CXI. THE KINETICS OF ALCOHOLIC FERMENTATION OF SUGARS BY BREWER'S YEAST.

I. EFFECT OF CONCENTRATIONS OF YEAST AND SUGAR.

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(Received February 28th, 1935.)

THE kinetics of alcoholic fermentation of sugars by living yeast was established by the work of Brown [1892] and Slator [1906; 1908]. These workers showed that the rate of fermentation is approximately constant over a wide range of sugar concentration and remains constant during the main part of the fermentation. Brown used a high seeding rate which, in the absence of added nutrients other than the sugar, remained fairly constant during the observations, and measured the rate of fermentation by the rate of disappearance of the sugar. On the other hand Slator used a low seeding rate and measured in a specially devised apparatus the velocity of CO_2 evolution over short periods of time during which no yeast increase could take place. He calculated the value of " n " in the equation

$$\frac{d[\text{CO}_2]}{dt} = k [\text{sugar}]^n,$$

and showed that it was approximately zero between the limits of sugar concentration 0.5 % and 10 %. Slator and Sand [1910] concluded that "...it is unlikely that conditions could be experimentally realised under which diffusion becomes a controlling factor in the rate of fermentation". Their deductions also indicated the importance of uniform shaking or stirring of the fermenting mixture.

Nord and Weichherz [1929] criticised Slator's apparatus and considered that,

(1) it had no adequate stirring device whereby continuity of reaction could be secured;

(2) it exhibited large temperature fluctuations;

(3) readings could not be made soon enough after the commencement of the fermentation.

Using the Iterson-Kluyver apparatus to measure the rate of evolution of carbon dioxide they investigated the effects of varying stirring rates and found that with 21 turns per minute or more the rate of fermentation exhibited three phases:

(1) a fairly rapid rise to a maximum;

(2) a short quasi-stationary period;

(3) a rapid asymptotical decrease to zero.

Phase (2) was absent when no stirring took place. The constancy of fermentation rate over a relatively long period of time, observed by Brown and Slator, was not observed in these experiments.

The method of stirring or agitation is important. Slator shook vigorously by hand at intervals. Nord and Weichherz assumed that all diffusion gradients in

the sugar solution are eliminated by stirring at the rate of 21 turns per minute, and attributed the further increase in maximum rate of fermentation consequent upon increased rate of stirring to an apparent increase in the membrane surface due to the motion of the cells relatively to the medium. But in the fermentations performed by them, the yeast rate was so high (2 g. of pressed yeast in 20 ml. total volume) that the cells, if uniformly distributed, would be separated from each other by a distance considerably less than one diameter. It seems probable that diffusion gradients and vortex effects would occur even at higher stirring rates. However, by maintaining a steady stirring rate, comparable results were obtained. Weichherz [1929; 1931] from mathematical considerations concluded, among other things, that proportionality between rate of reaction and dry weight of yeast employed does not necessarily hold. It may not hold in such cases as the above, but it was found by Slator to hold for low concentrations of yeast, and this has been confirmed by us (Exp. 2) over a wide range of yeast concentration.

In further experiments, Nord and Weichherz, using a stirring rate of 100 turns per minute, investigated the effect of glucose concentration on the maximum rate of fermentation and found the latter to increase up to 10 % glucose, whereas Slator had found little deviation over the range 0.5–10 %, and the maximum at 4 %.

A closer examination of the results, however, indicates that they may be interpreted to agree with those of Slator. In each of the fermentations recorded by Nord and Weichherz, a large yeast rate was employed and a large time lag (phase (1)) ensued before the maximum rate was attained. During this lag an appreciable amount of sugar was decomposed, and this amount can be calculated from the graphical data furnished. For example, in the case of 0.5 % initial glucose, the maximum rate was attained after fermentation had decreased the sugar concentration to 0.31 %. The maximum rate may therefore be taken to apply to this concentration and not to 0.5 %. Similar results calculated from the other cases are included in the table below.

Table I.

