THE FERMENTOMETER

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In 1906, Slator described a little instrument to determine the rate of yeast fermentation in short intervals. He measured the pressure of CO_2 produced in a closed container by means of a mercury manometer. The method was very simple and gave the possibility of determining the rate of fermentation in five to ten minutes. The only essential deviation from Slator's method in the "Fermentometer" described here is that Slator evacuated the apparatus and measured the decrease of vaccuum while in the following experiments, no vacuum was applied and the pressure measured was surplus pressure caused by the carbon dioxide produced by the yeast.

The apparatus consists in a flask or bottle closed with a perforated rubber stopper (ground glass would be better) with a glass tube which is connected by rubber tubing with the manometer. This is a simple glass tubing manometer, open at both ends, with a glass stopcock to release the pressure. In order to be able to speak of this instrument in one word, we have called it fermentometer, since Slator did not give it any name.

This little instrument has proved very convenient for the study of the influence of various factors on the rate of fermentation. Since in most cases, the total observation times are short, not more than a few hours, and since large quantities of yeast will always be used to get rapid development of pressure and to prevent multiplication, it is permissible for most experiments to use the ordinary yeast cake which is not a pure culture, but sufficiently pure as far as gas formation is concerned.

The advantage of this instrument is the possibility of determin-

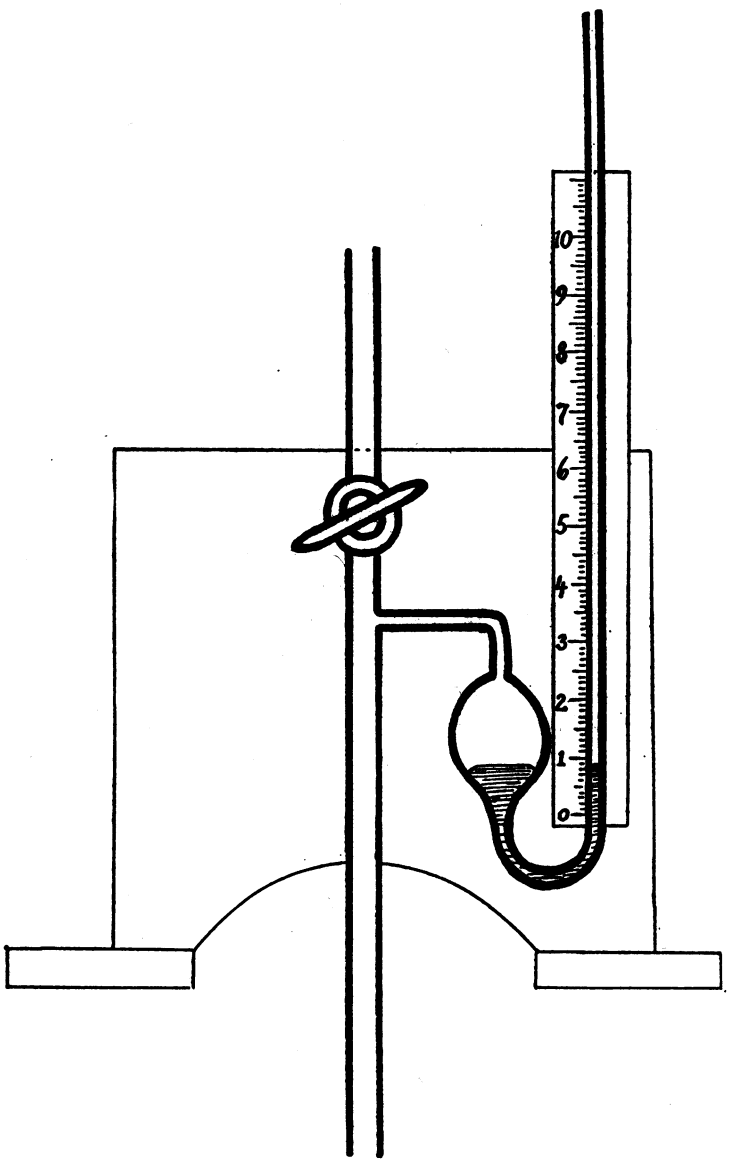

FIG. 1. THE FERMENTOMETER

ing rates of fermentation in a very short time, much shorter than with any other fermentation, and entirely independent of multiplication. In most fermentations, the products formed in a given time interval are produced by an increasing number of cells, and it takes a complicated formula to compute the fermenting capacity of the single cell as a measure of the rate of fermentation independent of multiplication (Rahn, 1911; Buchanan and Fulmer, 1918). Besides, neither of these two formulas is really correct, because they are based on the assumption that bacteria multiply in a strictly exponential way, in the order $a \cdot 2^x$; while this is correct for the first part of growth in a new culture, it is doubtless not correct for that part of the curve where most of the fermentation is observed, i.e., that part when the growth curve has reached its point of inflexion and the rate of growth is decreasing.

