In the Laboratory

Production of Penicillin X in "Submerged" Surface Cultures

EDITH STICE and ROBERTSON PRATT[1]

Division of Microbiological Research, Cutter Laboratories, Berkeley, California

Several references to the potential therapeutic importance of penicillin X have been published (*1, 2, 3, 6*). This compound is of no less interest and importance to plant physiologists and students of mold metabolism than to the clinician, but unfortunately it is not generally available at the present time. The apparatus described below is deemed valuable, since cultures grown in it consistently produce relatively large proportions of this antibiotic agent. The unit was originally devised in an attempt to incorporate in surface cultures the advantages of the deep-vat method of fermentation.

The apparatus is simple in design and may be constructed easily. It will produce a continuous supply of penicillin for several weeks without resterilization or reinoculation. The essential features are shown schematically in Fig. 1 (not drawn to scale).

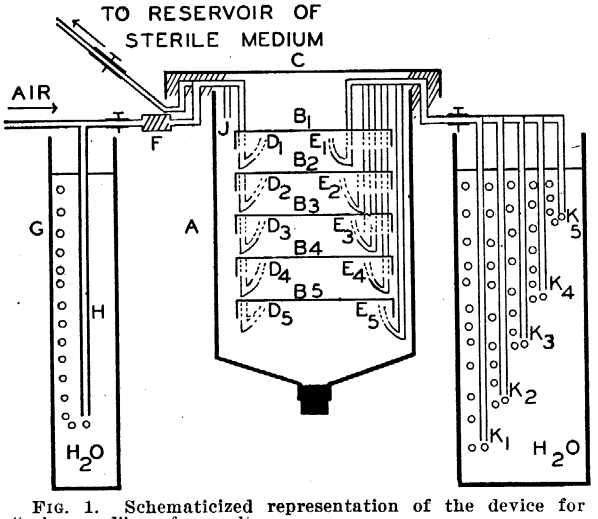

FIG. 1. Schematicized representation of the device for "submerged" surface cultures.

The fermenter, A, may be made any desired capacity, and the rest of the apparatus then may be built accordingly. A convenient laboratory-scale working model has been made from an inverted 10-liter bottle with the bottom removed. Several inverted trays (large Petri dishes or aluminum cake pans have been found practical), B_1, B_2, . . . B_n, are supported by a frame (not shown) consisting of three vertical rods, at the inner periphery of the cylinder, which are attached to a horizontal basal plate anchored by suitable means to the rubber stopper inserted in the mouth of the bottle. Rubber stoppers or sections of heavy rubber tubing slipped around the supporting rods hold the trays in the desired positions. The cover, C, consists of an inverted aluminum cake pan, the inner rim of which is padded with cotton (shown by diagonal lines). Aeration is effected by means of a separate inlet tube, D, and outlet tube, E, for each surface. Tubes and the supporting frame mentioned above may be of glass, stainless steel, aluminum, or Inconel. Incoming air from cylinders or house line passes through the sterile cotton filter, F. The pressure-control device, G, is not essential but is often useful, especially when the pressure on the air line is subject to fluctuation. If the level of tube H is properly adjusted, considerable fluctuation of the pressure of incoming air may occur without causing serious changes in the fermenter and consequent back or forward surging of the fermentation liquor. Samples are withdrawn aseptically from any desired level in the tank by means of a siphon tube (not shown). When not in use for sampling, the exposed end of this tube may be protected by a sterile test tube plugged with cotton. If large quantities are withdrawn, air inlet and outlet tubes should be closed until the initial volume of liquid has been restored by addition of fresh medium. After the mats are well formed, almost all of the solution can be drained from the tank without interfering with subsequent activity of the mold.

In our experiments the dry, sterile fermenter is inoculated by the "dry spore" (*4*) technique with the cover slightly raised at one edge (as for Petri dish inoculation), and then the sterile culture medium is siphoned into it through the J tube, causing the spores to rise and float on the surface of the liquid. During the siphoning operation the air inlet manifold and the outlet tubes, E_1 . . . . E_n, are closed by means of pinch clamps. Consequently, as the liquid rises, an air pocket forms under each plate, providing a surface upon which the mold mat develops. After the fermenter is charged with culture medium, the open ends of the leveling tubes, K_1 . . . . K_n (connected by rubber tubing to E_1 . . . . E_n, respectively), are submerged in water to a depth greater than that necessary to hold the medium under each plate in the fermenter at the desired level, and the tubes are clamped in place. Then the pinchcocks controlling the several

[1] Currently on leave from the University of California, College of Pharmacy, San Francisco.

tubes are opened, and each tube, beginning with the one from the lowest plate and progressing upward, is raised to a point slightly higher than that at which bubbles first appear. Following this adjustment, release of the proper amount of compressed air into all plates simultaneously from the inlet manifold is accompanied by a steady flow of outgoing air from under each plate without a change in the level of the medium and without undue agitation of the surface of the liquid. As long as the apparatus is in operation a stream of air continuously passes over the mold under each plate. During the first three days of operation or until penicillin can be detected in the liquor, the outgoing air is characterized by a foul odor.