Initial glucose %	Time taken to attain maximum rate (mins.)	Glucose remaining at time of maxi- mum rate (%)	Rate of fermentation ml. CO ₂ /min.
0.5	10	0.31	1.40
2.0	30	0.96	2.45
5.0	38	3.48	2.85
10.0	70	6.14	3.05
30.0	82	26.11	2.90

By plotting these maximum fermentation rates against the appropriate concentrations of glucose, a curve is obtained (Fig. 1) which shows close agreement with the corresponding curve of Slator [1906] and, in fact, demonstrates the "approximate independence" of sugar concentration more clearly than Slator's original curve. A deduction in support of this may be made from the equation derived from first principles by Weichherz [1929; 1931] who found that the velocity of fermentation

$$f = \frac{\mu k}{k - \mu} \cdot S_0 (e^{-\mu t} - e^{-kt}),$$

where S_0 = the initial substrate concentration in the outer medium,
 k = the unimolecular constant of the reaction proceeding in the cell,

and $\mu = \frac{F\alpha D}{v\delta},$

in which F = cell (membrane) surface,

δ = membrane thickness,

αD = diffusion coefficient of the substrate within the membrane (this diffusion coefficient is derived from the free diffusion coefficient (D) in the total system filled with solution, and α the degree of permeability of the membrane),

and v = cell volume.

The above equation can be solved for k and μ if sufficient values of f and t are known. Let us suppose the following values are known:

$$\begin{array}{ll} t=1 & f=f_1 \\ t=2 & f=f_2 \\ t=3 & f=f_3. \end{array}$$

By substituting these values in the equation, followed by usual algebraical methods, it can be shown that

$$\begin{aligned} e^{-\mu} + e^{-k} &= f_2/f_1 \\ e^{-\mu} \times e^{-k} &= (f_2/f_1)^2 - f_3/f_1, \end{aligned}$$

whence we can calculate k , the true unimolecular constant of the reaction proceeding inside the cell. Application of this theorem to the results of Nord and Weichherz revealed that the value of k decreased with increase in initial concentration of glucose. Although the reaction proceeding within the cell is unimolecular, it does not proceed unimolecularly in the presence of any appreciable external sugar concentration since the velocity of reaction, $f = ka$, is maintained by continuous replacement of sugar by diffusion.

It was considered desirable to investigate the following points which arise out of the above discussion;

- (1) the effect of stirring or shaking (Exp. 1);
- (2) the relationship between concentration of yeast and rate of fermentation (Exp. 2);
- (3) the relationship between concentration of sugar and rate of fermentation (Exp. 3).

Fig. 1.

Fig. 1a.

EXPERIMENTAL.

The apparatus used was essentially that described by Slator [1906] in which the fermentation vessel is connected with an exhaust pump and a mercury manometer, change of pressure being observed as fermentation proceeds. Modifications, some of which were designed to overcome the objections advanced

by Nord and Weichherz, included a two-way tap connecting to the exhaust pump or the air, and a shaking device for rocking the vessel to and fro and imparting a jerk, the rate of shaking being controlled by a rheostat in conjunction with the motor. Shaking was adopted in preference to stirring. The fermentation vessel, 250 ml. in capacity, was completely immersed in a constant temperature bath and the sugar, in solution (mutarotated), contained in a small beaker supported on a float on the surface of the yeast suspension previously placed in the vessel. Glass beads were present to assist dispersion of the yeast. When the whole system had settled down to the required temperature (30° in most experiments, as also adopted by Slator and Nord and Weichherz) the apparatus was exhausted and the shaking commenced; the beaker discharged its contents into the yeast suspension and readings on the manometer scale were taken from the beginning of the fermentation. These readings were plotted against time in each experiment, the rates of fermentation at selected arbitrary times deduced from the slope of the curve and calculated to the units, mg. of CO_2 per minute, as expressed in the various tables¹. For this purpose the apparatus was calibrated as follows. 2 ml. of solution of pure sodium carbonate containing 65.8 mg. of CO_2 were placed in a small beaker floating on the surface of 98 ml. of sulphuric acid, of such concentration as to be exactly equivalent, contained in the fermentation vessel of the apparatus. When the system had attained the desired temperature, the pressure was reduced, the reaction flask shaken and the change of pressure on liberation of CO_2 was observed. In the case described, 100 ml. of solution in the flask in liberating 65.8 mg. of CO_2 at 30° brought about an increase of pressure equal to 5.8 cm. of mercury. Similar calibrations were performed for other volumes of liquid in the reaction vessel and for other temperatures as required.

