

Gas-Tight Flask for the Concurrent Measurement of Gas Metabolism and Growth in Methane-Oxidizing Bacteria

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A flask is described for use in conjunction with a gas chromatograph and a spectrophotometer to follow methane and oxygen uptake, carbon dioxide production, and cell growth concurrently in methane-oxidizing cultures.

Identification of methane-utilizing microorganisms is often difficult because of the requirement for demonstrating growth unequivocally as a function of methane oxidation (2-6). As a solution to the problem, we have designed a flask (i) that is leak tight under vacuum, (ii) whose component parts are sterilizable and interchangeable, (iii) that can be flushed and filled aseptically with appropriate gas mixtures, and (iv) that can be sampled repetitively for gas phase composition and culture turbidity without removing liquid or disturbing the gas phase.

The flask was constructed as follows (Fig. 1): A heavy walled Erlenmeyer flask was modified by adding a tube to the sidewall for turbidity measurements (Bausch & Lomb Spectronic 20 spectrophotometer). A 1.25-in (3.18 cm) length of 1/4-in (0.635 cm) outside diameter glass tubing (beveled) was added to the top of the flask. The flask sampling valve was a Hi-Vac Teflon valve (Kontes Glass Co., Vineland, N. J.) with 1-in (2.54 cm) lengths of 1/4-in glass tubing added to both sidearms. A Cajon Ultra-Torr union (1/4-in diameter; Cajon Co., Solon, Ohio) was used to join the flask to its sampling valve (Fig. 1).

For gas chromatographic analysis a column 25-ft long (7.62 m) and 0.04-in (0.102 cm) inside diameter, packed with 2.5 g of Porapak Q (Waters Assoc., Framingham, Mass.) was used in conjunction with a double loop, 100- μ liter volume microsampling valve and a microbead thermistor detector (Carle Instruments Inc., Fullerton, Calif.). The carrier gas was high purity helium at a pressure of 100 lb/in². With this system, hydrogen, methane, oxygen, carbon dioxide, krypton (internal standard to correct for pressure changes), and nitrogen (marker for air contamination) can be separated effectively at room temperature (1). The internal volume of the flasks was determined with water at room temperature.

The following procedures were used to sterilize, assemble, and add gases to the flasks. The main body of the flask containing the appropriate liquid medium was sterilized by autoclaving. The sampling valve sidearm, packed loosely with cotton, and the 1/4-in union were autoclaved separately in glassine bags. The entire unit was then assembled aseptically under a transfer hood after inoculation. The flask, attached to a vacuum-gas manifold fitted with a 1/4-in Ultra-Torr union, was alternately filled with helium and evacuated four times then filled with the appropriate gases to a final pressure of approximately 740 mm of Hg by using an absolute pressure gauge (Wallace & Tiernan, Bellville, N.J.). The flask sampling valve was closed, and the flask was equilibrated at room temperature for 30 min and then attached to the gas chromatograph microsampling valve via a 1/4-in Cajon union.

The technique for gas sampling was as follows. (i) A vacuum of 10-20 millitorr was established in both the sample loop of the gas chromatograph microsampling valve and the connection to the experimental flask. (ii) After closing the vacuum line between the vacuum pump and the sample loop, the flask sampling valve was opened, permitting 100 μ liters of head space gas to enter the evacuated sample loop. (iii) The flask sampling valve was closed and the gas chromatograph microsampling valve was switched, allowing carrier gas to inject the sample onto the column.

The data that can be obtained with this system by using the obligate methane-oxidizing bacterium *Methylocystis parvus* OBBP (6) as the test organism are shown in Fig. 2. It can be seen from concomitant measurements of the gases utilized and cell growth that *M. parvus* OBBP can be verified unequivocally as a methane utilizer. Growth ceased after 8 days of incubation when methane and oxygen were re-

