APPARATUS FOR COLLECTING AND MEASURING THE GASES EVOLVED DURING FERMENTATION

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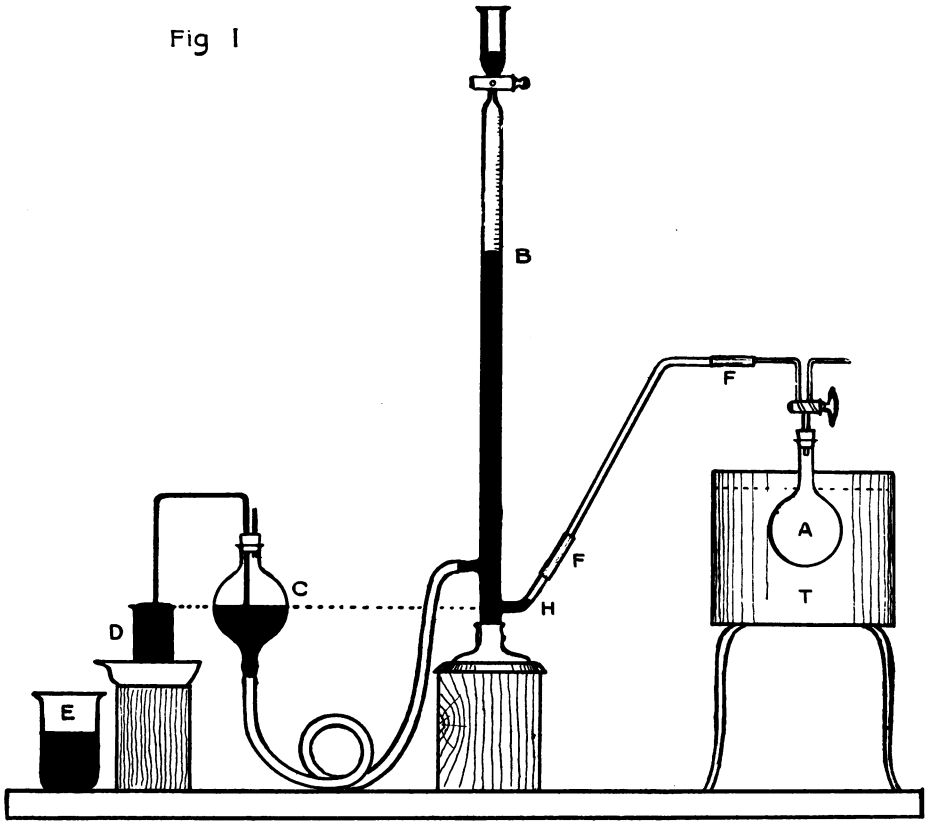
(Received August 2nd, 1910)

In measuring the total quantity and rate of evolution of gases evolved during fermentation, it is essential to keep the pressure in the fermentation flask as nearly constant as possible throughout the experiment. Two forms of apparatus have been in use in this laboratory for some time for this purpose, and have been found to be very simple and convenient.

1. The first form is intended for the measurement of comparatively small volumes of gas, such as those evolved in the fermentation of sugars by yeast juice,[1] and is illustrated in the accompanying figure 1.

Fig 1



1. Harden and Young. *Roy. Soc. Proc. B.*, 1906, 77, 406.

The fermentation flask (A) of 100-200 c.c. capacity, placed in the thermostat (T), is fitted with a three-way tap connected by glass tube and rubber with a Schiff's azotometer of 100 c.c. capacity, graduated to 0·2 c.c. It is essential that the rubber connections (F, F) should be as short as possible, owing to the high permeability of rubber for carbon dioxide, but at the same time it is necessary to allow so much play that the flask can be removed from the bath and shaken by hand, or mechanically agitated in the bath. The reservoir (C) of the azotometer is connected by a syphon, passing through a doubly-bored stopper, with a small cylinder or beaker (D) which stands in a flat dish of glass or porcelain provided with a spout from which mercury can flow into a collecting vessel (E). The effect of this arrangement is that the mercury displaced from B passes through the syphon into D and overflows into the porcelain dish and so into E. The mercury at C and H, therefore, remains at the level of the top of D, and this is adjusted once for all, so that the mercury just extends to the bend of the tube H, and consequently the pressure in the flask is maintained at that of the atmosphere.

The volume of gas is read on B at the reduced pressure corresponding to the height of the column BH, and is corrected by means of a table. (See below.)