Pressed washed brewer's (top) yeast from the same source was used in all experiments. By using yeast one day old, autofermentation was invariably found to be negligible.

Exp. 1a. Variation of rate of shaking.

Glucose 0.5 g. (1 %). Yeast, 8 g.
Temp. 35° . Total volume 50 ml.

Table 1 A. *Progressive rates of fermentation expressed as mg. CO_2 per minute.*

Time (mins.)	...	2	5	10	15	20	25	30	35	40	45
No shaking	—	1.17	3.76	3.63	3.50	3.24	2.98	2.72	2.46	2.32	
25 shakes per minute	—	1.43	2.98	3.50	3.63	3.50	3.50	3.37	3.32	3.24	
45 shakes per minute	1.55	3.37	4.02	4.02	3.94	3.89	3.76	3.76	3.63	3.37	
70 shakes per minute	2.09	3.50	4.02	4.02	3.89	3.89	3.89	3.89	3.76	3.50	

It can be seen from the graph (Fig. 1a) that the rate of shaking is important. The effect of shaking is presumably most marked when high yeast rates are employed, as in this case. However, 70 and 45 shakes per minute gave similar results. It is reasonable to suppose that intermediate rates would do the same, but in all subsequent experiments shaking was maintained at approximately 70 shakes per minute.

The quasi-stationary periods were much longer than the corresponding ones observed by Nord and Weichherz, although the yeast rate was even higher than that employed by them.

¹ An experimental error of, e.g., 1 mm. in the scale reading, spread over 30 mins. in the graphical procedure outlined above, amounts to less than 0.04 mg. CO_2 per minute.

Exp. 1b. Effect of intermittent shaking.

Parallel fermentations were performed:

- (a) with continuous shaking at 70 shakes per minute;
- (b) with alternate periods, without shaking and with vigorous shaking (over 70 per minute);
- (c) without shaking.

In all cases of intermittent shaking, the shaking periods were continued until the accelerated rate of gas evolution had ceased and the normal rate with shaking was attained.

Glucose 0.5 g. Yeast 5 g.
Temp. 30°. Total volume 50 ml.

The results are shown graphically in Fig. 1 *b*.

Exp. 1c. The Exp. (1 *b*) was repeated for conditions (a) and (b), but in the case of (b) longer time intervals, 14 minutes, were allowed to elapse between the periods of shaking. The object of this was to exaggerate any effect due to intermittent quiescent periods. The concentration of glucose adopted in the latter experiment was 2 % instead of 1 % whereby a greater rate of fermentation was secured (*vide* Exp. 3). This also exaggerates the difference between continuous and intermittent shaking. The results are shown graphically in Fig. 1 *c*.

Fig. 1 *b*.

Fig. 1 *b*. —○—○— Periods without shaking. -○---○- Periods with shaking.
— · — · — Apparent curve for conditions (b).

Fig. 1 *c*.

Fig. 1 *c*. —○—○— Periods without shaking. -○---○- Periods with shaking.
— · — · — Apparent curve for conditions (b).

It will be seen that intermittent shaking with only short time intervals, Exp. 1 *b*, the conditions adopted by Slator, gives results almost identical with those obtained by continuous shaking at 70 shakes per minute. The fermentation is proceeding at about the same rate in each case; a certain amount of supersaturation occurs between the shakings, but after a short agitation the readings approximate to those obtained with continuous shaking. Far more serious

errors are involved if continuous shaking at the rate of, *e.g.*, 25 per minute, is adopted (Exp. 1a). Apparently this rate is inadequate to prevent a certain amount of supersaturation. If conditions are exaggerated as in Exp. 1c the results obtained after each agitation indicate a somewhat low rate of fermentation.

Exp. 2. Variation of yeast rate.

Glucose 0.5 g. (1 %). Yeast 2, 3, 4, 5 g. respectively.
Temp. 30°. Total volume 50 ml.

