NOTES

Mass Spectral Measurement of Residual Oxygen in the Atmosphere of an Anaerobic Culture Chamber

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A simple and reproducible procedure has been devised for sampling gases and for analysis of the residual oxygen in the atmosphere of the anaerobic culture chamber by mass spectrometer.

Awareness of the importance of anaerobic bacteria in health and disease has led to the introduction of various improved culture techniques for their isolation. A most convenient method is based on the glove box isolator (2), which is now used increasingly in research and clinical laboratories. The atmosphere in the chamber consists of a mixture of gases (85% nitrogen, 10% hydrogen, and 5% carbon dioxide).

Despite measures to keep oxygen tension low in the chamber by the use of palladium "D" catalyst (1), build up of oxygen does occur by penetration through the neoprene material of the gloves and the polyvinyl material of the chamber. Anaerobiosis can be monitored by the use of chemical indicators based on redox indicator dyes such as methylene blue (3), resazurin, and phenosafranin, or more accurately by measuring the oxygen tension directly in the chamber with a trace oxygen analyzer (1). We, on the other hand, have developed a simple gas sampling technique which gives a quick measurement of the oxygen tension in the chamber by the use of a mass spectrometer.

Apparatus and method. A Varian MAT model M86 mass spectrometer was modified for continuous gas sampling. The conventional reservoir inlet was replaced by a glass capillary (length, 10 cm; outer diameter, 6 mm; inner diameter, 0.5 mm). One end was attached to the mass spectrometer inlet port, and the other was sealed with epoxy resin in a 5-cm length of stainless-steel hypodermic tubing (inner diameter, ca. 0.2 mm). The metal capillary tube was then crimped to restrict the gas flow through the mass spectrometer to such an extent that a pressure of 5×10^{-6} torr was recorded. With no gas flowing through the orifice, the background pressure in the spectrometer was about 10^{-6} torr.

A special gas sampling tube was designed for use with this inlet. It consisted (Fig. 1 and 2) of three 4-mm Springham diaphragm vacuum taps, a Springham 4-mm quick-connect vacuum flange, and a 6-mm (outer diameter) side arm which terminated in a 0.25-inch (0.64-cm) Swagelok vacuum union closed by a silicone rubber septum.

With all three taps open and the side arm closed, the tube was connected to a dual-stage forevacuum pump and evacuated to a pressure of 10^{-3} torr. The tube was then filled through the side arm with oxygen-free nitrogen and re-evacuated. This procedure was repeated twice, and then all taps were closed, leaving the reservoir and interspace A under vacuum.

The apparatus was then introduced into the flexible polyvinyl anaerobic chamber via the air lock. Once the sampling apparatus was inside the chamber, the three valves were opened, which caused gas to be drawn in from the chamber atmosphere to replace the vacuum in the apparatus. The valves were closed, and the apparatus was removed from the chamber. A sample of the culture chamber atmosphere was then contained in the reservoir and interspace A.

The tube was then connected by using the Swagelok vacuum union to the glass capillary of the mass spectrometer inlet, with the sampling orifice protruding into interspace B. (Preliminary experiments in which the septum cap was simply pierced by the hypodermic tubing of the sampling orifice did not yield consistent results, due to insufficient sealing between the hypodermic tube and the septum). The pumpout line was connected as shown in Fig. 2, and interspace B was evacuated, flushed with the contents of interspace A, and reevacuated. Valves 2 and 3 were then closed, and a background mass spec-

trum was obtained (Fig. 3). A portion of gas from the reservoir was then transferred to interspace A by opening valve 1 for a brief interval and then reclosing. By opening valve 2, the gas was admitted to the mass spectrometer through the sampling orifice. A second mass spectrum was then obtained. The whole procedure could be repeated several times until the quantity of gas in the reservoir became exhausted.

Measurement of oxygen tension. Quantitative data could be obtained by comparing the peak heights of the oxygen ions (mass, 32) with those of nitrogen. Since the signal from the most abundant isotopic form of nitrogen ($^{14}\text{N}_2$, mass,

FIG. 1. Gas sampler.

28) is "off-scale" under the present measuring conditions, we used the less abundant naturally occurring form ($^{14}\text{N}^{15}\text{N}$, mass, 29) which occurs to a 7.63×10^{-3} of the $^{14}\text{N}_2$. A correlation is made for the different relative sensitivity of the mass spectrometer toward oxygen as compared with nitrogen. Finally, the concentration of oxygen in

FIG. 2. Schematic representation of the gas sampler and its attachment to mass spectrometer.

FIG. 3. Mass spectral tracing of $^{14}\text{N}^{15}\text{N}$ (mass, 29) and O_2 (mass, 32) in background gas and in two runs from reservoir gas. Calculated oxygen = $(80/30) (7.63 \times 10^3 \times 0.85/30)$ ppm = 575 ppm.

the culture chamber atmosphere is obtained by knowing the relative proportion of nitrogen, using the formula: $^{32}\text{O}_2 = \text{height } ^{32}\text{O}_2 / \text{height } ^{29}\text{N}_2 \times \chi \text{ ppm}$, where $\chi = (7.63 \times 10^{-3} \times a/b) 10^6$, a = percent N_2 in the chamber (i.e., 85%) and b = the amplification factor of the sensitivity (i.e., $\times 30$) of mass spectrometer.

Although oxygen analysis is not absolutely necessary in routine use, it is important in research applications, for which oxygen tension in the chamber must be known with precision (1). This is particularly important when quantitative bacteriological work is undertaken and when comparative work between different laboratories is carried out. This is because the obligately anaerobic organisms are strongly inhibited or killed by exposure to molecular oxygen (4).

This procedure, therefore, provides a simple

and reproducible way of sampling gases and may be applied in centers which have access to a mass spectrometer.

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