When the azotometer is full of gas, the tap on A is closed, the syphon removed, the reservoir filled with the mercury from E, and the gas displaced by raising the reservoir. The syphon is then replaced, and started by means of a pressure bulb attached to a short tube passing through the stopper of the reservoir. The tap on A is then re-opened, and the collection of gas resumed as before. If required, a sample of the gas can be taken for analysis by means of the mercury cup at the top of the azotometer.

The table of corrections is constructed for an atmospheric pressure of 760 mm. by measuring the height of each cubic centimetre graduation above H, subtracting this from 760, and then calculating the corrected volume corresponding with each graduation. The corrected numbers for the intermediate graduations are found by interpolation. A table of this kind is sufficiently accurate for most purposes; when greater accuracy is required the readings must be specially corrected for the prevailing atmospheric pressure.

The rate of fermentation of a sugar by yeast-juice is determined by

the aid of this apparatus in the following matter. The azotometer is filled with mercury and the syphon adjusted in C. The desired mixture of yeast-juice, sugar and toluene is placed in A, brought to the temperature of the thermostat and saturated with carbon dioxide by removing the flask, filling it with carbon dioxide from a Kipp, replacing the stopper carrying the tap and shaking, and repeating this process until saturation is effected. If desired, the flask can be fitted with inlet and outlet tubes and the carbon dioxide blown through without removing the flask from the bath, but this is not so convenient in the case of yeast-juice as the method described above.

Since yeast-juice readily becomes supersaturated with carbon dioxide, the flask is vigorously shaken for about half a minute before each reading. The flask is then replaced in the thermostat, allowed to stand for one minute to re-establish temperature equilibrium, and the volume of gas in the azotometer read. At the expiration of the desired interval from the time of first shaking, the process is exactly repeated and the volume of gas evolved during the interval thus ascertained.

So strong is the tendency to supersaturation in the case of yeast-juice that scarcely any gas is evolved during 5-10 minutes, the whole volume produced during this time being retained in the liquid and only evolved when this is vigorously agitated.

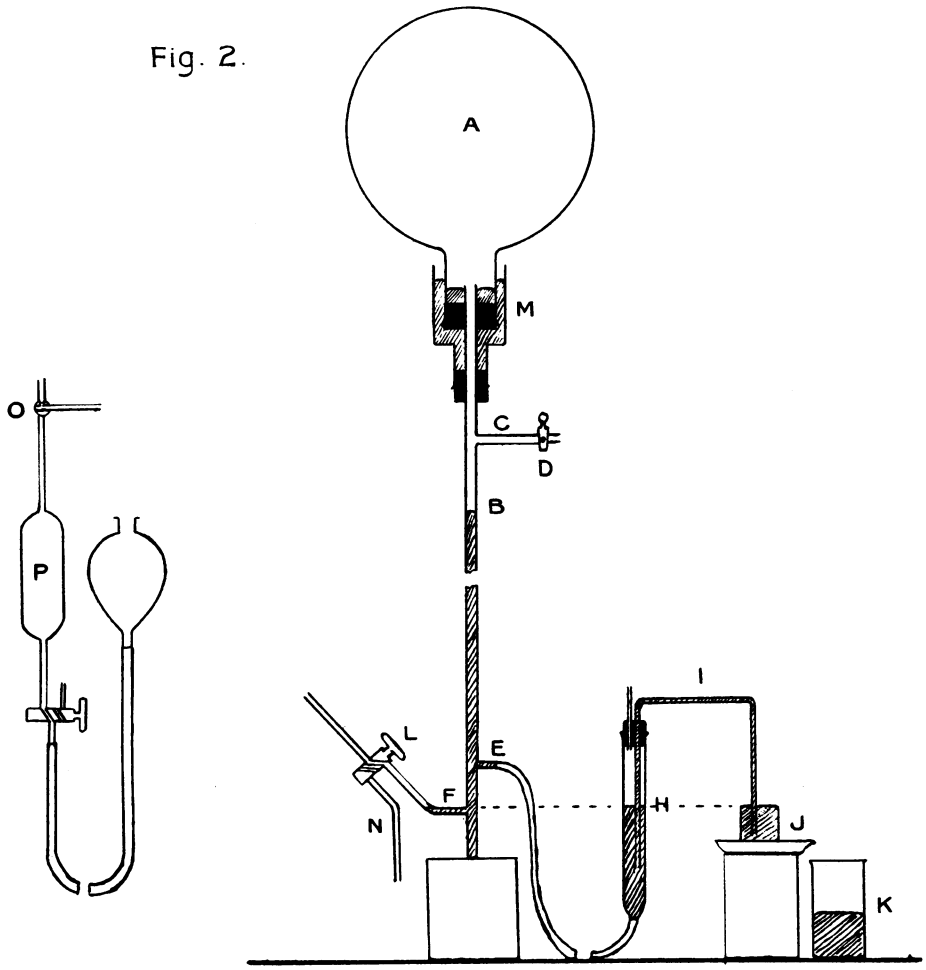
This method works extremely well so long as the volume of gas evolved does not exceed about 30 c.c. per 5 minutes, and does not fall below about 0·5 c.c.