Table II. *Progressive rates of fermentation expressed as mg. CO₂ per minute.*

Time (mins.) ...	1	2	3	4	5	10	15	20	25	30	35
2 g. yeast	—	1.30	2.34	—	3.43	3.51	3.48	3.43	3.38	3.38	—
3 g. „	—	2.60	4.42	—	5.07	5.20	5.20	5.20	4.94	4.42	3.90
4 g. „	—	—	5.59	6.63	6.97	6.97	7.02	6.36	5.66	4.94	4.16
5 g. „	0.78	—	6.11	—	8.71	8.71	7.67	6.65	5.49	4.68	3.90
Time (mins.) ...	40	45	50	55	60	65	70	75	80	90	100
2 g. yeast	3.15	—	2.99	—	2.62	—	1.98	—	1.30	0.86	0.52
3 g. „	3.51	3.07	2.60	2.13	1.69	1.30	0.78	0.26	—	—	—
4 g. „	3.48	2.73	2.00	1.25	0.52	—	—	—	—	—	—
5 g. „	2.96	1.95	1.09	—	—	—	—	—	—	—	—

Fig. 2a.

Fig. 2b.

The graph, Fig. 2a, shows the rate of fermentation plotted against time, and Fig. 2b the maximum rates plotted against yeast concentrations. It will be seen that the latter show exact proportionality.

Exp. 3a. Variation of initial glucose concentration.

Glucose 0.25–20 %. Yeast 4 g.
Temp. 30°. Total volume 40 ml.

Table III A. *Progressive rates of fermentation expressed as mg. CO₂ per minute.*

Time (mins.) ...	1	2	3	4	5	8	10	15	20	25	30	35	40
0.25%	2.29	2.80	—	—	3.57	3.31	2.80	1.43	0.25	—	—	—	—
0.50 „	2.55	3.31	—	—	4.13	—	3.97	3.16	2.14	1.17	—	—	—
1.0 „	3.31	4.08	4.84	5.10	5.25	—	5.20	4.74	4.28	—	—	—	—
2.0 „	—	4.84	5.35	—	5.81	—	5.86	5.81	5.61	5.25	5.10	—	—
5.0 „	—	—	—	—	6.02	—	6.37	6.37	6.37	6.24	6.12	5.99	5.86
10.0 „	—	—	—	—	6.37	—	6.84	7.04	7.14	7.14	7.14	7.14	7.19
20.0 „	—	—	—	—	5.10	—	5.56	5.61	5.66	5.71	5.71	5.76	5.86

In this experiment the yeast rate was the same as that employed by Nord and Weichherz. If the rates of fermentation are plotted against time it will be seen that with 0.25 % glucose the curve is typically compound exponential, but with increasing concentrations of glucose it flattens out and becomes quite flat with 5 % and 10 % glucose. With this yeast rate the highest maximum and constant rate was exhibited by the 10 % glucose solution and when at most only 0.6 % had been removed by fermentation.

Exp. 3 b.

Glucose 1.0-30 %. Yeast 2 g.
Temp. 30°. Total volume 40 ml.

Table III B. *Progressive rates of fermentation expressed as mg. CO₂ per minute.*

Time (mins.)...	5	10	15	20	25	30
1.0 %	3.16	3.21	3.21	3.16	2.96	2.80
2.0 "	3.21	3.62	3.67	3.52	3.47	3.31
5.0 "	3.42	3.82	3.95	4.05	4.05	4.05
10.0 "	4.18	4.31	4.31	4.31	4.33	4.33
15.0 "	3.31	3.87	3.87	3.87	3.90	3.90
20.0 "	2.55	3.06	3.26	3.31	3.31	3.36
30.0 "	1.02	1.33	1.53	1.58	1.63	1.66

Exp. 3 c.

Glucose 1.0-30 %. Yeast 2 g.
Temp. 30°. Total volume 80 ml.

Table III C. *Progressive rates of fermentation expressed as mg. CO₂ per minute.*

Time (mins.)...	5	10	15	20	25	30
1.0 %	3.01	3.56	3.61	3.61	3.50	3.28
2.0 "	3.50	4.10	4.10	4.10	4.05	3.72
5.0 "	3.78	4.54	4.82	4.82	4.82	4.82
10.0 "	4.38	4.93	4.93	4.93	4.93	4.98
15.0 "	4.24	4.44	4.44	4.44	4.49	4.49
20.0 "	2.74	3.12	3.12	3.12	3.18	3.18
30.0 "	1.37	1.53	1.64	1.70	1.70	1.75

The results of Exp. 3 b resemble those obtained in Exp. 3 a. The highest maximum and constant rate was apparently at a concentration of glucose approaching 10 %. In Exp. 3 c the yeast rate corresponded to that employed by Slator. At all concentrations of glucose from 1 % to 30 %, approximate constancy of rate of fermentation was observed over a period of time. By graphical procedure it can be seen that the concentration of glucose corresponding to maximum rate is about 6-8 %.

