

A LABORATORY SCALE APPARATUS FOR THE CONTINUOUS  
CULTURE OF MICRO-ORGANISMS WITH AUTOMATIC  
pH CONTROL

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INTRODUCTION

Since the publication of the now classical work of Monod (1950) continuous culture methods for the propagation of micro-organisms have attracted widespread interest and attention. (Monod, 1950; Novick & Szilard, 1950; Maxon, 1955; Herbert, Elsworth & Telling, 1956).

Various problems peculiar to continuous systems have emerged, primarily as a result of the demand for the avoidance of contamination for long periods of time. In many cases, the difficulties have been overcome by designing special apparatus for a particular purpose. Evidence that the challenge has been taken up in many laboratories is indicated by the variety of publications on this subject (Zubrzycki & Spaulding, 1958; Kubitschek, 1954; Perret, 1957; Rotman, 1955) and the fact that the "chemostat" introduced by Novick & Szilard (1950) has been developed further by Kubitschek and Rotman (*loc. cit.*).

However, the various types of apparatus described in the literature have proved unsuitable for our purposes, especially in experiments over long periods. We have accordingly developed our own modification of the "chemostat". This modified instrument has given satisfaction in this laboratory in runs lasting several weeks. In addition, like the chemostat, it has the merit of simplicity, being easily assembled in any laboratory with elementary glassblowing facilities.

Feeling that the apparatus may be useful to other workers in this field the entire assembly, as used in our laboratory, including the culture vessel and automatic pH control system designed, is described. (See also Weyland & Serfontein, 1959, in press.)

FEEDING SYSTEM

A 10 litre flask (A) is mounted with its bottom above the surface of the bench and connected to the pressure regulator (Z) (Fig. 1) by means of two pieces of latex rubber tubing. One of these leads from a small length of glass tubing, through the rubber stopper in (A) to the left hand top exit tube in (Z) while the other connects the bottom left exit tube of (Z) to the delivery tube in (A) as shown in Fig. 1.

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The pressure regulator consists of a 30 mm. internal diameter pyrex glass tube (length 60 cm.) closed at the bottom and with four side tubes (8 mm. internal diameter) of which two are connected to (A) as described above.

The upper end of (Z) accommodates a sterile seal arrangement (D, Fig. 1) shown diagrammatically in Fig. 2.

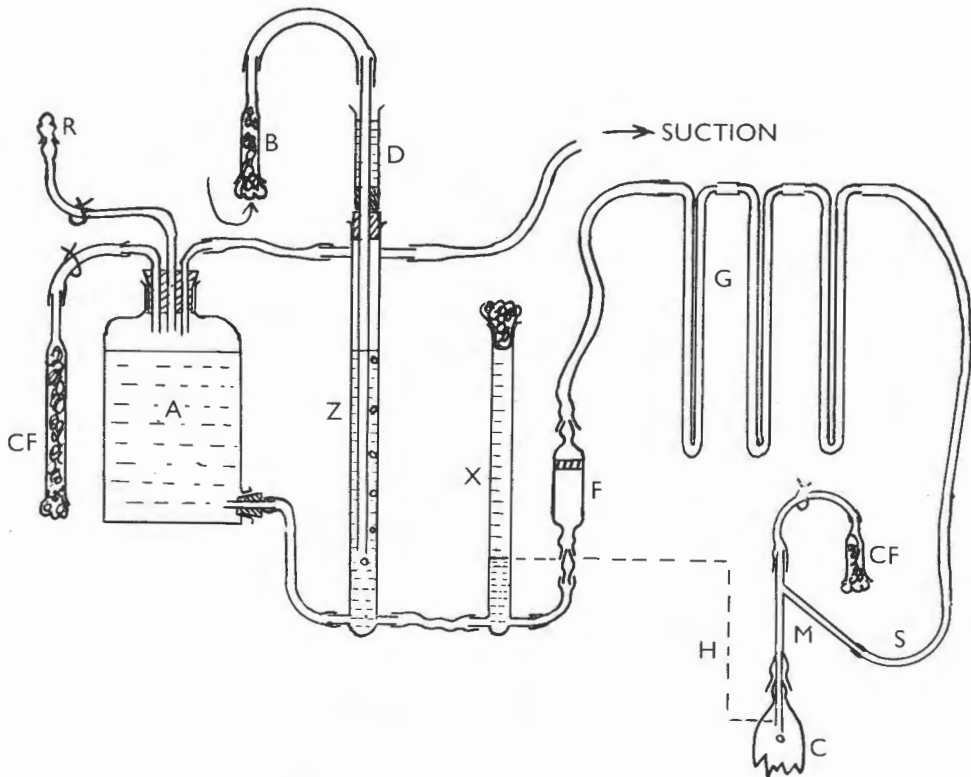


FIG. 1.—Feeding system (diagrammatic).

