LX. STUDIES IN GAS PRODUCTION BY BACTERIA.

I. APPARATUS FOR THE MEASUREMENT OF THE RATE OF GAS PRODUCTION.

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BACTERIAL activity is normally associated with the absorption or evolution of gases. The production of gases may be due to ordinary respiratory evolution of CO_2 , or to the disintegration of nutritive compounds whereby gaseous by-products are formed. The absorption of gas is usually to be correlated with respiration where oxygen is absorbed from the atmosphere.

When bacteria cultivated in broth absorb or evolve relatively large amounts of gas, volume changes may be measured with considerable accuracy, and thus may throw some light on the metabolism of the organisms. In particular, regular observations of volume changes over a period of time should determine the activities of the bacteria in various stages of the life of the culture.

In the course of researches on bacterial denitrification, three experimental methods were devised. The results of this work are published in the succeeding paper; the present paper describes the apparatus used.

I. THE PYKNOMETER METHOD.

The first method was devised for use in an incubator, where compactness is an advantage. A culture tube *A* (Fig. 1), of volume approximately 10 cc., is filled to the neck with a nitrate- or nitrite-broth and sterilised. After inoculation it is attached by means of pressure tubing, *B*, to a pycnometer, *C*, which is initially filled with pure dry mercury. The culture tube *A* has a narrow neck for convenience in attaching the tubing; milk centrifuge tubes were found to be suitable.

When the culture attacks the nitrate or nitrite, the gases evolved displace some of the mercury into a collecting tube, *D*, which is changed periodically. The displaced mercury is weighed and its volume calculated. Hence the volume of gas produced during any desired interval of time is known.

The following precautions are adopted.

(i) The pyknometer is thoroughly cleaned with hot chromic acid, and carefully dried before use in each experiment; the mercury also is washed and dried. These operations were designed to make the mercury sensitive to even slight increases of pressure due to gas evolution. In practice, it was found that the mercury was displaced spasmodically in quantities of about 0.03 cc. at a time, equivalent to about 5 drops.

(ii) The tube *A* is filled with broth to the neck, to minimise the air space above. A small production of gas thus causes a maximum increase of pressure on the mercury, and renders the apparatus more sensitive to small volume changes. A minimal air space is an advantage in processes such as denitrification where gas production is reduced or inhibited by the presence of air.

The whole apparatus is placed after assembly in an incubator automatically regulated to the desired temperature.

Fig. 1.

II. THE CAPILLARY TUBE METHOD.

This method was devised for making simultaneous observations on a number of cultures under the same physical conditions. The effect of varying other factors in a series of cultures could thus be determined.

The broth culture is normally contained in a centrifuge tube of about 10 cc. capacity, and connected by means of rubber pressure tubing to a glass capillary tube about 150 cm. in length. The culture tube is then completely immersed in a thermostat at the desired temperature, which should not vary during the experiment by more than 0.05°.

The capillary tube is first calibrated, thoroughly cleaned with hot chromic acid, washed, and carefully dried. A thread of mercury is then inserted to indicate gas evolution. The capillary tube is supported horizontally, lying on a paper scale by which the movement of the mercury thread can be recorded (Fig. 2). A movement of the thread of 1 cm. corresponded to an alteration in volume of 0.004–0.04 cc., according to the bore of the tube used. The total volume of gas produced by the denitrification of 25 mg. of KNO_3 —about 3 cc.—could be contained in 100 cm. or less of tubing, and its production with time could be measured with an accuracy of 0.01 cc.

When it is desired to measure the rate of gas production over short intervals of time, the small tube may be replaced by a boiling-tube of about 100 cc. capacity. If desired, mechanical stirring of the broth can be accomplished by using a tube with a mercury seal [Findlay, 1923]. By filling the culture tubes as completely as possible, the influence of variation in barometric pressure is minimised, and is usually negligible. Readings of the barometric pressure were taken systematically in all experiments extending over 24 hours, but corrections are necessary only when fluctuations are great, and when more than 3 cc. of gas is produced. The influence of fluctuations of room temperature on the volume of the gas are negligible; they were found to be of the order of $\frac{1}{2}$ %.

Fig. 2.

The less the volume of the air space above the culture, the more sensitive is the method. The movement of the mercury thread depends on the difference of pressure on its two ends, so that the minimum volume change sufficient to move the mercury thread is inversely proportional to the total volume of the air space.

III. LARGE-SCALE METHOD.

The third experimental procedure adopted was designed to determine the nature of the gases produced at various stages in the process of denitrification. The object therefore was to obtain quantities of gas sufficient for analysis at each of these stages rather than to obtain accuracy of measurement. However, it is possible by this method to measure the volume of gas produced in any given interval of time.

Samples of the broth can also be withdrawn periodically so that bacterial counts, p_H determinations, and chemical tests can be made.

The apparatus is shown in Fig. 3. A 5-litre flask containing the broth is fitted with a two-holed rubber stopper. Samples of the broth can be withdrawn into the gas washing bottle *C* by means of the tube *B* which extends into the middle of the flask. The gas evolved is collected in the gas-burette *D* by displacement of mercury. This vessel can be changed frequently, the

screw-clip *E* being closed during the operation. The whole apparatus is placed in an incubator.

The gases so obtained can then be analysed in a Haldane apparatus.

Fig. 3.

SUMMARY.

Simple methods are devised to measure the rate of production of gas continuously during the growth of a broth-culture.

REFERENCE.

Findlay (1923). Practical physical chemistry (London, 4th ed.), p. 242.