TECHNIQUE

In order to get a measurable increase in pressure, it seems necessary to have as small an air space in the apparatus as possible. The author preferred small Erlenmeyer flasks holding when filled to the rim about 100 cc. of water. The connecting rubber tubing should not be longer than necessary to allow vigorous shaking. This shaking is necessary to drive the excess carbon dioxide out of the supersaturated solution. The author followed Slator's method by putting one teaspoonful of glass beads into each flask. By using the 100 cc. flask with glass beads and 50 cc. of sugar solution to which 5 cc. of a suspension of 1 yeast cake (about 12 grams) in 50 cc. of water, i.e., 1 to 1.5 grams of yeast per flask was added, the author obtained pressures of 10 to 25 mm. in five minutes, depending upon temperature and other conditions. This is about the right pressure for measurements.

After trying various ways, it finally was decided to leave the yeast culture undisturbed for four and one-half minutes, shake the culture for half a minute vigorously avoiding however, as far as possible, entrance of the liquid into the glass and rubber tubing, read the manometer, and wait for another four and one-half minutes to repeat the procedure. This makes it possible to observe 5 fermentometers in one experiment simultaneously.

TABLE 1

Parallel experiments, 1 gram yeast in 55 cc. glucose solution

TIME AFTER ADDING YEAST TO SUGAR SOLUTION		MERCURY PRESSURE IN 5 MINUTES					AVERAGES				
		A	B	C	D	E	A	B	C	D	E
hours	*minutes*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*					
	0–5	15.5	17	16.5	12	8					
	5–10	20.5	15	14.5	18	7					
	10–15	18	15.5	15.5	18	7					
	15–20	17.5	15.5	15.5	17.5	New					
	20–25	17	15	13.5	17	rubber stopper	Pressure released after every reading				
	40–45	18	15	Lost	16	14					
	45–50	17.5	15.5	13	18.5	15					
	50–55	18	15	14	18.5	17.5					
	55–60	13.5	12.5	13	13.5	12.5	13.5	13.0	13.0	13.25	12.25
	60–65	13.5	13.5	13	13	12					
2	0–5	21	15	15.5	20	15					
	5–10	21.5	18	15.5	20	17	Pressure released after every reading				
	10–15	21.5	19	17.5	21	19					
	15–20	22.5	17	17.5	20.5	19					
	20–25	13	13.5	14	15.5	12					
	25–30	15.5	15.5	14.5	13.5	13					
	30–35	14	13	14.5	15.5	13.5	14.6	14.2	14.4	14.9	13.2
	35–40	15	15	14	15	13.5					
	40–45	14.5	14	15	15	14					
	45–50	15.5	Broken	14	15	13					
5	0–5	18		21	19	20					
	5–10	24.5		23	24	23	Pressure released after every reading				
	10–15	24.5		21.5	24	22					
	15–20	13.5		13.5	16	13.5					
	20–25	14.5		15	14	14.5					
	25–30	15		14	14	15	14.9		14.4	15.0	14.3
	30–35	15		15	15	13.5					
	35–40	15		14	16	14.5					
	40–45	16		15	15	15					
7	30–35	22.5		18	25	19.5	Pressure released after every reading				
	35–40	25		20.5	24	20.5					
	40–45	12.5		12.5	14	12.5					
	45–50	12.5		12.5	13.3	13.2					
	50–55	13		12.5	13.3	13.2					
	55–60	12.5		12.5	13.3	13.2	13.1		12.9	13.3	13.2
	60–65	13.5		14.5	13.3	13.2					
	65–70	14.5		12	13.3	13.2					
	70–75	13.5		13.5	13.3	13.2					

The frequency of shaking and reading and of releasing the pressure will of course depend upon the rate of fermentation.

