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Use of Hydrogen-Sensitive Pd-MOS Materials in Biochemical Analysis

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Abstract

A metal oxide semiconductor sensitive to hydrogen was used to monitor H_2 production from immobilized *Clostridium acetobutylicum* cells and from immobilized hydrogenase on a continuous basis.

Index Entries: Hydrogen-sensitive Pd-MOS materials; Pd-MOS materials, hydrogen-sensitive; MOS-Pd materials, hydrogen sensitive; semiconductor, hydrogen-sensitive; hydrogenase, Pd-MOS monitoring of; *Clostridium acetobutylicum*, immobilized.

Introduction

Hydrogen- and ammonia-sensitive MOS-structures (Metal Oxide Semiconductor) coated with a very thin layer of catalytically active metal such as palladium have previously been evaluated for leak and smoke gas detection (*1*, *2*). Recently, the application of an ammonia-sensitive Pd-MOSFET to follow biochemical reactions producing ammonia was described (*3*). Our current studies involve evaluation of different semiconductor structures as sensors for gaseous compounds of biochemical interest as a complement to the enzyme thermistor (*4*). There are many important biochemical routes leading to hydrogen evolution or consumption. As an ex-

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ample, the hydrogen produced by a preparation of an immobilized microorganism (*Clostridium acetobutylicum*) was monitored by a hydrogen-sensitive Pd-MOS-capacitor. The enzyme, hydrogen dehydrogenase (*5*), is capable of reducing a great number of substrates by heterolytic cleavage of molecular hydrogen, including $NAD(P)^+$ in the reaction:

$$NAD^+ + H_2 \rightleftharpoons NADH + H^+$$

This reaction is reversible and can be utilized both for the determination of NAD^+ and for NADH. This fact widens the field of application of the technique to include all dehydrogenase reactions.

Hydrogen Determinations with Pd-MOS-Capacitors

Since the biochemical systems studied here operate in solution, the hydrogen to be measured must be transformed from the buffer phase to a carrier gas and transported past the sensor. The experimental arrangement employed is shown in Fig. 1. Buffer, containing dissolved H_2, is continuously pumped at a flow rate of 0.5 mL/min with a multichannel peristaltic pump (Gilson Minipuls, France), which is also used to introduce the carrier gas (2.5 mL/min of air) to a mixing chamber. A gas permeable membrane (Fluoropore, Millipore, Mass., USA) is used to separate a part of the gas phase. By this arrangement hydrogen dissolved in the buffer down to a concentration of 0.05 m*M* can be measured. A schematic cross-section of a Pd-MOS capacitor is shown in Fig. 2.

Hydrogen Gas Production of Clostridium acetobutylicum

This microorganism is of great industrial interest since it is used to produce butanol and butyric acid from a diet of glucose. It has an interesting potential future use, since it also produces hydrogen gas. The microorganism was immobilized in alginate beads and 5 mL of the preparation were packed into a small column in the experimental setup shown in Fig. 1. Substrate (0.16*M* glucose) was continuously pumped through the column under anaerobic conditions at a flow rate of 0.4 mL/min. The hydrogen production could thus be followed for several days (Fig. 3), showing that this component can be used continuously over long periods.

NAD(H) Measurements Employing Immobilized Hydrogenase

The number of potential applications of a hydrogen determination method can be considerably increased by the combination with hydrogen dehydrogenase (hydrogenase, E. C. 1.12.1.2) through which all other dehydrogenase reactions can be made accessible for determination. Hydrogenase is involved in most of the reactions in which hydrogen gas is consumed or evolved, and is therefore found in many different microorganisms and algae. The enzyme can thereby differ much in

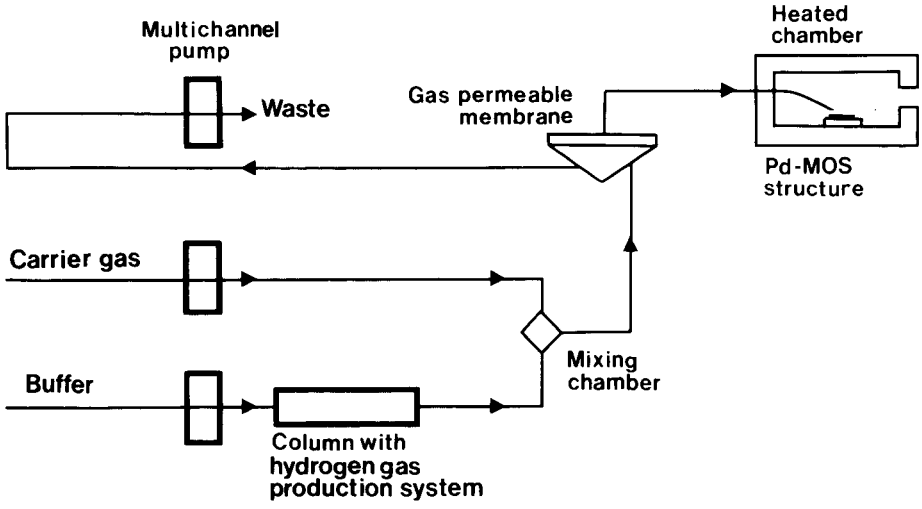

Fig. 1. Experimental arrangement.

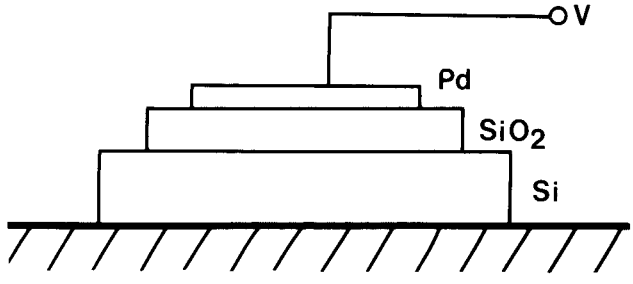

Fig. 2. Schematic cross-section of a Pd-MOS capacitor.

