IN THE LABORATORY

A Technique for Aeration of Sterile Liquid Culture Medium

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Sterile aeration of liquid culture medium can be accomplished simply by inserting a sterile tube through the cotton plug at the mouth of the tube and introducing sterile air through this tube. This method, however, does not allow for convenient manipulation of the culture flasks, making transfers, or moving the flask from the sterile air connection. In order to provide a more convenient and efficient means of aerating sterile root cultures the flask illustrated in Fig. 1 was constructed.

Sintered glass filters were prepared according to the method

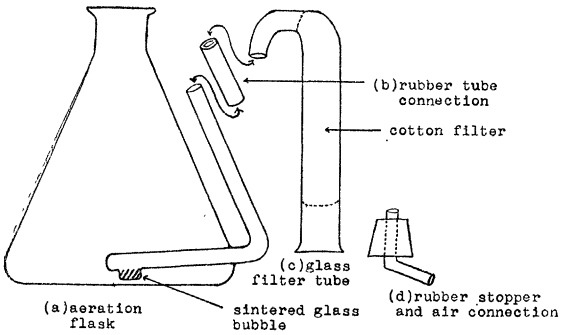

FIG. 1

of Kirk, Craig, and Rosenfels (*1*) and fused to the inside wall of the Erlenmeyer flasks as illustrated (a). An external arm, long enough to allow ample room for the culture medium to flow out without overflowing if the air pressure were cut off, was fused in place at the exit of the filter tube and bent up about parallel to the side of the flask. The sintered glass bubble was placed, open face down, in about the center of the flask bottom. Erlenmeyer flasks (250 ml.) were used.

To provide sterility, a glass tube was made in the shape shown (c), filled with cotton, and fitted with a one-hole rubber stopper and glass tube (d) in the outer end and a rubber connection (b) on the small end to hook to the glass side arm of the flask.

This flask was made and connected in sets of 10 to 14 with a glass manifold, the pressure and air flow in each flask being regulated with a screw clamp on the short rubber tube between the glass tube (d) and the manifold. Some special care had to be taken to attach the cotton filter tube to the side arm of the flask, but, once set up, the flasks were quite substantial. The cotton filter tubes were placed so that they lay against the flask. To avoid the danger of strains at the base, the flasks must be annealed well when the sintered glass filter tubes are inserted. It is also important to test the filters carefully for consistency in bubble size and porosity if the flasks are to be used in sets; otherwise, it is difficult to adjust the air flow evenly.

Further precautions were taken to ensure sterility by passing the laboratory compressed air used through a 2-l. suction flask of sterile water, protected on each side by glass, cotton-filled tubes (15 x 1 inches), before passing it into the glass manifold with the individual take-offs. The water, in saturating the air, prevented the cultures from drying out. Cotton plugs were used at the top of the flasks as usual. The air intakes may be detached and reconnected without contaminating the cultures.

Excised root cultures were kept sterile and aerated in these flasks over a period of three months and could have been kept longer if desired.

Reference

1. KIRK, P. L., CRAIG, R., and ROSENFELS, R. S. *J. ind. eng. Chem.* (Anal. ed.), 1934, **6**, 154–155.

Studies on the Stability of Streptomycin in Solution

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In the process of developing satisfactory assay methods in this laboratory, preliminary studies have been made of the stability of the F.D.A. streptomycin working standard (*2*) when held in solution in different concentrations and at three pH levels. The working standard, which was established on November 20, 1945, has a potency of 400 μg. of activity/mg. when streptomycin base is assigned a value of 1,000 μg./mg. It was established early that streptomycin is considerably more stable in solution than penicillin, and it appeared to be particularly desirable to determine the most efficient concentration and pH level that could be utilized for the establishment of a working standard solution. Accordingly, the working standard was dissolved in sterile 0.05M potassium phosphate buffer at pH levels of 6.0, 7.0, and 8.0 in quantities sufficient to give solutions of 100 μg./ml. and 1,000 μg./ml. at each pH level. The two concentrations at each pH level were divided into 100-ml. amounts and tightly stoppered to minimize evaporation and chances of mold contamination. The solutions were held at 10°C. and assays[1] performed by both the turbidimetric and plate methods (*2*) every week for a period of three months. The data obtained were subjected to statistical

[1] These solutions were assayed against a standard solution prepared monthly to contain 100 μg. of the working standard/ml. in .005M potassium phosphate buffer—pH 6.