The fermenter, air filter with inlet manifold, siphon tubes for introducing media and withdrawing samples, and the outlet tubes are sterilized as one unit. Since the outlet tubes are under continuous positive pressure during a run, it is unnecessary to provide filters for them, but of course they must be connected sterilely to the respective leveling tubes.

In frequently repeated trials with a working model of this apparatus, 40–45 per cent of the penicillin synthesized by *Penicillium notatum* 1249.B4[2] on a medium recommended for surface production of penicillin (*5*) was insoluble in $CHCl_3$ (two extractions in the cold at pH 2.2) but was soluble in amyl acetate. Differential assays were not performed on these samples, but their solubility characteristics were indicative of penicillin X. Yields of 60–70 Oxford units/ml. may be obtained readily in six or seven days on the above medium, and moderate-sized portions of approximately equal potency may be withdrawn at frequent intervals over a period of at least three weeks provided the initial volume of liquor is restored each time by addition of sterile medium. The same strain of mold on the same medium in the conventional bottle method of surface production run simultaneously consistently produced about 85–90 Oxford units/ml., 15–20 per cent of which was insoluble in $CHCl_3$.

The mean ratio of $CHCl_3$ insoluble/$CHCl_3$ soluble penicillins obtained in the apparatus for "submerged" surface cultures (about 10 runs) was 0.44. The lowest ratio obtained was 0.40, and the highest, 0.47. The range in conventional bottle production was 0.14–0.25 with a mean of 0.18.

Results of a typical run with a 10-liter laboratory model of the "submerged" surface fermenter are shown in Fig. 2. For this run the apparatus was set up with four plates and was charged with six liters of medium. At intervals samples were withdrawn from a level between the second and third plates.

[2] This strain is a variant of *P. notatum* NRRL 1249.B21.

The points on the curve represent the total number of units of penicillin in the fermenter at the time indicated plus the total number withdrawn previously for assays, extractions, or other purposes; thus, a drop in the curve indicates that destruction of penicillin exceeded production during the period involved.

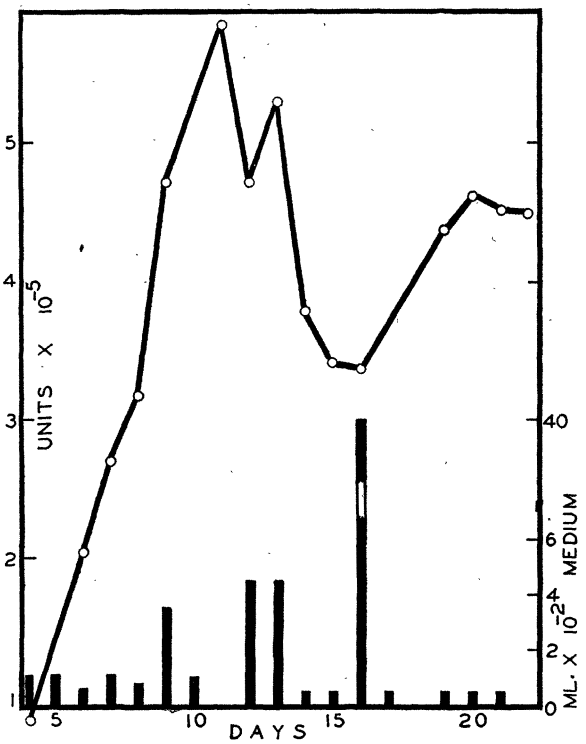

FIG. 2. *Curve.* Summary of penicillin production in run No. 7 with "submerged" surface fermenter. Ordinates show hundreds of thousands of units. *Bars.* Volume liquor removed and fresh medium added during run.

The vertical bars show the volume of liquor removed and replaced with fresh medium each day. The effect of addition of fresh medium is reflected for the first time in the assay for the following day. To facilitate visualization of this effect the bars have been displaced one day to the right on the abscissa. Thus, the volumes recorded for any given day were actually added to the culture on the preceding day.

It is interesting to note that the rapid destruction of penicillin evidenced after Day 11 was on two occasions counteracted and exceeded by increased production brought about by removal of considerable volumes of liquor and introduction of fresh medium. This experiment was discontinued at the end of the twenty-second day.

The optimum rate for removal and renewal of the culture liquor for maximum production of penicillin and of penicillin X has not yet been determined. Variables to be considered include geometry of the fermenter, particularly with respect to surface/vol-

ume ratios, rate of aeration, temperature, strain of mold, composition of the medium, and pressure to which the mold mat is subjected.

