Bioinstrumentation and Biosensors

edited by

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Preface

This important reference text is directed to those interested in the newly emerging field of instrumentation which couples both modern biotechnology and advanced electronics. The text consists of contributed chapters prepared by experts directly carrying out research and development in this new field of bioinstrumentation and biosensors. Bioinstrumentation and biosensors are the terms used to describe the unique joining of advanced microelectronics with modern biotechnology. Chapters included here present novel biotechnology-based microelectronic instruments, such as those used for detection of very low levels of hazardous chemicals, as well as new medical diagnostic instruments. In the medical diagnostics area, bioinstrumentation and biosensors are described that provide for *direct* assay and readout of antibody/antigen binding information. Bioinstrumentation and biosensors for direct readout of such information differ substantially from present traditional immunological systems in which an electrical instrument is used simply as an observer.

Those conducting research and development work in the rapidly emerging field of biotechnology have observed the unique joining of advanced electronics with modern biotechnology. Often this merging of electronics and biotechnology has been directed toward some selected sensor. Some new biotechnology-based electronic devices are used for analysis, as a laboratory or process control instrument. Other systems relate to measurements in the body and in the environment—thus, the terms *bioinstrumentation* and *biosensors*. The point to be made is that the direct combining of biotechnology and electronics is a *new* area (otherwise, every lab instrument used for an assay or measurement in the life science field could be termed a bioinstrument or a biosensor). Perhaps a few examples will be useful.

One area involving biosensors is that of detection of very low levels of chemical agents (very toxic chemicals a foe might use to cause injury or immobilization). Because many chemical agents, by design, act on the central nervous system, it is practical to investigate enzymes that react or bind with these chemical agents (in this case, the chemical

agents are called nerve gases). If these enzymes can be made to function outside the body, they may have practical applications. It may be possible to use these enzymes as very selective sensors by combining or attaching them to electronic devices (field effect transistors, for example). Thus, when a chemical agent binds to the enzyme—attached to an electronic device—an electrical signal is given. This electrical signal can sound an alarm to indicate the presence of the particular chemical agent. Because the device so described combines modern biotechnology with advanced electronics to sense or detect something, the term *biosensor* is used.

Other chapters included in the text describe applications such as "sniffing" explosives or drugs by using the understanding of the biotechnology of the olfactory senses (i.e., smell) and directly combining this sensor into an instrument package. Also, studies into very modern high-speed computers are beginning to explore in more detail the potential for biotechnology. Thus, those developing artificial intelligence systems may soon recognize more fully the contribution that a better understanding of neurology will bring to advanced electronic systems. Further, work on using biologically based peptides as molecular-sized wires and switches is underway. These, too, may appropriately be considered the bioinstrumentation of the future.

This text will be of keen interest to a very wide audience including instrument manufacturers, the electronics industry, and many of the increasing number of firms in the field of biotechnology, especially those firms looking for additional applications of their biotechnology-based expertise. University professors and government officials, as well as industrial executives, all working in the areas of modern biotechnology, advanced electronics, and instrumentation will find this text to be extremely valuable.

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Microbial Sensors for Process and Environmental Control

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1.1 INTRODUCTION

A biosensor can be produced using an immobilized biocatalyst such as enzymes and fixing them onto an electrode. Many types of enzyme sensors have been developed and used in clinical analysis and fermentation control. Enzymes are generally expensive and unstable when used for fermentation and wastewater monitoring. Therefore, we have developed microbial sensors, composed of immobilized microorganisms used as recognition elements combined with an electrochemical device. These perform measurements using the assimilation capacity of microorganisms as an index of respiratory or metabolic activity. One example of this is the combination of an alcohol-assimilating microorganism (yeast) immobilized membrane and an oxygen electrode as the basis of an alcohol. Oxygen consumption can then be measured at an oxygen electrode. In this chapter we describe microbial sensors used in process and environmental control.