The seal consists of a rubber stopper ( $S_1$ ) fitted with a 10 cm. length of glass tubing ( $G_1$ ). A second, very much longer glass tube ( $G_2$ ) (length 80 cm.) passes through  $G_1$ , as well as through a 6 cm. length of latex tubing (L) which also fits  $G_1$ , thus serving as a seal through which the longer tube  $G_2$  may be moved up and down.

Finally, an inverted rubber stopper ( $S_2$ ) fitting over  $G_1$  and holding the tube (T) (Fig. 2) into which 70 per cent alcohol is poured, serves to maintain the above-mentioned rubber seal (L) and sliding tube ( $G_2$ ) sterile.

The pressure regulator is then connected to the "manometer" (X) (Fig. 1) where the actual liquid pressure (i.e. height of column) may be accurately observed on a scale and checked for constancy.

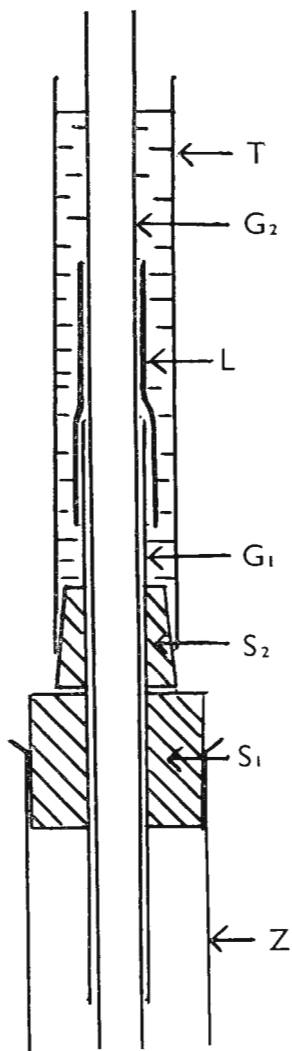


FIG. 2.—Sterile seal.

The manometer (X) is connected by means of latex tubing to a modified sintered glass funnel (F) where the medium is filtered before entering the capillary resistance (G). These consist of 1 mm. internal diameter, thick-walled pyrex glass tubing bent in U-form so that several lengths may be joined to each other with latex tubing in such a manner that the U-bends may be immersed in a constant temperature water bath while the joints remain above the water surface. The danger of contamination is thereby materially reduced. Three to four lengths, each 155 cm. long, have been found sufficient. From the capillary resistance the feeding line enters the culture vessel (C, Fig. 1).

In order to facilitate the expulsion of air from the system at the beginning of an experiment and to eliminate the necessity of having to let the air escape through the capillary inflow tube, a special arrangement (M, Fig. 1) has been adopted at the port of entry on the culture vessel. In the following diagram (Fig. 3) (*a*) is a two-piece glass tube of which the upper half (*e*) consists of 20 cm. of glass tubing (7 mm. internal diameter). This is joined at the point (*f*) to a 20 cm. long 1 mm. capillary tube which fits into the ground glass socket (*d*) by means of a length of rubber tubing (*c*) as shown in Figure 3.

A 1 mm. capillary glass tube (*b*) [joined to (*a*) at the point (*f*)] is connected to the medium supply line (S, Fig. 1) and introduces medium into (*a*). Any air bubbles introduced into (*a*) with the medium, rise in (*e*) and may be expelled through the cotton wool filter (F) (Fig. 1 and 3) by opening the clamp (K) (Fig. 3). The whole assembly fits on to the culture vessel (C) via the ground glass joint as shown in Fig. 3.

