Technical methods

A simple, inexpensive semi-continuous or continuous culture apparatus

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The need for a small, semi-continuous culture apparatus became apparent during the course of certain trial experiments in our laboratory. Representative literature (Monod, 1950; Novick and Szilard, 1950; Málek and Fencl, 1966; Málek, Beran, Fencl, Munk, Řičica, and Smrčková, 1969) generally describes an abundance of designs for complex, expensive units but none that could meet our budgetary requirements and immediate needs. Accordingly, we designed an apparatus that is both compact and of small volume (200 to 300 ml), easy to sterilize and assemble, convenient for sampling, and inexpensive.

General Description

The apparatus consists of two modified, calibrated Pyrex separatory funnels. The larger of the two (500 ml) serves as the reservoir and the other (250 ml) as the reaction vessel. A pressure-equalizing tube (A) and a micro-drip Teflon stopcock (B) are added to the reservoir. These modifications allow a free flow of medium from the reservoir to the reaction vessel and permit finer regulation of the flow rate in a closed system. Extension of the drip tube (C) into the reaction vessel facilitates accurate determination of the flow rate. A port (D) attached to the upper side of the reaction vessel permits the introduction of a sterile, disposable, plastic, 1 ml pipette for periodic sampling.

The reservoir may be filled intermittently through a port (E). Both ports (D and E) are flamed to assure sterility during the sampling and filling procedures. Representative samples for assay are assured by

using a small magnetic stirrer² (F) to maintain continuous gentle mixing of the culture. A constant temperature is maintained by placing the entire apparatus in a conventional incubator of an appropriate size. Observation of the apparatus and monitoring of flow rates are facilitated by employing an incubator equipped with inner glass doors.

The initial flow is regulated by adjusting the drop rate. This rate can be verified periodically by observing the total flow volume of the receding medium level in the precalibrated reservoir. The location of the port (D) permits sampling without removal of the apparatus from the incubator. Additional ports may be introduced to accommodate other monitoring apparatus, such as pH, O₂, and CO₂ electrodes.

Comment

The use of this apparatus has exceeded our expectations for semi-continuous culture experimentation in our laboratory. However, if a strictly continuous culture system is desired, the outflow stopcock of the reaction vessel may be left open and a collection

Tri-R Instruments, Rockville Centre, New York (model 1 MS-7).

Fig. 1 Diagrammatic representation of continuous culture apparatus:

- (A) Pressure equalizing tube
- (B) Micro-drip Teflon stopcock
- (C) Drip tube
- (D) Sampling port
- (E) Reservoir filling port
- (F) Magnetic stirrer and stirring bar

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flask positioned to receive the effluent.

Since the basic component glassware is readily available and inexpensive, repair and replacement costs and time lost in experimentation have been minimized. These facts should permit anyone with a moderate budget to use a similar apparatus.

As in the use of all semi-continuous or continuous culture devices, caution must be exercised in order to avoid contamination. However, if the principles governing sterile technique are observed, contamination does not pose a problem, because the entire system is essentially closed except during the short (one- to two-minute) sampling period.

References

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An adjustable, semi-automatic dispenser for use with dry absorbents in protein-binding techniques

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The assay of steroid hormone levels in plasma often requires the addition of precise amounts of a dry absorbent, such as florisol, to separate the free from the protein-bound components. To achieve this various dispensers have been constructed and described, ranging in complexity from the simple calibrated spoon described by Murphy (1967) to the more elaborate semi-automatic dispenser described by Castro, Klein, and Cryderman (1970). Modification of the dispenser described by Bayard, Beitins, Kowarski, and Migeon (1970) has resulted in a semi-automatic dispenser which is robust, has a high degree of precision, is of simple construction, and has the further advantage of being adjustable to deliver various amounts of the dried material.

Construction

The dispenser was made from a 250 ml separating flask fitted with a teflon stopcock. The stopcock was removed and its existing channel widened to 6 mm. At one end the channel was widened to a diameter of 10 mm to form a recess which was 2.5 mm deep. Using 10 mm diameter teflon rod a plug was made as shown in Figure 1. The diameter of the shaft was 6 mm, the head 10 mm in diameter and 3 mm thick. The plug was inserted into the stopcock and pushed home. The head of the plug was then shaped to match the contour of the stopcock. A punch mark was placed on the stopcock and the plug to allow correct alignment of the plug for subsequent insertions.

Using a scalpel blade the shaft of the plug can be reduced in length until the desired amount of absorbent is delivered (a shaft length of 7 mm delivered about 40 mg of florisol). With this technique plugs have been made which, when inserted into the stopcock, will deliver approximately 40 mg, 60 mg, and 90 mg of florisol. One of the side arms of the