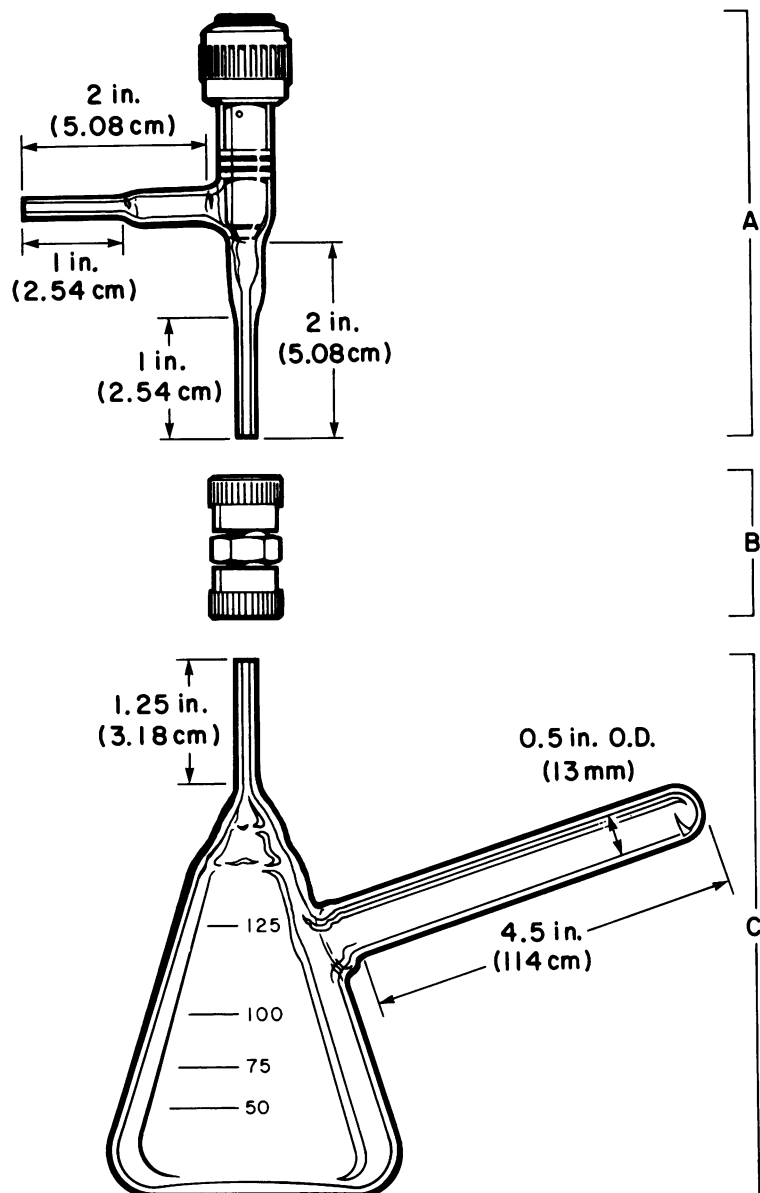


FIG. 1. Gas-tight flask design. Flask sampling valve (A). Cajon Ultra-Torr union $\frac{1}{4}$ -in (0.635 cm) diameter (B). Modified 125-ml Erlenmeyer flask (C).

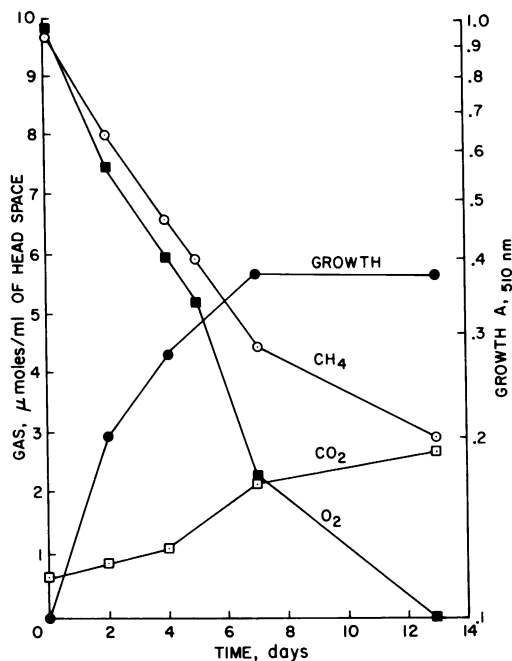


FIG. 2. Gas utilization and growth of *M. parvus* OBBP (6). Conditions: Nitrate mineral salts medium (6) 30 ml, 30 C, standing culture, 1-ml inoculum, volume of head space 116.54 ml. The zero time gas composition was CH₄, O₂, CO₂ as indicated above, Kr as internal standard, balance He.

duced in pressure, perhaps as a result of nutrient limitation due to diminished gas-mass transfer rates, other nutrient limitations, or accumulation of toxic products.

This system should also be useful for screening enrichments of pure cultures of aerobes or anaerobes for growth and specific gas utilization or production under a controlled gas atmosphere. Moreover, utilization rates and ratios for the different gases can be established for cultures during growth.

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