

# ADVANCES IN BIOSENSORS

*A Research Annual*

Editor: ANTHONY P.F. TURNER  
*Biotechnology Centre  
Cranfield Institute of Technology  
Bedford, England*

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VOLUME 2 • 1992



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**ELECTROCHEMICAL BIOSSENSORS**

**APPLICATION TO SOME REAL PROBLEMS**

**ENZYMATIC AMPLIFICATION AND**

**ELIMINATION IN BIOSSENSORS**

*Florian Schubert, Ulla Wollenberger*

*Dorothea Pfeiffer and*

*Ernst W. Scheller*

**BASIS BASES BASED ON MODIFIED**

**ELECTROELECTROCHEMISTRY A**

**PROACH TO BIOSENSING**

*Primož P. Čerušič and*

*Anthony P.F. Turner*

**ELECTROCHEMICAL APPROACH**

**TO BIOSENSING**

*Anthony P.F. Turner, H. Brian Halsall and*

*William R. Heineman*



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Cranfield Institute of Technology  
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## INTRODUCTION

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Biosensors have captured the imagination of the world's scientific and commercial communities by combining the multidisciplinary skills of biologists, physicists, chemists and engineers to provide innovative solutions to analytical problems. Biosensors are applicable to clinical diagnostics, food analysis, cell culture monitoring, environmental control and various military situations. Ever increasing demands for rapid and convenient analyses of a wide variety of materials in diverse locations has led to intense interest in this fusion of biology and electronics which mimics our principal concern: the effect of materials and environments on living systems.

A biosensor may be defined as an analytical instrument containing a sensing element of biological origin, which is either integrated within or in intimate contact with a physicochemical transducer. The usual aim is to produce a continuous electronic signal which is directly proportional to the concentration of a chemical or set of chemicals present in a sample. Sensing elements may be biocatalytic (e.g. enzymes, organisms, tissues) or affinity systems (e.g. antibodies, nucleic acids, cell receptors). Optical, electrochemical, calorimetric or piezoelectric transducers are used to convert the biological recognition event into a processable signal. Regardless of the precise definition, however, tangible results of endeavour in this general technological area are becoming increasingly evident with an



exciting range of novel analytical devices emerging onto the marketplace.

The series *Advances in Biosensors* will present a unique compendium of research-level publications, which do not have a place in conventional journals, but have an increasingly important role to play in completing the primary research literature and offering a more incisive alternative to the full blown, exhaustive review article. In these papers represented by eight chapters in this inaugural volume, eminent authorities in the field of Biosensors provide an up-to-date overview of their laboratory's contribution, summarizing the primary research as it has appeared, possibly scattered, in the journal and conference literature and reflecting on their findings. This produces an innovative synthesis of such smaller research efforts into an overall perspective on the topic, which is difficult for the reader to glean from the multifarious original publications. There is latitude for the inclusion of detail that may have been excised from the original publication and for speculation on future possibilities. The net result is intense, yet highly readable accounts of the state of the art at this leading edge of analytical technology.

Cranfield, Bedford

Anthony P.F. Turner  
Professor of Biosensor Technology  
*Series Editor*

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# WHOLE-ORGANISM BASED BIOSENSORS AND MICROBIOSENSORS

Izumi Kubo, Koji Sode and Isao Karube

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## 1. INTRODUCTION

While advances in biosensors have begun to be appreciated in various analytical fields such as diagnostics, process control and food production, the original conception of the scientists in this field has still not been fully realized. The aim of this chapter is to introduce these new ideas, especially those concerned with whole-organism-based biosensors and microbiosensors.

Whole organisms such as microorganisms possess enzyme complexes. Artificial reconstruction of such enzyme complexes is not easily carried out, but the enzyme complexes can themselves be utilized by use of the intact cell. This advantage is what lies behind the study of biosensors based on microorganisms.

Compared with conventional biosensors, microbiosensors have definite advantages. Only tiny amounts of sample are required for measurement, and implantation of the biosensors is made possible. By integration, multi-functionalized biosensors are also a possibility. Not only have immobilized enzymes been incorporated but also microorganisms. Whole-organism-based biosensors and microbiosensors are not necessarily a clearly defined group of biosensors, as can be seen from Table 1. In this chapter our review of biosensors utilizing whole organisms and/or miniaturized electrochemical devices will be concentrated on those listed in the table.