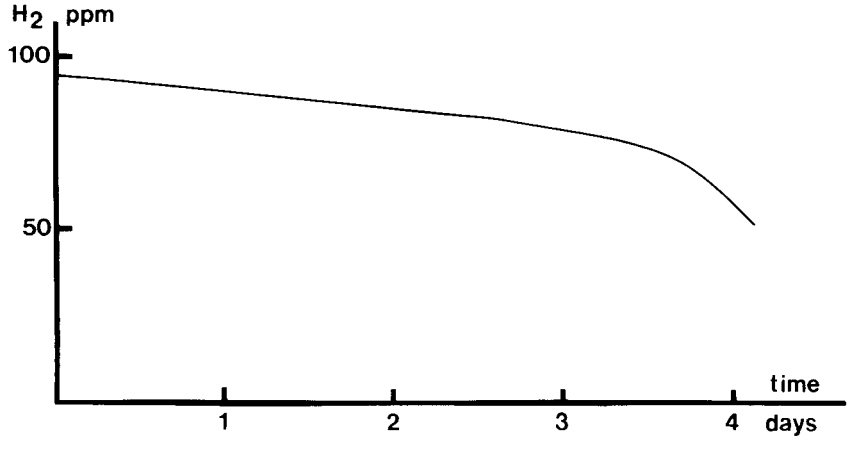

Fig. 3. Time course of the hydrogen concentration in the effluent from a 5 mL columnn of *C1. acetobutylicum* immobilized in alginate beads.

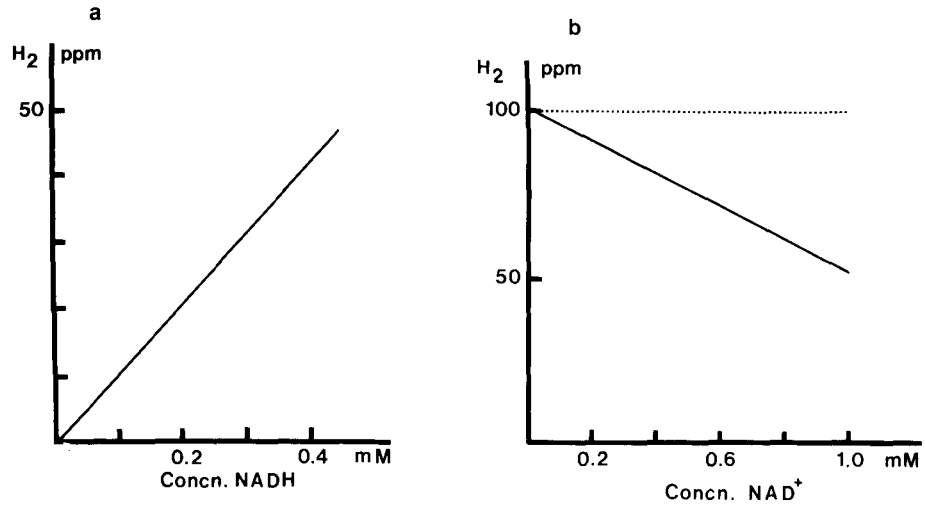

Fig. 4. (a) Response curve of NADH obtained with 1 mL samples at a flow rate of 1 mL/min. The amount of hydrogen developed is expressed as ppm of the carrier gas. (b) Response curve of NAD^+ obtained with 1 mL samples at a flow rate of 1 mL/min. The hydrogen intially present in the buffer corresponded to a hydrogen concentration of 100 ppm in the carrier gas.

molecular weight, subunit composition, thermal and oxygen stability, although they are all iron sulfur proteins that can cleave hydrogen molecules heterolytically and thereby reduce NAD^+ and a number of other substrates reversibly. In this study a hydrogenase from the bacterium *Alcaligenes eutrophus H16* was purified (*7*) and immobilized on controlled pore glass (CPG-10, mean pore diameter 200 nm, 80–120 mesh, Corning Glass Works, Corning, NY, USA) using a previously described technique (*8*). The specific activity of the soluble enzyme preparation was increased from 0.6 to 25 IU/mg. In the experiment reported here, 500 units were applied to 2 mL of CPG and, after coupling, 200 units were recovered bound to the CPG. The hydrogenase–CPG was packed into a 5 × 30 mm column that was adapted to the experimental setup shown in Fig. 1 and the activity of the enzyme was retained for about 2 weeks. The Pd-MOS capacitor was calibrated by introducing known amounts of hydrogen dissolved in buffer.

Figure 4a shows a standard curve for NADH obtained with 1 mL samples. The response curve was linear over the range tested, 0.1–0.8 m*M*. It is also possible to measure NAD^+ by a similar technique, although in this case the buffer solution passed through the system must contain a constant and sufficient concentration of hydrogen. A sample of NAD^+ will consume a corresponding amount of hydrogen by the action of the hydrogenase and a negative recorder deflection will be observed. As can be seen from Fig. 4b, the response curve is also linear in this case and the sensitivity is about the same as that obtained for NADH.

A more generalized procedure, including a primary dehydrogenase step, was exemplified by determination of ethanol. The ethanol samples were incubated with appropriate amounts of NAD^+ and soluble alcohol dehydrogenase and subsequent determination as above of the NADH produced. These experiments indicated that

this technique can be used as a general method for following dehydrogenase reactions producing NADH.

Conclusion

The advantages of the present technique are the independence of the optical properties of the sample, the great number of reactions that can be assayed, the simplicity of the equipment, its direct applicability in continuous assay, and the comparatively high sensitivity despite its early stage of development.

Acknowledgments

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