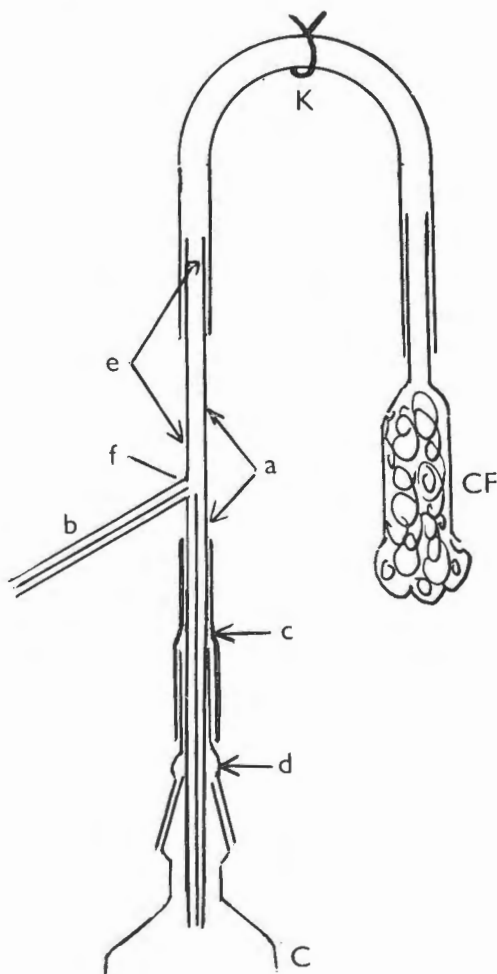


FIG. 3.—Medium inlet system.

The medium reservoir (A) may be replenished when empty by connecting the ground glass joint (R) (Fig. 1) to its counterpart leading to a fresh supply of medium, with due regard to the usual precautionary measures to avoid contamination. During re-filling, (A) may be connected via cotton wool filter (CF) to the atmosphere by opening a clamp.

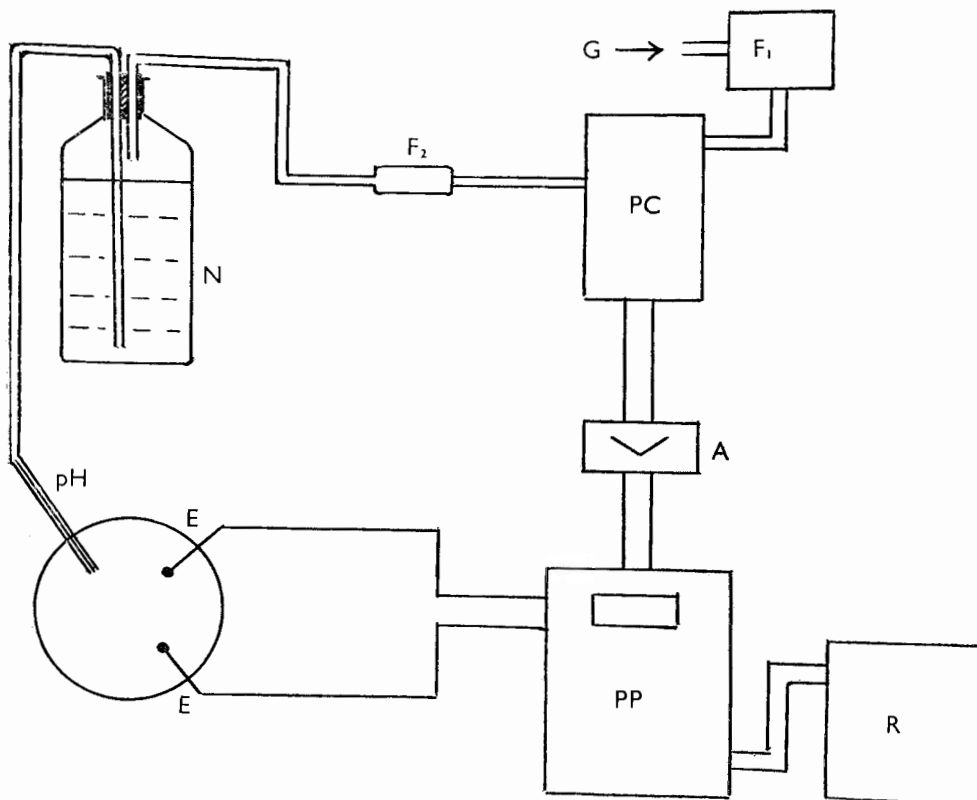


FIG. 4.—pH Control system (diagrammatic)

The various units of which the apparatus consists are joined by means of ground glass joints. This not only facilitates re-assembly of the various parts after sterilization, but also permits replacement of any broken or defective parts during operation over long periods with little danger of contaminating the whole system. Silicone grease (heat resistant) is used for the ground glass joints.