2. The second form of apparatus is intended for the collection and measurement of the comparatively large volumes of gas which are evolved by the action of bacteria on quantities of about 20 grams of sugar, the object being the collection and measurement over mercury of a large volume of gas (3-6 litres) without employing an unmanageable amount of mercury. The apparatus is shown in fig. 2.

A large inverted bolthead (A) (3-7 litres in capacity) is fitted with a rubber stopper carrying a vertical tube (B) about 1 metre in length and 10 mm. in external diameter. This tube projects about 2 cm. inside the stopper of the flask, and carries near the top a side piece (C) fitted with a good mercury sealed glass tap (D). B is sealed at the lower end, near which are two side tubes arranged as in Schiff's azotometer (E, F). At E is attached a rubber tube and a cylindrical mercury reservoir (H) about

20 mm. in diameter and 180-200 mm. in length, and this is fitted with a syphon and overflow cylinder (I, J K) exactly like those employed for apparatus 1. The side tube (F) carries a three-way tap (L).

Fig. 2.



In order to prevent leakage, the rubber stopper of the flask A is immersed in mercury contained in a cup (M) carried on the vertical tube B by means of a rubber stopper. In order to prepare the apparatus for use, mercury is poured into the reservoir (H), and the lower part of the tube B and side tube F up to the tap are filled with mercury, which is also allowed to flow out of the upper end of B and fill the space immediately above the stopper of the bolthead. This prevents the gas from coming into

contact with rubber after it has once passed into the apparatus. The side tube C is then connected with a Fleuss pump or a mercury pump and the whole apparatus exhausted. Mercury then rises in B to the barometer height above that in H. As soon as the exhaustion is as complete as possible, D is closed; H is fitted with its syphon, and J is adjusted so that the level of the mercury is at the same height as the point at which F enters B. The height of the column BF subtracted from the height of the barometer gives the residual pressure, if any, in the apparatus, and is measured by a vertical millimetre scale. The fermentation flask, placed in an appropriate incubator or thermostat, is connected by rubber to the tube carrying the tap L, and the air of the space above the liquid in it is swept out, if this is desired, by a suitable gas through the limb N. The tap L is then turned so as to connect the flask with B, and the collection of the gas then proceeds regularly until the experiment is interrupted. Any mercury carried up by the bubbles of gas strikes against the concave surface of the bolthead and falls back again into the tube. The diameter of the side tube F must be decidedly less than that of B, or the bubbles of gas entering B drive too much mercury before them and cause violent oscillation of the column. At the close of the experiment the pressure and temperature of the gas are read off and its corrected dry volume ascertained by calculation from the known capacity of the apparatus. The calibration of the large flask is effected by means of water, and that of the tube B by means of mercury. It is necessary to know the volume of the bolthead as arranged for the experiment, and of the side tube C and tube B down to a fixed point, and the capacity per centimetre below this. The actual internal volume of the apparatus when the mercury is standing at any height above H is then easily ascertained.

A sample of gas for analysis is taken at D by means of the pipette P, of about 100 c.c. capacity. This is attached at D, and the air from the intervening tube removed by using the pipette and its reservoir as a mercury pump, the gas removed being swept out of the three-way tap O. After this process has been completed, the reservoir of the pipette is lowered and the taps D and O are opened; gas flows from A to P, and as soon as no more gas passes over, the taps are closed and the pipette disconnected. The sample can then readily be brought into a gas analysis apparatus. The total error in the measurement of the volume need not exceed 0·5 per cent.

The space above the liquid in the fermentation flask is kept as small

as possible, and should not be more than 100-200 c.c. when 1 litre of liquid is employed. The composition of the gas remaining in this space can be ascertained with sufficient accuracy by rapidly removing a sample with the pipette from N; the slight error due to evolution of carbon dioxide or other gas from the liquid in the flask during this process being as a rule negligible. Finally, in calculating the volume of gas actually evolved during the experiment, it is necessary to allow for the volume of gas originally present in the flask; for the gas dissolved in the liquid in the flask; and for the gas corresponding with any residual pressure in the apparatus at the commencement of the fermentation.