Table 1 gives a typical experiment showing a comparative test of 5 fermentometers. All flasks were treated exactly alike containing each 50 cc. of an 8 per cent glucose solution and 5 cc. of yeast suspension (10 grams yeast cake in 50 cc. of water), so that each flask received about 1 gram of yeast. All cultures stood in the 30° incubator room in water baths of 27.5°C.

The first five minutes cannot be counted as full because some of the CO_2 produced is dissolved in the medium, and only after this is saturated, can the actual measurement begin. It takes less than five minutes to develop sufficient CO_2 to saturate the medium. The first measurements show no very good agreement, even if we omit the last observation which was spoiled by a fine scratch in the rubber stopper. In the beginning, pressure was released after every reading, then, after fifty minutes, pressure was not released, with the result that the data became at once fairly constant.

The yeast was then left to itself for an hour, with the glass stopcock of the manometer open. Two hours after the first mixing of yeast and sugar solution, the pressure was measured again, with the same result that by letting the pressure accumulate, the results became more stable. The same was true after five hours. The reason is probably that with pressure at zero, the manometer does not indicate the pressure accurately while as soon as pressure is developed, the readings are reliable.

Another bothersome feature has not been fully accounted for as yet, that is the gradual increase in the rate of fermentation as evidenced by the average results of the first experiment. It does not seem to be caused by the pH adjustment because this slow increase has been observed at all pH values tried. The average of all 5 experiments is 10 per cent higher at two hours than at one hour, and 11.7 per cent higher after five hours, while after seven hours, the rate of fermentation decreases on account of the accumulation of the alcohol. This increase in the rate of fermentation may be due to a rejuvenation process in the old yeast, though no nitrogenous material was given nor could any dead cells be detected by the methylene blue test which might be the source of nitrogen.

For most experiments, it is sufficient to compare the pressure readings directly, provided that the flasks are of uniform size. Occasionally, however, the absolute quantities of CO_2 produced during the experiment may be wanted. This can be ascertained by measuring the total volume of the enclosed gas in the apparatus, i.e., in flask, rubber tubing and fermentometer. By filling the flask to the rim with water, and then placing the rubber stopper and glass tube in their usual position, and by filling also rubber tubing and manometer with water to the same amount which is ordinarily filled with gas, the water volume gives the total en-

TABLE 2

Corrected volumes of CO_2 produced by yeast

		A	B	C	D	E
Total volume of flask, stoppered, + glass tube, cc		103	103	103	99	104
Gas volume of manometer		7.5	3.5	6.5	7	10
Gas volume of rubber tubing		2.5	2.5	2.5	2.5	2.5
Volume of culture liquid		55	55	55	55	55
Volume of glass beads		2	2	2	2	2
Total enclosed airspace		56	52	55	51.5	59.5
Real volume of CO_2 produced per 5 minutes	After 1 hour	0.995	0.89	0.94	0.90	0.96
	After 2 hours	1.08	0.97	1.04	1.01	1.03
	After 5 hours	1.10		1.04	1.02	1.12
	After 7.5 hours	0.965		0.93	0.90	1.03

closed volume. From this, we have to deduct the volume of the solution used in the experiment, including the glass beads. The remaining volume is the enclosed gas space over the culture, which we shall call v. By the formation of x cc. of CO_2, the pressure increases p mm. Since pressure times volume is constant, we must have

$$(v + x)\ 760 = v\ (760 + p)$$

This equation means that the original gas volume, increased by x cc. of CO_2 at normal pressure, will be reduced to the original gas volume v if the pressure is increased by p mm.

The total CO_2 formed is therefore $x = \frac{v.p.}{760}$ cc.

Applying this to the above experiment, we find the results given in table 2.

This means that each flask containing about 1 gram of yeast produces about 1 cc. of CO_2 in five minutes which corresponds (uncorrected for temperature and moisture) to 3.95 mgm. This means 47.4 mgm. of CO_2 per gram yeast per hour, and about the same amount of alcohol. This quantity in 55 cc. corresponds to 0.086 per cent per hour.

This little instrument has been used by Slator to measure the influence of temperature, of sugar concentration and of stimulants upon the rate of alcoholic fermentation. The author has used it to study the influence of temperatures above the optimum, and the influence of alcohol upon the rate of fermentation; he expects to use it for the study of the theory of chemical stimulation.

The fermentometer will prove a useful, time-saving and inexpensive little instrument wherever the rate of alcoholic fermentation is being studied from a physiological viewpoint.

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