Exp. 3 d.

Glucose 0.1-15 %. Yeast 1 g.
Temp. 30°. Total volume 100 ml.

Table III D. *Constant rates of fermentation expressed as mg. CO₂ per minute.*

Initial concentration of glucose %	0.1	0.2	0.3	0.4	0.5	1.0	2.0	4.0	6.0	8.0	10.0	15.0
Rate of fermentation	0.71	1.10	1.31	1.44	1.55	1.77	1.95	2.10	2.17	2.12	2.05	1.85

(The progressive rates of fermentation in this experiment are stated in Part II, Exp. 2.)

With this low concentration of yeast the rate of fermentation became definitely constant for long periods of time. The maximum rate was exhibited by the solution of which the initial concentration of glucose was 6 %.

Exp. 3 e. Concentrations of glucose below 0.10 %.

For this experiment a different apparatus and procedure were required, as Slator's apparatus is not sufficiently sensitive. Indeed it was at the suggestion made privately by Dr Slator that this experiment was performed. The fermenting vessel and stirring arrangements employed were similar to those described by Nord and Weichherz [1929] and consisted of a 200 ml. flask with a stirrer passing through a mercury seal. The evolved CO_2 was swept out of the fermenting vessel by a current of CO_2 -free air and passed through Pettenkofer tubes containing standard baryta. By the judicious use of two-way taps the CO_2 evolved was estimated at regular intervals by back-titration, the current of gas being diverted through a second tube while the first was being titrated and replaced. A control autofermentation was performed side by side with each experiment in an exactly similar apparatus, the fermenting vessel being immersed in the same thermostat, the appropriate deductions being made from each titration.

Table III E.

Glucose 0.04–0.08 %. Yeast 9 g.
Temp. 30°. Total volume 165 ml.
(Results in ml. 0.0944 *N* HCl.)

Initial conc. of glucose %	Time from addition of sugar mins.	Period of absorption of CO_2 mins.	Back-titration in fermentation tube ml.	Back-titration in auto-fermentation tube ml.	Difference ml.	Total fermentation of sugar ml.	Unimolecular velocity constant <i>k</i>
0.0848	10	10	28.9	33.1	4.2	4.2	0.0292
	20	10	25.5	38.4	12.9	19.1	0.0372
	30	10	31.8	36.3	4.5	21.6	0.0355
	40	10	35.9	37.35	1.45	23.05	0.0311
	50	10	37.65	38.75	1.1	24.15	0.0299
	60	10	37.85	38.35	0.5	24.65	0.0281
	70	10	38.4	38.7	0.3	24.95	0.0265
	80	10	38.8	39.1	0.3	25.25	0.0276
	100	20	38.8	39.0	0.2	25.45	—
0.0636	10	10	30.35	33.8	3.45	3.45	0.0288
	20	10	31.5	40.0	8.5	11.95	0.0323
	30	10	36.85	40.0	3.15	15.1	0.0291
	40	10	39.7	41.0	1.30	16.4	0.0253
	50	10	40.5	41.5	1.00	17.4	0.0239
	60	10	40.9	41.5	0.6	18.0	0.0227
	70	10	41.2	41.6	0.4	18.4	0.0219
	80	10	41.4	41.7	0.3	18.7	0.0217
	90	10	41.4	41.7	0.3	19.0	0.0238
0.0424	10	10	29.4	32.1	2.7	2.7	0.0414
	20	10	31.5	37.2	5.7	8.4	0.0372
	30	10	35.6	37.6	2.0	10.4	0.0327
	40	10	36.9	38.4	1.5	11.9	0.0354
	50	10	37.8	38.2	0.4	12.3	0.0332
	60	10	37.8	38.0	0.2	12.5	0.0311
	70	10	38.3	38.5	0.2	12.7	0.0337