In this system, like others (Kubitschek, 1954), the application of suction instead of pressure (Chemostat) has the advantage that the system is not affected by atmospheric pressure and temperature changes.

Suction is applied through a cotton wool filter (not shown) connected to the right hand top entrance to (Z) (Fig. 1). As a result air filtered through (B) enters the medium in (Z) (approximately 1 bubble/5 sec.) through (G<sub>2</sub>) (Fig. 2) which simultaneously functions as pressure regulator by virtue of its up and down movement through the sterile seal into (Z).

The flow rate depends on the effective pressure head (H) (Fig. 1) which may be controlled by means of the pressure regulator as described.

The feeding system differs in one important aspect from other similar types: The gas or air that is bubbled through the medium with the object of establishing the desired pressure in the outflowing liquid, is not led through the main medium reservoir, (Kubitschek, 1954) but through a separate communicating vessel (Z). As a result only the small amount of medium in (Z) is in contact with the gas or air used to bubble through, thereby reducing possible decomposition of the medium. In addition, if gas is bubbled through the main reservoir flask, any sediment that might form is stirred up. In our system any doubt regarding the exact composition of the medium added to the culture vessel and the possibility that the sediment particles would tend to clog the resistance (G), the filter (F) and the in-flow capillary tube on the culture vessel (Fig. 1), are removed.

Further advantages arise from the arrangement whereby the pressure regulating system is separated from the main supply vessel (A). The range over which variations in pressure (and therefore flow rate) may be effected, is large in our system and the desired pressure for any particular purpose may be conveniently selected and measured.

#### *The Culture Vessel*

The standard culture vessel designed in this laboratory, is used in all continuous culture work. It is described in detail elsewhere (Weyland & Serfontein, 1959).

Briefly it consists of a pyrex tube with various ground glass joint entrances which accommodate medium supply line, special nutrient supply, pH correcting fluid and various electrodes for automatic measurement and regulation of pH (and if necessary, redox potential). A sintered glass plate in the bottom of the vessel serves to break up the gas supplied from below for stirring purposes into fine bubbles to ensure maximal stirring effect.

A constant volume is maintained inside the culture vessel by means of an obliquely attached capillary side arm which communicates with a small flask from which the overflow may be either collected in a sample tube for analysis, or deviated directly to the waste flask.

#### *pH (and/or redox potential) Control System*

The desirability of having the pH value in continuous cultures separately controllable is evident from theoretical discussions on the subject (Serfontein & Weyland, 1959).

Various systems for this purpose have been devised for use in conjunction with continuous cultures. Most of these seem to be more or less adaptations of industrial scale instruments where automatic pH control is established routine. The main disadvantage of those systems examined was that the addition of the pH corrective fluid was not continuous. In other cases, where the control mechanism was electrically operated via solenoid valves, the system did not seem sensitive enough for use in small scale laboratory apparatus.

In our own efforts we received technical advice from Messrs. Metrohm A. G. Herisau, Switzerland. The system that we finally adopted is particularly sensitive and capable of continuous addition of base or acid as pH corrective. The apparatus is also well adapted to laboratory scale continuous culture work and in addition provides for the continuous registration of pH and/or redox potential. It is not unduly expensive if its general use in the microbiological laboratory as well as that of the units separately be considered.

The pH control system is shown diagrammatically, in Fig. 4. The culture vessel (C) holds the electrodes (glass and calomel) (E) immersed in the growing culture. The electrical potential from the electrodes is transmitted to the precision potentiometer (PP) where the pH is directly indicated, as well as continually registered on the recorder (R) operated from the precision potentiometer.

In addition, the electrical impulse from the electrodes is transmitted to an amplifier (A) which in turn operates the pressure controller (PC). In this instrument, a gas pressure proportional to the electrical impulse received is imposed through bacterial filter ( $F_2$ ) on the flask (N) containing the pH corrective fluid (acid or base). At a rate depending on the magnitude of the pressure, the acid or base is forced out of (N) into the culture vessel (C) via a capillary inlet tube (pH). Purified nitrogen (G) supplied from a cylinder through the filter and pressure reducer ( $F_1$ ), serves as the source of gaseous pressure which is regulated by (PC).

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