Monitoring the Atmosphere in an Anaerobic Chamber

SARA Z. SUDO AND PAUL A. HERSCH

Division of Periodontology and Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

Received for publication 28 May 1974

The Couloximeter, a fuel cell designed to measure trace amounts of oxygen, was used to monitor the atmosphere in an anaerobic chamber. The device, easy to operate and to maintain, allowed both major and minor fluctuations in oxygen concentration to be measured. Using a hose attached to the outlet within the box, defective (ruptured) gloves were consistently distinguishable from intact gloves.

This report describes the use of the Couloximeter (coulometric oxygen sensor) for monitoring the oxygen content of the atmosphere in a glove box used for the isolation and handling of oxygen-sensitive bacteria from dental plaque. Trace oxygen analyzers, such as the one used on occasion in this context by Aranki et al. (1) are available, but they are expensive, intricate, and bulky. The attachment of the Couloximeter to the glove box required only simple accessories and did not interfere with normal usage of the box.

The Couloximeter (2, 3; P. A. Hersch U.S. Patent 3223597, 1965), is basically a fuel cell that receives oxygen directly from the gas phase. The cell, (Fig. 1) is contained in a tubular housing with a fourway bypass valve. The cell itself consists of three parts: (i) a core of nickel supporting active cadmium (negative electrode) surrounded by (ii) an inert fabric impregnated with aqueous KOH, which separates the active cadmium from (iii) an outer carbonaceous layer, the positive electrode. The two electrodes have leads connecting to a direct current measuring device with low impedance, on the order of 10 ohm. When a gas stream carrying trace amounts of oxygen is allowed to flow into the cell and over the carbonaceous layer, virtually all the oxygen is captured. Electrons flow through the circuit from the cadmium to the oxygen, reducing it to hydroxyl ion (equations 1 and 2).

\[ \frac{1}{2} O_2 + H_2O + 2e^- \rightarrow 2OH^- \]  
\[ Cd + 2OH^- - 2e^- \rightarrow Cd(OH)_2 \]  

The current is proportional to, and solely dependent on, the mass flow of oxygen. Under normal ambient conditions a volume flow of 40 cc/min with 1 ppm O_2 generates 10 \( \mu A \). This can be checked by generating known quantities of oxygen in a micro-electrolyzer as described elsewhere (2). However, practice has shown that calibration is not required during the life cycle of the sensor. After months of continuous use, it shows a slow down of response rather than a loss of sensitivity. The sensor can then be rejuvenated in situ through charging, like a storage battery.

MATERIALS AND METHODS

Glove box. The glove box (Coy Manufacturing Co.) was maintained according to the instructions of the manufacturer. It consisted of a plastic chamber (65 in long by 32 in deep by 40 in high) (ca. 164.6 by 81.28 by 101.6 cm) equipped with one pair of gloves, an entry lock, and two heated catalyst boxes (1). Two rubber stoppers, each containing two outlet tubes, were located opposite the entry lock. Only one outlet was necessary for use of the Couloximeter. The gas used to fill the chamber and for the final flushing of the entry lock consisted of 80% \( N_2 \), 10% \( CO_2 \), 10% \( H_2 \). The chamber was maintained at 37 C by adjusting the heaters in the catalyst boxes.

Measuring system. The apparatus used in this study is pictured in Fig. 1. It consisted of a bubbler, the Couloximeter with its bypass valve and microampereter, a flowmeter, a “blowin” flow stabilizer, and a small suction pump (Romulus Industries Corp.) lined up in that order. The bubbler contained aqueous KOH (ca. 24 g/100 g of solution) to retain the bulk of the \( CO_2 \) from the sampled gas. Continuous exposure of the oxygen-sensing element to concentrations of \( CO_2 \) as high as 10% would eventually impair its sensitivity and speed of response.

The gas sample stream left the glove box via a glass pipe placed in a rubber stopper in one of the walls of the enclosure. The connections between this pipe and the bubbler and between the bubbler and the sensor valve were short sleeves cut from PVC (Tygon tubing). The nature and length of the connections between the sensor valve and the flowmeter and further downstream are irrelevant.

The flow rate of the sample stream is determined by two factors: (i) the columns of liquid in the flow

582
stabilizer and bubbler and (ii) the overall flow resistance of the analysis train. The latter can be adjusted by a needle valve (or simply a pinch clamp) immediately preceding or following the flowmeter. We adjusted the flow to 40 cc/min so that each ppm of O₂ was shown as a deflection of 10 μA.

Operation. After the initial installation, or with fresh KOH solution, the system was flushed for 60 to 90 min. During this procedure, the electrochemical cell was bypassed by means of the four-way valve. Thereafter, the system was flushed for only 10 min before the cell was engaged. A shunt resistor was left in parallel to the microammeter when the apparatus was not in use and during the initial period of reading. With the shunt in place the scale of the microammeter represented 0 to 500 ppm oxygen. After about 15 s, when it was ascertained that the gas contained less than 50 ppm oxygen, the shunt was removed, expanding the scale of the ammeter to 0 to 50 ppm oxygen. The reading reached 90% of equilibrium value in less than 2 min. During the 2 months the device was used, the only maintenance consisted of changing the KOH solution biweekly.