## 2. BIOSENSORS BASED ON WHOLE ORGANISMS

### 2.1 CO<sub>2</sub> Sensor Based on Autotrophic Bacteria

The potentiometric pCO<sub>2</sub> electrode has found widespread use in biological and industrial applications, and has been used as the sensing element in enzyme electrodes [1]. The response character-

Table 1. Constituent of whole-organism based biosensors and micro-biosensors

Sample analysed	Biocatalysts	Transducer	Reference
CO <sub>2</sub>	<i>Pseudomonas</i> S-17	Oxygen electrode	[7, 8]
L-tyrosine	<i>Pseudomonas</i> S-17 L-tyrosine decarboxylase	Oxygen electrode	[9]
BOD	Thermophilic bacteria	Oxygen electrode	[11]
CO <sub>2</sub>	<i>Pseudomonas</i> S-17	Micro-oxygen electrode	[12]
Urea	Urease	ISFET	[17]
HSA	Anti-HSA	ISFET	[18]
ATP	H <sup>+</sup> -ATPase	ISFET	[15]
Acetylcholine	Acetylcholine receptor	ISFET	[21]
Ethanol	Cell membrane of acetic acid producing bacteria	ISFET	[22]
Hypoxanthine	Xanthine oxidase	a-ISFET	[30]
Inosine	Nucleoside phosphorylase	a-ISFET	[31]

istics of the pCO<sub>2</sub> electrode have been evaluated from many viewpoints [2], but major problems are associated with the potentiometric method of detection. Various ions and some organic and inorganic volatile acids affect the potential of the inner pH electrode and the gas-permeable membrane of the pCO<sub>2</sub> electrode [1–6]. In addition, the precision of the conventional potentiometric pCO<sub>2</sub> electrode is somewhat restricted because of the Nernstian type of response. In contrast, amperometric biosensors consist of an oxygen electrode and a biochemical reaction which consumes oxygen, and exhibit excellent sensitivity. The amperometric oxygen electrode generates a current which is directly proportional to the chemical reaction, hence it possesses far greater theoretical sensitivity.

Along this line of thought, a novel microbial amperometric CO<sub>2</sub> sensor based on an autotrophic bacterium was developed by Suzuki *et al.* [7]. The autotrophic bacterium (which can grow with carbonate as the only source of carbon) was obtained from The Fermentation Research Institute, Japan. This bacterium was isolated from soil obtained in Akita Prefecture, Japan, and is named S-17. It is thought to belong to the genus *Pseudomonas*; S-17 is a 1 × 4–5 μm Gram-negative rod. S-17 was incubated under aerobic conditions at 30°C, for one month on inorganic medium.

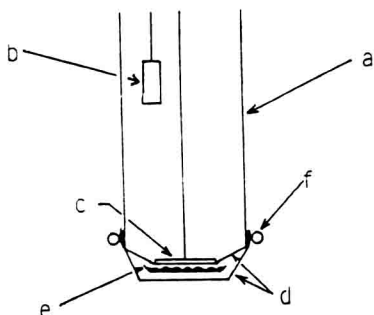


Figure 1. Schematic diagram of the CO<sub>2</sub> sensor: (a) dissolved oxygen electrode, (b) Pb anode, (c) Pt cathode, (d) gas-permeable membrane, (e) immobilized whole cells, (f) —.

### 2.1.1 Procedure for CO<sub>2</sub> Determination

A CO<sub>2</sub> sensor was constructed as shown in Figure 1. Bacterial cells retained on a cellulose nitrate membrane (Toyo Roshi Co. Tokyo; pore size 0.45  $\mu\text{m}$ ) were placed around the platinum cathode of a galvanic-type oxygen-sensing electrode (Ishikawa Seisakujo Co., Tokyo; diameter 1.7 cm). The cathode and the immobilized cells were then totally covered with a Millipore polytetrafluoroethylene (PTFE) membrane (pore size 0.5  $\mu\text{m}$ , type FH). The PTFE membrane acts as a gas-permeable membrane. The determination of CO<sub>2</sub> was carried out with a batch system, at pH 5.5.

When a sample solution containing CO<sub>2</sub> is injected into the external buffer solution, CO<sub>2</sub> permeates through the gas-permeable membrane, and is assimilated by the bacteria. The respiratory rate of the bacteria is consequently increased, causing the consumption of oxygen. If the decrease in the oxygen concentration is measured with an oxygen-sensing electrode, the concentration of CO<sub>2</sub> can be measured indirectly. Thus, an amperometric CO<sub>2</sub> sensor can be constructed.

CO<sub>2</sub> was supplied by acidification of a potassium carbonate solution mixed with the same buffer as contained in the cell, so as not to create a change in oxygen concentration. Because the CO<sub>2</sub> solution was continuously stirred, the CO<sub>2</sub> concentration came to a definite equilibrium state. Then 100  $\mu\text{l}$  of potassium carbonate solution was injected into the sensor cell from a microsyringe at constant time intervals. The CO<sub>2</sub> sensor was stored in the buffer solution at 4°C when not in use.