Graphical inspection of the results given in Table III E indicated, as was anticipated, that the reactions were proceeding unimolecularly, provided that the induction period of about 7 minutes was neglected. To calculate the values of *k*, the constant, involved a little difficulty in deducting from the values of *t* the appropriate allowance for this induction period. This period could not be judged exactly enough from the graphs. The allowances made in the respective experiments, 7.3, 7.0 and 7.5 minutes, were judged by inspection. The reasonable

constancy in the values of k justifies the allowances made and also the conclusion that at initial concentrations of glucose between 0.04 and 0.06 % if not also 0.08 %, the fermentation proceeds unimolecularly.

DISCUSSION.

The initial concentration of glucose corresponding to maximum constant rate of fermentation varies between 9 % or over in the case of the highest yeast rate (Exp. 3 *a*) and 6 % in the case of the lowest yeast rate (Exp. 3 *d*). With the low yeast rates, the rate of fermentation varies comparatively little between 2 % and 10 % glucose, whereas with the high yeast rates greater variations and more definite maxima are exhibited.

The results of Exp. 3 *d* lend themselves to mathematical treatment in two ways.

(1) *Theory of enzyme action of Michaelis and Menten [1913].*

Assuming a single enzyme action to limit the rate of fermentation within the yeast cell, application of the theory gives us the relationship

$$\frac{1}{v} = \frac{K_m}{V} \cdot \frac{1}{x} + \frac{1}{V},$$

where v = velocity of breakdown of enzyme-substrate compound, *i.e.* velocity of fermentation,

V = value of v when the enzyme is fully saturated with substrate and wholly combined with it,

K_m = dissociation constant of the enzyme-substrate compound (*ES*),

x = concentration of substrate.

In this equation V and K_m are constants. Hence if the fermentation conforms to the equation, the graph obtained by plotting $\frac{1}{v}$ against $\frac{1}{x}$ should be a straight line. In Table IV are tabulated values of x , v , $\frac{1}{x}$ and $\frac{1}{v}$ from the data in Table III D and interpolations from a graph drawn from them.

Table IV.

x = conc. %	v = rate	$1/x$	$1/v$
0.2	1.09	5.0	0.917
0.4	1.44	2.5	0.695
0.6	1.62	1.67	0.617
0.8	1.72	1.25	0.582
1.0	1.77	1.00	0.565
1.2	1.83	0.83	0.547
1.4	1.87	0.71	0.535
1.6	1.90	0.63	0.526
1.8	1.92	0.56	0.521
2.0	1.95	0.50	0.513
2.5	2.00	0.40	0.500
3.0	2.04	0.33	0.490
3.5	2.07	0.29	0.483
4.0	2.10	0.25	0.476
4.5	2.12	0.22	0.472
5.0	2.14	0.20	0.468
8.0	2.12	0.125	0.472
10.0	2.05	0.10	0.488

The results are plotted in Fig. 3 *a*. Between the limits 0.2 % and 5.0 % the graph is a straight line. From this it may be deduced that the Michaelis and

Menten theory holds for alcoholic fermentation of glucose within this range of conditions.

According to Haldane [1930] if an enzyme action follows the Michaelis and Menten equation three cases can arise.

(a) The substrate concentration remains so high until very near the end of the reaction that the enzyme is fully saturated.

Fig. 3a.

Here, if the products of the reaction do not inhibit, y (the amount of substrate transformed in time t) $= Vt$. The curve for 2 g. yeast (Fig. 2 a) illustrates this case.

This is actually the condition under which Slator examined fermentation kinetics, and under which it is also easy to understand that the rate of fermentation is proportional to the concentration of yeast.

(b) The substrate concentration is so low that the amount of enzyme combined is proportional to the substrate concentration. Here

$$\begin{aligned} \frac{dy}{dt} &= (a-y) V/K_m, \\ \text{i.e.} \quad t &= K_m/V \cdot \log_e (a/a-y) \\ & \quad (a = \text{initial substrate concentration}), \end{aligned}$$

and the reaction is unimolecular. These are the conditions investigated in Exp. 3 e.

