, M. AND GUILBAULT, G.G. (1974). Enzyme electrode for L-amino acids and glucose. *Analytica chimica acta* **73,** 367–373.

NANJO, M., VON STROP, L.M. AND GUILBAULT, G.G. (1973). Enzyme electrode for glucose, based on an iodine membrane sensor. *Analytica chimica acta* **66,** 443–455.

NEGOIU, D., IONESCU, M.S. AND COSOFRET, V.V. (1981). Determination of novocaine in pharmaceutical preparation with novocaine selective membrane electrodes. *Talanta* **28,** 377–381.

NEWMAN, D.P. (1976). US Patent, No. 3979274.

OKADA, T., KARUBE, I. AND SUZUKI, S. (1981). Microbial sensor system which uses *Methylomonas* sp. for the determination of methane. *European Journal of Applied Microbiology and Biotechnology* **12,** 102–106.

OSWALD, W. (1984). Die Wissenschaftliche Elektrochemie der Gergenwart und die Technische der Zukunft, 2. *Elektrochemie* **1,** 122–125.

PAN, R.L., BHARDWAJ, R. AND GROSS, E.L. (1983). Photochemical energy conversion by a thiazine photosynthetic-photoelectrochemical cell. *Journal of Chemical Technology and Biotechnology* **33A,** 39–48.

PAPARIELLO, G.J., MUKHERJI, A.K. AND SHEARER, C.M. (1973). A penicillin selective enzyme electrode. *Analytical Chemistry* **45,** 790–792.

PARDUE, H.L. (1963). Automatic amperometric measurement of reaction rates: Enzymatic determination of glucose in serum, plasma and whole blood. *Analytical Chemistry* **35,** 1240–1243.

PAZUR, J.H., KLEPPE, K. AND CEPURE, A. (1965). A glycoprotein structure for glucose oxidase from *Aspergillus niger*. *Archives of Biochemistry and Biophysics* **11,** 351–357.

PLOTKIN, E.V., HIGGINS, I.J. AND HILL, H.A.O. (1981). Methanol dehydrogenase bioelectrochemical cell and alcohol detector. *Biotechnology Letters* **3,** 187–192.

POTTER, M.C. (1911). Electrochemical effects accompanying the decomposition of organic compounds. *Proceedings of the University of Durham Philosophical Society* **4,** 260–274.

RECHNITZ, G.A. (1967). Ion Selective Electrodes. *Chemical and Engineering News* June 12th, pp. 146–158.

RECHNITZ, G.A., KOBOS, R.K., RIECHEL, S.J. AND GEBAUER, C.R. (1977). A bioselective membrane electrode prepared with living bacterial cells. *Analytica chimica acta* **945,** 357–365.

RIECHEL, T.L. AND RECHNITZ, G.A. (1978). Hybrid bacterial and enzyme sensor with NAD response. *Journal of Membrane Science* **4,** 243–250.

ROLLER, S.D., BENNETTO, H.P., DELANEY, G.M., MASON, J.R., STIRLING, J.L., THURSTON, C.F. AND WHITE, D.R., JR (1983). A biofuel cell for utilisation of lactose wastes. In *Biotech 83: Proceedings of the International Conference on the Commercial Applications and Implications of Biotechnology*, pp. 655–663. Online Publications, Northwood, London.

ROSS, J.W., RISEMAN, J.M. AND KRUEGER, J.A. (1974). Potentiometric gas sensing electrodes. *Pure and Applied Chemistry (IUPAC)* **36,** 473–487.

ROSSETTE, J.L., FROMENT, B. AND THOMAS, D. (1979). Glucose oxidase electrode measurements of glucose in samples exhibiting high variability in oxygen content. *Clinica chimica acta* **95,** 249–253.

ROURBACK, G.M., SCOTT, W.R. AND CANFIELD, J.H. (1962). Biochemical fuel cells. In *Proceedings of the American Power Sources Conference, Fort Monmouth, New Jersey, May 1962*, volume 16, pp. 18–21. Committee of the American Power Sources Conference, Fort Monmouth, New Jersey.

SATOH, I., KARUBE, I. AND SUZUKI, S. (1976). Enzyme electrode for sucrose. *Biotechnology and Bioengineering* **18,** 269–272.

SCHENIC, J.O., MILLER, E. AND ADAMS, R.N. (1982). Electrochemical assay for brain ascorbate with ascorbate oxidase. *Analytical Chemistry* **54,** 1452–1454.

SCOTT, W.R. AND COHN, E.M. (1962). *Proceedings of the Biochemical Fuel Cell Session, Santa Monica Pic-Bat 209/5, Power Information Center, Philadelphia*, p. 9.