1.2 MICROBIAL SENSORS FOR PROCESS CONTROL

1.2.1 Acetic Acid Sensor[1]

In the cultivation of microorganisms with acetic acid as the carbon source, precise monitoring and control of the acetic acid concentration are essential. A microbial sensor consisting of immobilized yeasts, *Trichosporon brassicae*, a gas-permeable Teflon membrane, and an oxygen electrode was developed for the determination of acetic acid. A porous acetylcellulose membrane with immobilized microorganisms was fixed onto the surface of an oxygen electrode. This was covered with a gas-permeable Teflon membrane, and the entire arrangement was held together with a nylon net. The system consisted of a jacketed flow cell, a magnetic stirrer, a peristaltic pump, an automatic sampler, and a current recorder (Fig. 1.1).

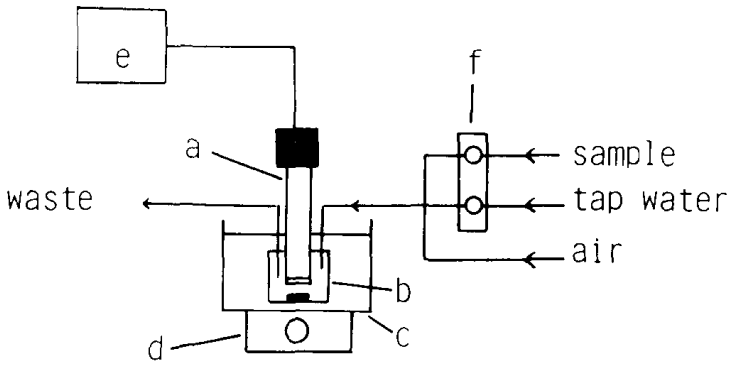

Fig. 1.1 Schematic diagram of acetic acid sensor. a, Microbial sensor; b, flow cell; c, water jacket; d, magnetic stirrer; e, recorder; f, peristaltic pump.

When the sample solution containing acetic acid entered the system, acetic acid permeated through the gas-permeable membrane to be assimilated by the microorganisms. Oxygen consumption by the microorganisms resulted in a decrease of dissolved oxygen around the membranes. The current decreased until it reached a steady state (Fig. 1.2). The total time required for an assay was 15 min.

The calibration graphs obtained showed linear relationships between the current decrease and the concentration of acetic acid up to 72 mg/L. The minimum concentration of acetic acid for the determination was 5 mg/L. The reproducibility of the current difference was within $\pm 6\%$ for an acetic acid sample containing 54 mg/L. The standard deviation was 1.6 mg/L over 20 experiments. The sensor did not respond to volatile compounds such as formic acid and methanol, or to nonvolatile nutrients such as glucose and phosphate ions.

The microbial sensor for acetic acid was applied to a fermentation broth of glutamic acid and the determined concentration of acetic acid compared with that obtained by a

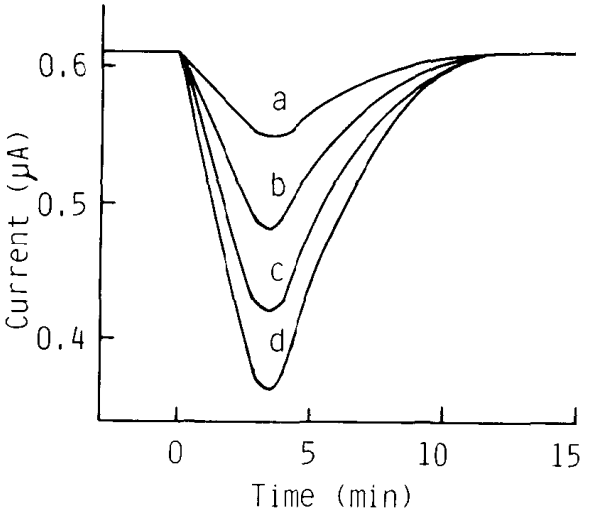

Fig. 1.2 Response curve for acetic acid. a, 18 mg/L; b, 36 mg/L; c, 54 mg/L; d, 72 mg/L.

gas chromatographic method. Good agreement was obtained and the regression coefficient was 1.04 for 26 experiments. Whole cells in the broth did not affect the electrochemical determination of acetic acid. The stability of the microbial sensor held up for more than 3 weeks and 1500 assays.