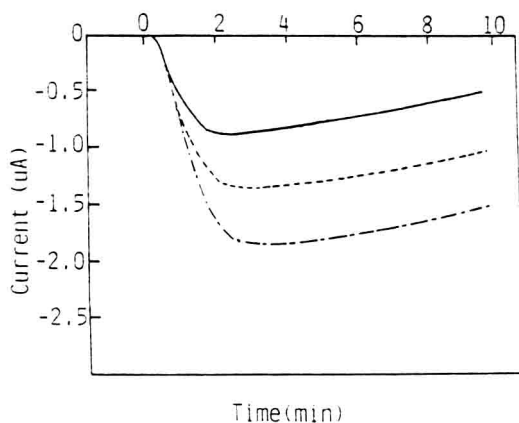


Figure 2. Typical response curves of the  $\text{CO}_2$  sensor: —, 2 mM, ----, 4 mM, — · —, 6 mM potassium carbonate, respectively. Conditions: 0.05 M  $\text{KH}_2\text{PO}_4/\text{NaOH}$  buffer, pH 5.5,  $30^\circ\text{C}$ .

### 2.1.2 Characteristics of the System

As shown in Figure 2 the current decreased rapidly, with a maximum response of 2–3 min after the injection of potassium carbonate; the current then gradually increased because of the escape of  $\text{CO}_2$  to the air. Reflecting the optimum growth temperature for S-17 of  $30\text{--}34^\circ\text{C}$ , the response of the sensor was low for temperatures below  $30^\circ\text{C}$ , and exhibited a maximum in the range  $30\text{--}32^\circ\text{C}$ , gradually decreasing above this level.

The concentration of dissolved  $\text{CO}_2$  is greatly affected by a change in pH and so the  $\text{CO}_2$  determination must be done at a constant buffered pH.  $\text{CO}_2$  and  $\text{H}_2\text{CO}_3$  reach an equilibrium state depending on pH:



However, as for the amperometric  $\text{CO}_2$  electrode, the  $\text{CO}_2$  ratio in the total carboxide compounds could not easily be determined because the buffer solution was stirred to ensure it was saturated with oxygen. As is well known, at lower pH values the ratio of  $\text{CO}_2$  is increased. But if the pH were too low, the rate of  $\text{CO}_2$  production in the buffer would exceed the rate of  $\text{CO}_2$  assimilation by the bacterium, and the decrease in current would not then be a true reflection of the  $\text{CO}_2$  concentration. As a compromise between the



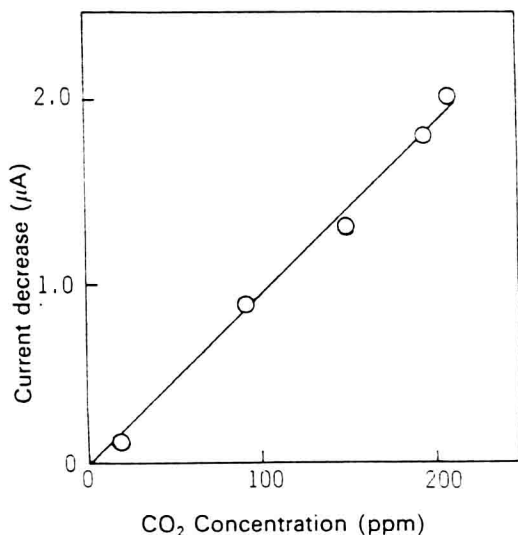


Figure 3. Calibration curve for the CO<sub>2</sub> sensor. Conditions: 0.05 M KH<sub>2</sub>PO<sub>4</sub>/NaOH buffer, pH 5.5, 30°C.

CO<sub>2</sub>-generating ability of the buffer solution and the experimental error incurred through loss of unassimilated CO<sub>2</sub>, the pH value was set at 5.5.

### 2.1.3 Calibration

The initial rate of carbon dioxide utilization did not depend on the carbonate concentration at values higher than 8 mM. The rate of CO<sub>2</sub> utilization appears to be limited by the uptake or metabolism of CO<sub>2</sub> by the microbial cells.

Figure 3 shows the calibration curve for the biosensor. The CO<sub>2</sub> concentration was determined separately by repeating the experiment with a conventional potentiometric pCO<sub>2</sub> electrode. A calibration graph was then constructed up to 200 mg l<sup>-1</sup> CO<sub>2</sub> (ca. 5 mM) relating to the CO<sub>2</sub> concentration measured potentiometrically to the sensor. The upper limit of the linear range was governed by saturation of CO<sub>2</sub> in the buffer solution and not by biochemical kinetics. Considering that the current fluctuation resulting from experimental errors is within 0.05 μA, the proposed sensor could detect a variation of 5 mg l<sup>-1</sup> CO<sub>2</sub> in the lower concentration range.