(c) The substrate concentration varies so that neither of these conditions is fulfilled.

$$\begin{aligned} \text{Here} \quad \frac{dy}{dt} &= (a-y) V/(K_m + (a-y)), \\ \text{therefore} \quad Vt &= \int_0^y ((a-y) + K_m)/(a-y) \cdot dy \\ &= y + K_m \cdot \log_e (a/(a-y)) \\ &= y \left(1 + \frac{K_m}{a} \right) + y^2 \frac{K_m}{2a^2} + y^3 \frac{K_m}{3a^3} + \dots \end{aligned}$$

If a is large compared with K_m the logarithmic term is at first negligible and the reaction proceeds uniformly, slowing down when y becomes appreciable. These are the conditions investigated by Nord and Weichherz. The curves for 5 and 4 g. yeast (Fig. 2 a) illustrate this case.

Hence, by application of the Michaelis and Menten theory, the results of Nord and Weichherz and those of Slator, apparently contradictory in part, are reconciled.

(2) *Mathematical treatment adopted by Slator.*

According to Slator the fermentation of sugars by yeast could be represented by the equation

$$\frac{d[\text{CO}_2]}{dt} = k [\text{conc. of sugar}]^n$$

(the yeast factor being kept constant),

or

$$v = k [\text{sugar}]^n$$

i.e.

$$\log v = \log k + n \log [\text{sugar}].$$

By plotting $\log v$ against $\log [\text{sugar}]$ we can, from the slope of the curve produced, derive the value of n . From the data of Exp. 3 *d* the values of $\log v$ and $\log [\text{sugar}]$ are tabulated in the following table (V) (first two columns) and plotted in Fig. 3*b*. From the slope of the curve are calculated the values of n tabulated in the fourth column, corresponding to concentrations in the third column, and plotted in Fig. 3*c*.

Table V.

$\log (\text{concentration})$ ($\log [\text{sugar}]$)	$\log (\text{rate of ferment-}$ $\text{ation}) (\log v)$	Concentration %	n
-0.699	0.037	—	—
-0.523	0.117	0.30	0.381
-0.398	0.158	0.40	0.323
-0.301	0.187	0.50	0.265
0	0.248	1.0	0.167
0.301	0.290	2.0	0.125
0.602	0.322	4.0	0.054
0.778	0.328	6.0	-0.022
0.903	0.326	8.0	-0.071
1.0	0.312	10.0	-0.13
1.176	0.274	—	—

Fig. 3*b*.

Fig. 3*c*.

It can be seen from Fig. 3*c* that between the concentrations 1 % and 10 % of glucose the value of n approaches zero, *i.e.* the "approximate independence" of Slator holds good. At very low concentrations of sugar, however, the value of n approaches unity and the reaction becomes one of the first order with respect to sugar (*cf.* Exp. 3 *e*), which has been explained on the basis of the Michaelis and Menten theory.

SUMMARY.

1. It has been shown that if all the conditions which influence the rate of alcoholic fermentation of glucose by living yeast are carefully considered the kinetics are precisely similar to those of an ordinary enzyme reaction. The fermentation conforms to the theory of Michaelis and Menten and the mathematical deductions made from the theory apply under their special conditions.

Thus the apparently contradictory results of Slator and of Nord and Weichherz were obtained from experiments investigating respectively two special cases out of three possible cases under the theory. Relatively low concentration of yeast leads to saturation of the enzyme and constancy of reaction velocity over a wide range of substrate concentration (Slator). Relatively high concentration of yeast and sufficiently low concentration of sugar, 0.04–0.07 % glucose in the case investigated, yield a unimolecular reaction. Conditions intermediate between these yield a maximum rate of reaction followed sooner or later by a slowing down (Nord and Weichherz). In all cases an induction period precedes the attainment of the maximum rate.

2. The rate, if not also the nature, of the stirring or shaking is important, particularly when high yeast rates are employed. To attain uniform results shaking was preferred to stirring and was adopted whenever possible, from 45 to 70 shakes per minute being adequate. Slower shaking, *e.g.* 25 shakes per minute, gave a low rate of fermentation. On the other hand, intermittent vigorous shaking gave good results provided that the intervals between the shakes were not more than three or four minutes in duration.

3. In the case of glucose concentration as low as 1% proportionality between yeast concentration and rate of fermentation was observed over a wide range.

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