1.2.2 Alcohol Sensor[2]

On-line measurements of alcohol concentrations in culture broth are essential in fermentation processes. A microbial sensor consisting of immobilized yeast cells or bacteria, a gas-permeable Teflon membrane, and an oxygen electrode were prepared for the determination of methanol and ethanol.

T. brassicae was used for the ethanol sensor. When the sample solution containing ethanol was injected into the system, ethanol permeated through the gas-permeable membrane and was assimilated by the immobilized yeast cells. Oxygen consumption by immobilized yeast began and caused a decrease in dissolved oxygen around the membrane. As a result, the electrode current decreased markedly with time until a steady state was reached. The steady-state currents depended on the concentration of ethanol. The response time was less than 10 min at 30 °C.

The assay can also be done within 6 min by the pulse method. A linear relationship was observed between the current decrease and the concentration of ethanol below 22.5 mg/L (Fig. 1.3). The minimum detectable ethanol concentration was 2 mg/L. The reproducibility of the response was within ±6% of the relative error when a sample solution containing 16.5 mg/L of ethanol was employed. The standard deviation was 0.5 mg/L over 40 experiments.

The selectivity of the ethanol sensor was then examined. The sensor did not respond to volatile compounds such as methanol, formic acid, acetic acid, or propanoic acid. The ethanol sensor applied to fermentation broths of yeasts. The correlation coefficient obtained by gas chromatography was 0.98 for 20 experiments. Tests showed that the sensor could be used for more than 3 weeks and 2100 assays. Therefore, the sensor's stability for ethanol measurements was well displayed.

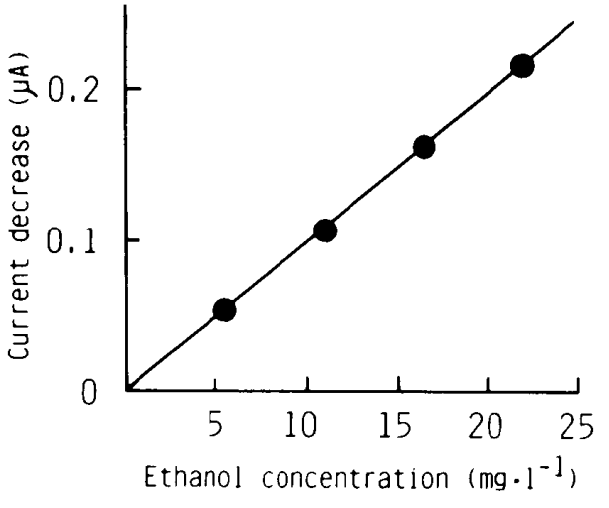

Fig. 1.3 Calibration curve of the microbial sensor for ethanol.

A methanol-utilizing bacterium (AJ 3993) was used for the determination of methanol. The determination was performed under conditions described for the ethanol sensor using the steady-state method. A linear relationship was also observed between the current decrease and the concentration of methanol. Therefore, the sensor can be used for determining methanol.

1.2.3 Glutamic Acid Sensor[3]

Glutamate decarboxylase catalyzes the decarboxylation of glutamic acid, which produces carbon dioxide and amine; however, the enzyme is expensive and unstable. Therefore, a microbial sensor for glutamic acid using immobilized microorganisms with a glutamate decarboxylase activity was developed in conjunction with a carbon dioxide gas-sensing electrode.

Freeze-dried cells of *Escherichia coli* were mixed with 1 drop of water and coated on both sides of a nylon mesh. This was placed on the surface of the silicone rubber membrane of the carbon dioxide electrode and then covered with a cellophane membrane to entrap the microorganisms between the two membranes. The cellophane membrane was fastened with a rubber ring.