RESULTS AND DISCUSSION

Minimal O₂ concentration. In the initial stages of the study, gas was drawn from the glove box via a short piece of glass tubing extending through the rubber stopper in the plastic wall. The outlet was, therefore, close to the perimeter of the box. Under these conditions, the O₂ concentration in the glove box which had been sealed overnight ranged from 4.5 to 6.2 ppm.

A 60-in (ca. 152.4 cm) latex hose was then attached to the outlet so that the gas at various places within the box could be sampled. The minimal concentration of O₂ at the center of the box ranged from 3.0 to 4.3 ppm. The oxygen concentration directly above the catalyst box was lower, that near the walls was slightly higher. The mean concentration of oxygen was lower when freshly regenerated catalyst was used; that is, O₂ permeating the plastic was reduced at a more rapid rate.

Passage of material through lock. The lock was evacuated, flushed twice with N₂ and once with the N₂-H₂-CO₂ mixture when material was passed into the box. This procedure raised the O₂ concentration in the center of the box by 0.5 to 1.0 ppm if the lock was immediately resealed. The minimal oxygen concentration was reestablished in 5 to 15 min. Passage of agar media containing sheep’s blood into the box raised the O₂ concentration a greater amount (depending on the volume of medium) and for a longer period, as the hemoglobin released bound O₂ into the atmosphere.

Removal of catalyst, faulty sealing of lock. Even brief absence of catalyst (or turning off the fans on the catalyst heaters) resulted in rapid increase in O₂ concentration in 15 to 20 min (Fig. 2A). The oxygen level fell rapidly when catalyst was returned to the trays (arrow, Fig. 2A).

The minimal O₂ concentration in Fig. 2A is nearly 10 ppm because the lock was not tightly sealed. If 3 to 5 lb/in²-vacuum was maintained on the lock, this value was lower. Release of the vacuum seal on the entry lock (Fig. 2B) occasionally, though not invariably, allowed O₂ to enter the chamber, though both doors on the lock were apparently tightly closed. Adjustment of the hing mechanism reduced but did not eliminate this problem. There was no other
The vacuum indication pressure.

2B). prolonged the which could through the doors and/or leaks then closed with metal cuff and rubber binder. The hose was admitted with the outside of the glove placed into the glove. The base of the glove was then closed with a rubber binder. This procedure reduced the effect of positive pressure in the chamber which minimized entry of oxygen through the break. The outlet was removed after 1 min, thus admitting a pulse of oxygen (Fig. 2C). The peak occurred at 5 min (shorter lags could be achieved by reducing the length and/or internal diameter of the hose). The height of the peak for an intact glove averaged 14.3 ± 1.6 ppm O₂ whereas that for a glove containing a pinhole leak was 26.6 ± 4.4 ppm O₂. We were consistently able to locate even pinhole leaks in the gloves by this method. The magnitude of the peak depended on the size and the location of the rupture. Larger ruptures often produced readings exceeding the scale of the microammeter. In these cases, damage to the instrument was avoided either by inserting the shunt resistor or simply isolating the sensor with the fourway bypass valve.

With additional modification, it may be possible to locate leaks by scanning the walls from the inside with the inlet end of a long sampling hose. Jones and Dever (4) recommend scanning the outside with a halogen detector used in vacuum work. However, this qualitative tool requires halogenated hydrocarbon inside the enclosure as a tracer, a type of compound that may interfere with the cultures.

The Couloximeter was a convenient and useful instrument for monitoring the concentration of O₂ within the anaerobic glove box. Although we did not use a recorder, a permanent record of O₂ concentration may be obtained by connecting a millivolt recorder to the sensor terminals, in parallel to the microammeter.

ACKNOWLEDGMENT

We thank L. E. A. Folke for the use of laboratory space during this investigation.

Addendum in Proof

After this manuscript was submitted, the Couloximeter experienced problems stemming from ineffi-
cient removal of CO$_2$ from the gas. Subsequently, we have been testing a gas-washing apparatus of a different design, which provides prolonged contact of the gas phase with the KOH solution. The apparatus appears to be an improvement over the one used in this study.

**LITERATURE CITED**

1. Aranki, A., S. A. Syed, E. B. Kenney, and R. Freter. 1969. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. Appl. Microbiol. 17:568–576.

2. Hersch, P. A. 1964. Galvanic analysis, p. 209-219. In C. W. Reilley, (ed.), Advances in analytical chemistry and instrumentation, vol. 3. Wiley-Interscience Inc., New York, N. Y.

3. Hersch, P. A. 1973. Coulometry. Amer. Lab. 5:29–36.

4. Jones, G. L., and S. M. Dever. 1974. Detection of gas leaks in an anaerobic glove box. Appl. Microbiol. 27:806.