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Improved Culture Flask for Obligate Anaerobes

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An improved flask system for the growth of extremely oxygen-sensitive bacteria in liquid culture is described. The improvement described utilizes an all-glass, neoprene-stoppered flask designed for growth of 50- to 1,000-ml cultures of bacteria with continuous gassing.

Several procedures have been described that are appropriate for the growth of anaerobic microorganisms (1-7). The Hungate tube technique with its modifications has proven to be an excellent method for isolating and maintaining small quantities of cells (3). However, continuous gassing and agitation is necessary for maximal growth of some hydrogen-oxidizing bacteria (8). The methods described by Bryant et al. (1) for culturing larger quantities of cells have proven acceptable for even the most oxygen sensitive of the anaerobes; however, this paper describes modifications of their 500-ml culture flask which improve its function, convenience, and durability.

Although of good design, the old-style flask suffered from several disadvantages: (i) small amounts of air leaked in through the top, due to slight movement of both the stopper in the top and the steel tubes in the stopper (this leaking was accentuated with age of the stopper assembly); (ii) it was somewhat unhandy and top heavy; and (iii) various parts (e.g., wire, spring, and stopper assembly) rusted or wore out with time. The improved flask has the following advantages: (i) it is much more immune to oxygen leakage since the only direct opening to the atmosphere is tightly plugged with a solid neoprene stopper which can be conveniently changed at will; (ii) the flask is convenient and compact; and (iii) the flask is good for many years of continuous use.

The flask shown in Fig. 1 and 2 is a modified 500-ml Erlenmeyer which is normally used with 200 ml of culture media. It can be constructed from Pyrex glass parts by a qualified glassblower. Gas enters through a glass tube partly filled with sterile cotton and passes into the flask via a short section of thick-walled latex hose (Scientific Products, no. R5330-4). Gas enters the culture as small bubbles from the constricted tip of the gas inlet. The 0.75-mm orifice of the gas inlet provides small bubbles for efficient gas transfer in the culture medium. The flask is equipped with four baffles on the bottom surface to increase turbulence (and thus gas mixing) while being agitated in a water bath shaker. Gas exits through the top and passes through a bubbler tube firmly attached to the side of the flask. The bubbler tube is filled with medium by tilting the culture flask or, alternatively, reducing agent can be added with a hypodermic syringe. This provides a moisturizer trap to alleviate media evaporation, an effective barrier to oxygen entrance at low gassing rates, and a simple device for estimating gas flow. A short glass tube filled with sterile cotton is attached to the final exit by using a section of latex hose. A glass neck opening protrudes at an angle of about 60° from the flask wall half-way up. The diameter of the opening is such that a no. 4 stopper will fit very tightly; the internal diameter is also decreased in a 20-mm distance from outside (23 mm) to inside (19 mm), assuring a completely closed system.

This flask can be equipped with any diameter of sidearm designed to allow turbidity measurements of the cells. The sidearm should be placed opposite the gas exit so that when the flask is tipped no culture will inadvertently enter the bubbler. A variety of sizes of flasks may be used, from 125 ml to 2 liters, allowing volumes of cultures from 50 ml to 1 liter. The flask is incorporated into a strictly anaerobic system similar to that described by Bryant et al. (1). Access to the flask may be accomplished by removing and replacing the stopper quickly or by insertion through the stopper with a syringe and a 22-gauge 1.5-inch (ca. 3.81 cm) needle. Sterility can be assured by flaming the stopper and glass neck surfaces. Care should be taken not to ignite the outlet gas from the flask, if it is flammable. The no. 4 neoprene stopper (Scientific Products) used in our lab completely maintains the anaerobicity of both flasks and an-

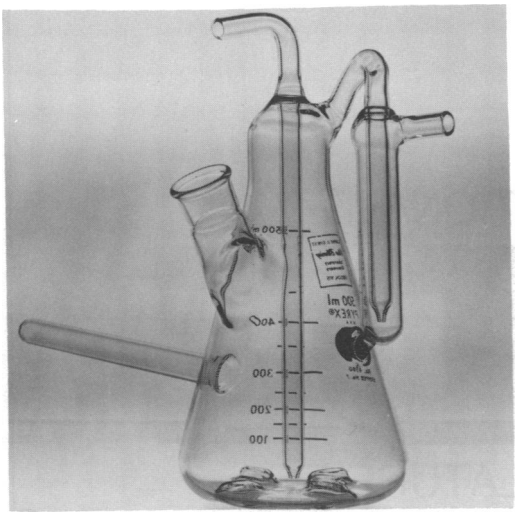

FIG. 1. *Photograph of the culture flask as constructed by a glassblower.*

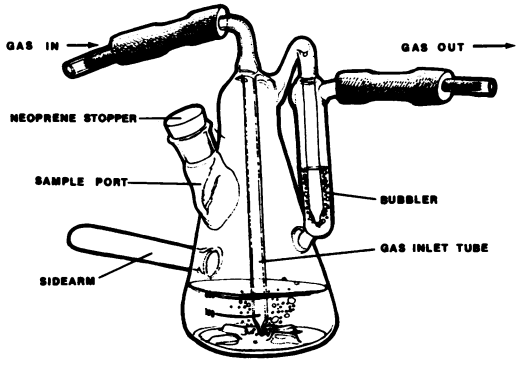

FIG. 2. *Anaerobic shake flask equipped for continuous gassing and measurement of culture density.*

aerobic culture tubes. The neoprene stoppers, as opposed to the black rubber stoppers, are easier to insert needles through and do not burn and smear when exposed to flame. Alternatively, butyl rubber serum bottle stoppers can be used (5) but must be replaced more often.

This method has been used in our laboratory to cultivate several species of extremely anaerobic bacteria (including *Methanobacterium thermoautotrophicum*, *Methanosarcina barkerii*, and other methanogenic bacteria, and several species of *Desulfovibrio* and *Clostridium*) in a wide variety of media.

We feel that the flask system described is excellent for the growth of the most oxygen-sensitive microbes, and that its use will be valuable in laboratories concerned with ecological, physiological, or medical aspects of anaerobic microbiology. Further, by the total exclusion of atmospheric gases, the content of the gaseous phase of cultures of any organism (aerobic or anaerobic) can be controlled rigorously with this system, given the proper mixture of bottled gases.

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