The initial constant potential (E) was obtained with the buffer solution only. When the sample solution containing glutamic acid was injected into the system, glutamic acid permeated through the cellophane membrane and was metabolized by the microorganisms to produce carbon dioxide:

$$\mathrm{HOOC{-}CH_2{-}CH_2{-}CH(NH_2){-}COOH} \xrightarrow[\text{decarboxylase}]{\text{glutamate}} \mathrm{HOOC{-}CH_2{-}CH_2{-}CH_2{-}NH_2} + \mathrm{CO_2}$$

The enzyme reaction was carried out at a pH of 4.4, which was sufficiently below the pK value (6.34 at 25 °C) of carbon dioxide to allow carbon dioxide concentration around the membranes to increase. As a result, the potential of the carbon dioxide gas-sensing electrode increased with time.

The plot of the maximum potential versus the logarithm of the glutamic acid concentration was linear over the range shown in Fig. 1.4, the slope over this range being approximately Nernstian. When a glutamic acid solution (400 mg/L) was measured repeatedly, the standard deviation was 1.2 mg/L (over 20 experiments). The sensor responded to glutamic acid and glutamine and very slightly to some other amino acids. The response to glutamine can be decreased, if necessary, using acetone-treated *E. coli*.

The microbial sensor was evaluated for the determination of glutamic acid in a fermentation broth. Satisfactory recovery data (99 to 103%) were obtained. The concentrations of glutamic acid in some fermentation broths were determined by the microbial sensor and by the AutoAnalyzer method for comparison. The results were in good agreement. Dissolved carbon dioxide in the culture broth at pH 4.4 was removed by passing nitrogen gas and therefore did not influence the determination.

A glutamic acid solution (240 to 800 mg/L) and fermentation broths of glutamic acid were used to test the long-term stability of the sensor. The response of the sensor was constant for more than 3 weeks and 1500 assays. Thus the microbial sensor appears to be very attractive for the determination of glutamic acid.

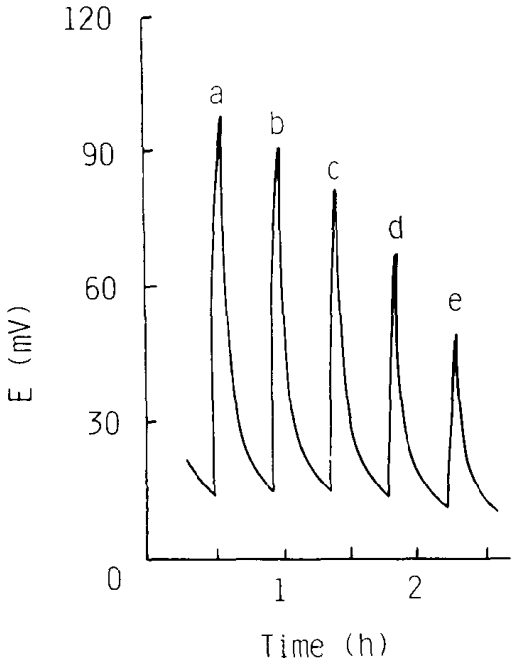

Fig. 1.4 Response curves for glutamic acid. a, 800 mg/L; b, 600 mg/L; c, 400 mg/L; d, 240 mg/L; e, 120mg/L.

1.2.4 Carbon Dioxide Sensor[4]

Determination of CO_2 is very important in industrial processes. The potentiometric pCO_2 device has found widespread use in biological and industrial applications. However, major problems are associated with the potentiometric method of detection because various ions and organic and inorganic volatile acids affect the potential of the inner pH electrode and the gas-permeable membrane of the pCO_2 electrode. Therefore, we developed a novel microbial CO_2 sensor based on amperometry.

The chemoautotrophic bacterium, which can grow with only carbonate as the carbon source, was used in this study. This microorganism was obtained from the Fermentation Research Institute, Japan. The bacterium was isolated from soil obtained in Akita prefecture, Japan, and named S-17. S-17 is presumed to belong to the genus *Pseudomonas*. It is a 1 × 4 to 5 μm gram-negative rod and grows both autotrophically and heterotrophically. Optimum temperature and pH for growth are 20 to 34 °C and pH 4.0 to 8.6, respectively. In this study, S-17 was incubated autotrophically under aerobic conditions at 30 °C for approximately 1 month in an inorganic medium.