SEVERINGHAUS, J.W. (1968). Measurement of blood glucose, O_2 and CO_2. *Annals of the New York Academy of Sciences* **148,** 115–132.

SHICHIRI, M., KAWAMORI, R., YAMASKI, Y., HAKAI, N. AND ABE, H. (1982). Wearable artificial endocrine pancreas with needle type glucose sensor. *Lancet* **ii,** 1129–1131.

SISLER, F.D. (1962). Electrical energy from microbiological processes. *Journal of the Washington Academy of Sciences* **52,** 181–187.

SITTAMPALAM, G. AND WILSON, G.S. (1982). Amperometric determination of glucose at parts per million levels with immobilised glucose oxidase. *Journal of Chemical Education* **59,** 70–73.

SUZUKI, S., KARUBE, I. AND MATSUNAGA, T. (1979). Application of a biochemical fuel cell to wastewaters. In *Biotechnology in Energy Production and Conservation. Biotechnology and Bioengineering Symposium*, volume 8, pp. 501–511. John Wiley and Sons, Inc., New York.

SUZUKI, S., SATOH, I. AND KARUBE, I. (1982). Recent trends of biosensors in Japan. *Applied Biochemistry and Biotechnology* **7,** 147–155.

SUZUKI, S., KARUBE, I., MATSUNAGA, T. AND KAYANO, H. (1980a). Biochemical energy conversion system. *Enzyme Engineering* **5,** 143–145.

SUZUKI, S., KARUBE, I., MATSUNAGA, T., KURIYAMA, S., SUZUKI, N., SHIROGAMI, T. AND TAKAMURA, Y., (1980b). Biochemical energy conversion using immobilised whole cells of *Clostridium butyricum*. *Biochimie* **62,** 353–358.

TRAN, N.D., ROMETTE, J.L. AND THOMAS, D. (1983). An enzyme electrode for specific determination of L-lysine: Real time control sensor. *Biotechnology and Bioengineering* **25,** 329–340.

TURNER, A.P.F., HIGGINS, I.J. AND HILL, H.A.O. (1982). Biological fuel cells. In *Fuel Cells* (G.E.G. Daggit, Ed.), pp. 107–122. Science and Engineering Council, Didcot, Oxon.

TURNER, A.P.F., RAMSAY, G. AND HIGGINS, I.J. (1983). Applications of electron transfer between biological systems and electrodes. *Biochemical Society Transactions* **11,** 381–384.

UPDIKE, J.W. and HICKS, J.P. (1967). The enzyme electrode. *Nature* **214,** 986–988.

VAN HEES, W. (1965). A bacterial fuel cell. *Journal of the Electrochemical Society* **112,** 258–262.

VERDUYN, C., VAN DIJKEN, J.P. AND SCHEFFERS, W.A. (1983). A simple, sensitive and accurate alcohol electrode. *Biotechnology and Bioengineering* **21,** 1049–1055.

VIDELA, H.A. AND ARVIA, A.J. (1975). The response of a bioelectrochemical cell with *Saccharomyces cerevisiae* metabolising glucose under fermentation conditions. *Biotechnology and Bioengineering* **17,** 1529–1543.

WEIBEL, M.K. AND DODGE, C. (1975). Biochemical fuel cells: demonstration of an obligatory pathway involving an external circuit for the enzymatically catalysed aerobic oxidation of glucose. *Archives of Biochemistry and Biophysics* **169,** 146–151.

WHO (1980). *WHO Expert Committee Report on Diabetes Mellitus.* World Health Organization, Geneva.

WINGARD, L.B., KATZIR, E.K. AND GOLDSTEIN, L., EDS (1981). *Analytical Applications of Immobilised Enzymes and Cells. Applied Biochemistry and Bioengineering,* volume 3. Academic Press, New York.

WINGARD, L.B., SHAW, C.H. AND CASTNER, J.F. (1982). Bioelectrochemical fuel cells. *Enzyme and Microbial Technology* **4,** 137–147.

WOLLENBERGER, U., SCHELLER, F. AND ATRAT, P. (1980). Microbial membrane electrode for the determination of cholesterol. *Analytical Letters* **13,** 825–836.

YAMAMOTO, N. AND HIROSHI, T. (1981). Immunoelectrodes. *Kagaku* **36,** 538–542.

YOUNG, T.G. (1965). British Patent 981,803.

YUDKIN, J. (1935). The reduction potentials of bacterial suspensions. *Biochemical Journal* **229,** 1130–1135.

ZHUKOV, A.F., ERNIE, D., AMMANN, D., GUGGI, M., PRETSCH, E. AND SIMON, W. (1981). Improved lithium ion selective electrodes based on a lipophilic diamide as neutral carrier. *Analytica chimica acta* **131,** 117–122.