Suitability of Butyl Rubber Stoppers for Closing Anaerobic Roll Culture Tubes

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Stringently anaerobic bacteria have been successfully cultured in rubber-stoppered roll tubes. Stoppers of certain types of rubber are so permeable to oxygen that they are unsuitable (Hungate, J. Bacteriol. 86:848, 1963). Butyl rubber is less permeable to gases than are many other types. The permeabilities of butyl stoppers and a common laboratory type to gases concerned in anaerobic culture work have been compared, under conditions comparable to those commonly employed for anaerobic cultures.

Both sets of stoppers were obtained from Arthur H. Thomas Co., Philadelphia, Pa. (HR-108 #00 recessed stoppers as supplied under catalog no. 8806); similar stoppers of butyl rubber were generously provided especially for this test by the company, and are now stocked as catalog item 8820-B.

Twenty-four glass culture tubes (16 by 150 mm; Bellco Glass, Inc., Vineland, N.J.) with narrow necks were freed from air by passing carbon dioxide gas through each tube and then seating the stopper without access of air. Eight additional tubes were filled with H₂. Half of the tubes in each group were closed with HR-108 stoppers, the other half with butyl rubber. The carbon dioxide and H₂ were freed from oxygen by passing the gases over reduced copper heated to 350 C. The gas in the tubes was initially at atmospheric pressure plus a slight additional pressure due to 1 to 2 ml of gas pushed in as the stopper was inserted. Samples of the gas in tubes 1-4 (for both sets of stoppers) were removed immediately with a 1-ml syringe (the dead space filled with CO₂), and were analyzed for oxygen by use of a Perkin-Elmer gas chromatograph with thermistor detectors and a silica gel column. None was found, though the method was sensitive to 0.025 μ liter of oxygen per ml of sample.

Tubes 5-8 were incubated in an atmosphere of oxygen at a temperature of 39 C for 10 days. The tubes were removed to room temperature, and the amount of water required to bring the pressure to atmospheric was measured by introducing water from a 5-ml syringe (fitted with a 1¼-inch 21-gauge needle) until atmospheric pressure was reached. The volume of water required to bring

each tube to atmospheric pressure is recorded in Table 1. In two tubes, little carbon dioxide had diffused out and the pressure was above atmospheric.

Tubes 9-12, after incubation in oxygen, were removed and the pressure in each was increased to 5 psi above atmospheric by introducing O₂-free carbon dioxide through the stopper with a syringe needle connected by a rubber tube to

TABLE 1. Amounts of water required to provide atmospheric pressure

Tube no.	HR-108 rubber	Butyl rubber
5	4.4	0
6	4.0	0
7	1.6	-1.5*
8	2.6	-1.0
Avg	3.2	-0.6

* Minus sign indicates positive pressure.

the reducing valve on a cylinder of CO₂ with the pressure set at 5 psi. This raised the contents above atmospheric pressure, and they could be sampled with little possibility that O₂ would enter during the sampling process. Samples were then removed and analyzed for oxygen (Table 2).

Since as little as 1 μ liter of oxygen can retard the growth of rumen methane bacteria (Smith and Hungate, J. Bacteriol. 75:713, 1958), the amounts of oxygen passing through the HR-108 rubber could be inhibitory.

Tubes 13-16, filled with hydrogen, showed positive pressures after incubation in the oxygen atmosphere. The excess gas above atmospheric pressure in both sets of tubes was measured (Table 3).

The butyl rubber was less permeable to hydrogen than was the HR-108. The amounts of hydrogen retained by both types of rubber suggest that they were less permeable to hydrogen than to carbon dioxide.

For anaerobic roll tube cultures, the butyl rubber is distinctly superior to the HR-108 stock. The impermeability to oxygen will permit growth of anaerobes especially sensitive to oxygen. The

TABLE 2. Amounts of oxygen (microliters) diffusing into tubes 9-12

Tube no.	HR-108 rubber		Butyl rubber	
	Per ml	Per tube	Per ml	Per tube
9	15.8	316	<0.025	<0.5
10	14.4	288	<0.025	<0.5
11	31.6	632	<0.025	<0.5
12	27.4	548	<0.025	<0.5
Avg		446		<0.5

impermeability to carbon dioxide will decrease the pressure drop in stored tubes. Reduced pressure increases the chance for mass entrance of air through any stab holes in the stoppers or

TABLE 3. Amount of gas (milliliters) above atmospheric pressure in H_2 -filled tubes incubated for 10 days in oxygen

Tube no.	Butyl rubber	HR-108 rubber	Difference
13	2.0	1.2	0.8
14	1.8	0.7	1.1
15	2.1	0.4	1.7
16	2.2	0.4	1.8
Avg	2.0	0.7	1.3

through leaks around them. The greater impermeability to both carbon dioxide and hydrogen will increase the retention of these gases in the tubes after the culture has grown, a feature of value if these fermentation products are to be measured.

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Reciprocal Recombination of Chromosome and F-Merogenote in *Escherichia coli*

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Volume 90, no. 6, page 1665, col. 2, line 9: Change "heterozygous for the z and the y genes" to "heterozygous for the z gene or the y gene, or both."

Methyl Dipicolinate Monoester from Spores of *Bacillus cereus* var. *globigii*

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Volume 90, no. 5, page 1503: Change the title to "Methyl Dipicolinate Monoester from Spores of *Bacillus subtilis* var. *niger*."