Bacterial cells immobilized on a cellulose nitrate membrane (pore size 0.45 μm) were placed in the vicinity of the cathode of a galvanic-type oxygen-sensing electrode. The cathode and the immobilized cells were then totally covered with a polytetrafluoroethylene (PTFE) membrane (pore size 0.5 μm, type FH) secured with a rubber O-ring. The PTFE membrane acts as a gas-permeable membrane.

Figure 1.5 represents the time course of the CO_2 sensor response to the addition of CO_2 (K_2CO_3). When a sample solution containing CO_2 is injected into the external buffer solution, CO_2 permeates through the gas-permeable membrane and is subsequently

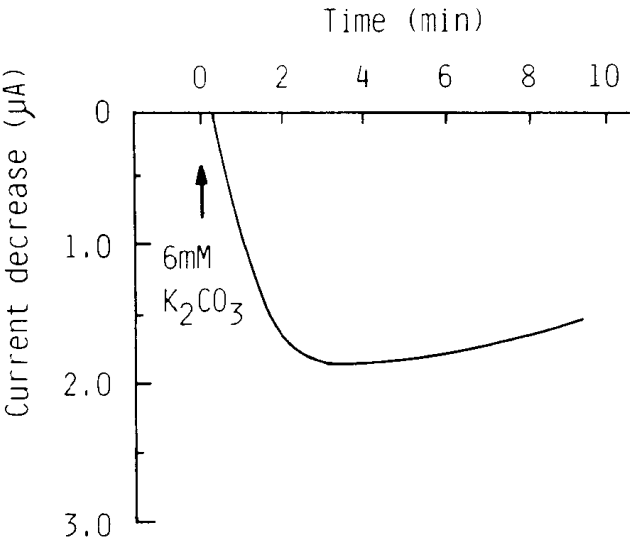

Fig. 1.5 Time course of CO_2 sensor response.

assimilated by the bacteria. The respiration of the bacteria consequently becomes elevated, giving rise to the consumption of oxygen. This experiment was executed, injecting 300 μL of 1 M K_2CO_3 solution into 50 mL of a KH_2PO_4/NaOH buffer solution (30 °C, pH 5.5). The current decreased rapidly and reached its maximum 2 to 5 min after injection of K_2CO_3 followed by a gradual increase of the current due to the escape of CO_2 to the air. For concentrations of K_2CO_3 below 6 to 8 mM, the current decrease was approximately proportional to the K_2CO_3 concentration. At higher concentrations, however, the current decrease plateaued because of system saturation with CO_2. The initial rate of carbon dioxide utilization does not depend on a K_2CO_3 concentration higher than 8 mM. The rate of CO_2 utilization appears to be limited by the uptake or metabolism of CO_2 by the microbial cells.

Figure 1.6 shows the calibration curve for the biosensor. The CO_2 concentration was determined by performing the same experiment as described previously but with a conventional potentiometric pCO_2 electrode. The results of this experiment permitted a linear calibration curve to be constructed relating to the CO_2 concentration and the current decrease of the novel CO_2 sensor. The linear range of the sensor was found to be below 200 ppm CO_2. The upper limit of the linear range, determined by the saturation of CO_2 in the buffer solution rather than by considering the current fluctuation resulting from experimental errors, is within 0.5 μA. This novel sensor could easily detect a variation of 5 ppm CO_2 (200 μM K_2CO_3) in the lower concentration range.

The selectivity of this sensor was also examined. Various aqueous solutions of volatile compounds such as acetic acid, citric acid, formic acid, ethyl alcohol, and butyl alcohol were added to the sensor system. Although the sensor responded slightly to acetic acid, all other compounds produced no observable response. Selectivity could also be improved by selecting a more suitable material as the gas-permeable membrane. The stability of the sensor was more than 3 weeks. This was considered to be very satisfactory for its application in practical use, especially in a flow cell configuration.