References

1. FLIPPIN, H. F., MAYOCK, R. L., MURPHY, F. D., and WOLFERTH, C. C. *J. Amer. med. Ass.*, 1945, **129**, 841.
2. LIBBY, R. L., and HOLMBERG, N. L. *Science*, 1945, **102**, 303.
3. ORY, E. M., MEADS, M., and FINLAND, M. *J. Amer. med. Ass.*, 1945, **129**, 257.
4. PRATT, R. *Amer. J. Bot.*, 1945, **32**, 528.
5. RAPER, K. B., ALEXANDER, D. F., and COGHILL, R. D. *J. Bact.*, 1944, **48**, 646.
6. WELCH, H., PUTNAM, L. E., RANDALL, W. H., and HERWICK, R. P. *J. Amer. med. Ass.*, 1944, **126**, 1024.

A Low-resistance Valve and Indicating Flowmeter for Respiratory Measurements[1]

LESLIE SILVERMAN and ROBERT C. LEE

Department of Industrial Hygiene, Harvard School of Public Health

Nutrition Laboratory, Carnegie Institution of Washington

In performing respiratory exchange experiments and measurements of respiratory air flow it is important that the valves for separating inspiration and expiration operate with low air-flow resistance and negligible back leakage. Dautrebande and Davies (*2*) have shown that the presence of appreciable breathing resistance in mask valves may alter the alveolar carbon dioxide concentration. Several low-resistance valves have already been described in the physiological literature, the principal ones being those of Pearce (*5*), Henderson and Haggard (*3*), and Bailey (*1*). Most of these valves may be suitable for measurements under resting or sedentary conditions but are not wholly satisfactory for working conditions when maximum air flows exceed 75 liters per minute. It is important to note that the maximum rate of air flow is specified here rather than the minute volume. The average inspiratory or expiratory air flow is approximately twice the minute volume during work, and the maximum air flows may be as high as three times the minute volume because of the shape of the respiratory air-flow curve *vs.* the time (pneumotachogram). The valve described in this article was designed primarily for experiments requiring heavy work rates and maximum effort either on a treadmill, bicycle ergometer, or hand ergometer.

In a recent article (*4*) we described a low-resistance valve (designated as a tubular valve) for use in a recording inspiratory air-flow instrument. While this valve was very satisfactory for experiments requiring low air-flow resistance and has a resistance of 0.1, 0.6, and 2.2 mm. of water at air flows of 25, 100, and 200 liters per minute, respectively, it is not entirely satisfactory when inspiratory and expiratory

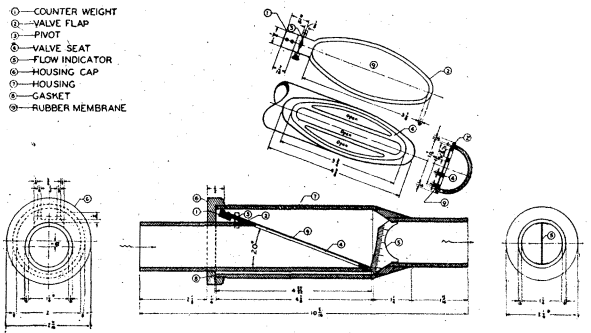

FIG. 1. Detail drawing of low-resistance valve construction.

measurements are made together because of back leakage and air-volume changes on closure. This tubular valve was designed primarily to prevent passage of expired air into the inspiratory line. The valve described below is very low in resistance and also does not have significant back leakage.

The principles involved in the operating of this valve are fundamental in the design of respiratory valves. Such valves, in order to be low in resistance, should have negligible opening pressure and should offer little change in direction so that air-turbulence losses are not induced. The valve seating surfaces should have a small contact area so that they do not adhere to each other when wet. Wet conditions in expiratory valves result from condensation of exhaled moisture upon the expiratory valve surfaces. Treatment of the seating surfaces to prevent moisture absorption and wetting aids materially, but not completely, in reducing the moisture effect; hence it is desirable to provide that such surfaces have a minimum contact area.

Construction. The details of the valve construction are shown in Fig. 1. The original models of the valve were made of brass with aluminum frames covered with rubber membranes for valve flaps and glass cylinders for valve housings. The present models are now made of methyl methacrylate resin (Lucite) plastic. The only parts not made of this plastic are the rubber membrane, the rubber housing gasket, the metal counterweight, the metal flow indicator, and the metal flap pivot. The complete valve, when made of plastic, weighs 7.4 oz. (210 grams). The chief advantages of the plastic material are its light weight and transparency. The transparent plastic allows

[1] This work was done in part under Contract No. OEMsr-306 between the President and Fellows of Harvard College and the Office of Scientific Research and Development, which assumes no responsibility for the accuracy of the statements contained herein.