Improved Automatic Inoculator for Bacterial Cultures

S. MAIER

Zoology Department, Ohio University, Athens, Ohio 45701

Received for publication 9 February 1970

A simple automatic inoculator which allows the simultaneous inoculation of several cultures with identical or different inocula is described. The use of Teflon spaghetti tubing allows great flexibility in handling and placing of components. The inoculator can be adapted to handle aerobic and anaerobic bacteria.

Several automatic inoculators have been described which provide vigorously growing cultures of predetermined age (1, 2, 4). The advantages of having cultures of specified age early in the morning are obvious. Limitations of inoculators previously described include incubation of inoculum and final culture at the same temperature (1, 2), anaerobic inoculum (1), narrow limits of the volume of inoculum (2), use of culture flasks larger than 500 ml only (2), risk of premature inoculation (2), and expenditure of labor and equipment to obtain multiple inoculations (4). The system described here is essentially a modification of Knolle's inoculator (3), but is easier to construct, provides greater flexibility in the arrangement and choice of components, and allows considerable variation in the volume of the inoculum. In addition, the system also works when the simpler method of inoculating the final shaker flask in the evening, and delaying shaking until a desired time during the night, fails.

Figure 1 shows the inoculator with a single-inoculum reservoir (D), inoculum line, and culture flask (E). Teflon spaghetti tubing [Bel-Art Products; 0.053-inch (0.13 cm) inner diameter; 0.012-inch (0.03 cm) wall] and 16-gauge hypodermic needles were used for air lines and for the transfer of the inoculum. The timer (A) activates the air pump (B) at the preset inoculation time, and air is blown through the air filter-manifold (C) into the top of the inoculum reservoir (D; also Fig. 2).

The resultant air pressure forces the inoculum through the line (i, Fig. 2) into the medium of the final culture flask (E, Fig. 1). Culture flask (E) and inoculum reservoir (D) are mounted on a shaker to provide aeration for the final culture. The timer can be programmed to turn off the air pump once the inoculum is transferred.

The inoculum reservoir (D, Fig. 1; and Fig. 2) consists of a 15-ml conical centrifuge tube, which is closed with a serum bottle stopper (s, Fig. 2). The inoculum line (i, Fig. 2) and air line needle (a, Fig. 2) pass through holes in the thin portion of the stopper. A bottle closed with a one-hole rubber stopper serves as both air filter and manifold (C). A piece of cotton-stuffed glass tubing admits filtered air, which leaves by way of hypodermic needles that pierce the stopper from the inside. A number of flasks may be inoculated simultaneously by providing the filter-manifold with several air lines and inoculum reservoirs. In multiple inoculations, the inocula can be varied independently of one another. The total number of flasks inoculated is limited only by the capacity of the air pump.

In practice, the assembled filter-manifold with attached air line, inoculum reservoir, and inoculum line (the distal end of the latter wrapped in aluminum foil) is autoclaved. For use, the empty inoculum reservoir is replaced with a similar one containing the desired kind and volume of...
inoculum; aseptic precautions are observed. The terminus of the inoculum line (i) is removed from its wrapping and inserted into the culture flask. The cotton stopper keeps the line in place.

For reproducible results, a standardized inoculum must be pipetted into the inoculum reservoir in the evening. Prior to discharge, the inoculum in the reservoir may be allowed to grow or may be kept in an ice bath. The flexible tubing does not limit the placing of components. Starting with Bacillus megaterium spores, for example, we found a sequence of 30 C Brain Heart Infusion (Difco) cultures satisfactory. A 24-hr culture was diluted 1:10 and incubated for 8 hr; the inoculum reservoir received 0.5 ml of a 1:100 dilution of the 8-hr culture; the inoculum was incubated and discharged 10 hr later (3 AM) into 20 ml of a glucose mineral medium in a 500-ml flask. At 9 AM, an exponential culture with an optical density of 0.3 (Coleman Nephocolorimeter, model 9, 430-nm filter, 19-mm side arm culture flask) was obtained. Varying the volume and density of the inoculum and the time of discharge will provide cultures of different optical densities.

With only slight modifications, this system can be used without a shaker. The culture flask is replaced by a culture tube. The air pump is not shut off after the transfer of the inoculum. The combination of tubing [¾ inch (0.8 cm) inner diameter] between pump and manifold and spaghetti tubing thereafter provides a sufficient pressure differential to supply comparable flow rates in multiple culture tubes. However, it is imperative to adjust the air pressure carefully to achieve transfer, yet prevent a "blow-out" in the culture tube. If the laboratory is equipped with an air line, a combination of pressure-reducing regulator and solenoid valve can replace the air pump.

Further modifications make inoculation of anaerobic organisms possible. A supply of inert gas must be available. Initially, line i (Fig. 2) must terminate above the inoculum culture. After the system has been flushed to remove oxygen, line i is pushed down to the bottom of the inoculum. When the timer activates the gas flow, the inoculum is transferred. Continued gas flow keeps the final culture anaerobic.

Grateful acknowledgment is made to J. V. Lawrence for helpful discussions and to J. Richardson for the illustrations.

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