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Apparatus for Metabolic Studies with Anaerobes

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An apparatus is described which allows metabolic experiments with obligate anaerobic bacteria to be performed with minimal disturbance of the E_h of the culture.

Continuous sampling of cultures during studies on the metabolism of anaerobes presents problems of which aeration of the culture is the most serious one. Certain microorganisms require increased CO_2 levels so that control of the gaseous environment of the culture during experimentation is essential. In the course of studies of the metabolism of vitamin K by *Bacteroides melaninogenicus*, the uptake of various amino acids and other compounds was determined. For this purpose, the apparatus shown in Fig. 1 was constructed. It consists of a 250-ml Erlenmeyer flask fitted with a screw cap which is sealed to the side of a Pyrex anaerobic jar (Brewer jar). A small tube projects from the base of the flask to the exterior of the jar. This tube is sealed with a vial cap.

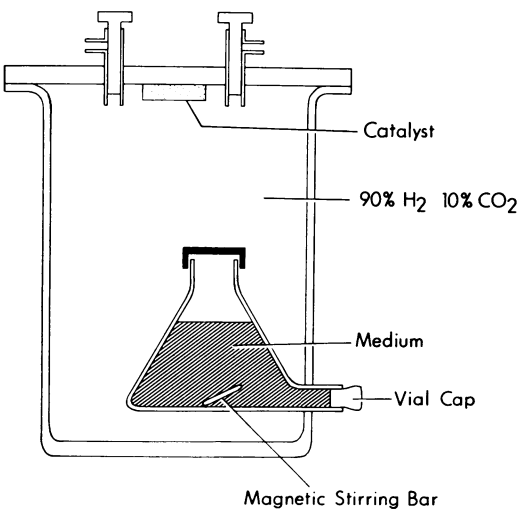

FIG. 1. *Diagram of modified anaerobic jar.*

In use, 200 ml of medium is placed in the flask containing a magnetic stirring bar and sterilized by autoclaving. After cooling, with the cap of flask loose, the top is placed on the jar which is then evacuated and filled with a mixture of 90% H_2 and 10% CO_2 to a positive pressure of 30 inches of water.

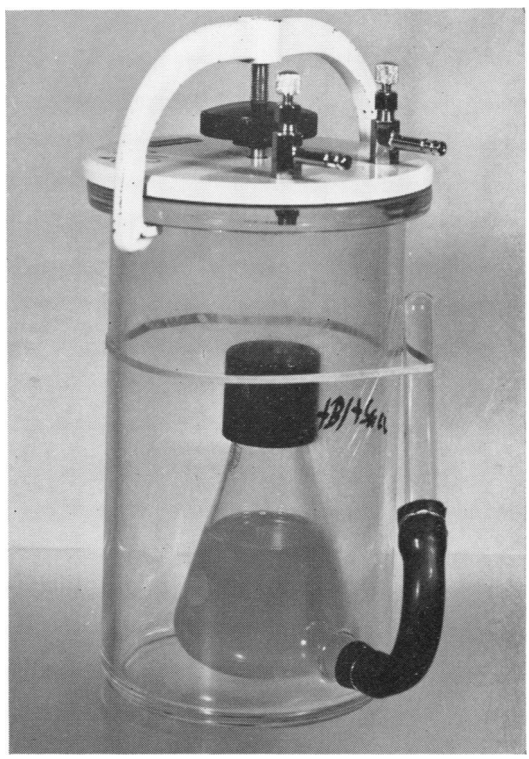

FIG. 2. *Modified anaerobic jar with nephelometer tube attachment.*

Inoculations are made into the medium with a syringe through the vial cap. Material to be injected into the medium may be prereduced if necessary. Samples are removed in a similar fashion, and the optical density and other parameters are measured.

A variation on the above model is illustrated in Fig. 2. A nephelometer tube is attached to a short rubber tube which is in turn attached to the side tube leading to the Erlenmeyer flask. During the experiment, the culture is incubated with the nephelometer tube in an upright position; the culture is stirred, and the tube is lowered, filled with culture, and read. The culture in the nephelometer tube is then returned to the reservoir flask by raising the tube and incubated further.

This apparatus has been in use for 3 years and, besides uptake studies on radioactive amino acids, uridine, thymidine, etc., by *B. melaninogenicus*, pulse-chase experiments were performed with this apparatus. Thus, with this equipment, metabolic experiments with minimal disturbance of the E_h of the culture can be routinely done.

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