LXXI. FERMENTATION BY DRIED YEAST PREPARATIONS.

BY ARTHUR HARDEN.

From the Biochemical Department, Lister Institute, Chelsea, S.W. 1.

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SOME years ago the behaviour of zymin (yeast dehydrated by acetone) with different volumes of sugar solution was described by Harden and Young [Harden, 1911, p. 37].

When zymin is incubated with 10 cc. of approximately 10 % glucose solution at 25°, fermentation commences almost immediately, increases to a maximum rate in about 40 minutes and then very slowly decreases in rate. If, however, 25 cc. of the glucose solution of the same concentration be employed with the same weight of zymin, only a very slow fermentation occurs for the first hour and a half, after which the rate increases and finally becomes only slightly less than that in the presence of 10 cc. of the glucose solution. The curves *A* and *B* in Fig. 1 show this behaviour in typical manner, but the actual volume of gas evolved varies with different samples of zymin. A similar phenomenon is shown by dried yeast (Fig. 2) but the difference in behaviour is often still more marked. When larger volumes of glucose solution are taken the onset of fermentation is still further delayed or may even be entirely prevented. This is shown by the following results:

A. 1 g. zymin + 5 cc. of 10 % glucose solution + 0.2 cc. toluene.

B. 1 g. zymin + 20 cc. of 10 % glucose solution + 0.2 cc. toluene.

Fermentation commenced at once in A, at the rate of about 0.6 cc. per 5 minutes, and at the end of 7 hours 66 cc. of CO₂ had been evolved, whereas no fermentation was observed in B, even after 23 hours.

The autofermentation of zymin is affected in a similar manner. Thus 2 g. zymin (1) + 10 cc. H₂O, and (2) + 25 cc. H₂O at 25° gave the results shown in curves *A* and *B*, Fig. 3.

These observations have evidently an important bearing on those discussed by Abderhalden and Fodor [1921] and subsequently studied by Sobotka [1924].

The thesis maintained by Abderhalden and Fodor is that the complex chemical action of yeast on sugar is only reproduced by preparations such as acetone-yeast (zymin), yeast-juice, maceration extract or dried yeast, in so far as the original colloidal condition of the protoplasm of the yeast cell is retained. Without at the moment discussing this interesting and important conclusion, for and against which much can be said, attention may be directed to one of the arguments adduced which appears to be founded on a misunder-

Fig. 1. 2 g. zymin in each + 0.2 cc. toluene.

A. 10 cc. of 10 % glucose	} Solutions added direct.
B. 25 cc. "	
C. 25 cc. "	} Solutions added after preliminary moistening for 1 minute.
D. 25 cc. " ; 0.2 M NaC ₂ H ₃ O ₂	
E. 25 cc. " ; 0.1 M "	
F. 25 cc. " ; 0.05 M "	

(The curve for 0.025 M acetate is identical with E.)

Fig. 2. 2 g. dried yeast + 0.2 cc. toluene.

A. + 10 cc. of 10 % glucose	} added direct.
B. + 25 cc. " "	

standing. One of the marked differences between living yeast and yeast preparations is alleged to be that these preparations are incapable of fermenting dilute sugar solutions (p. 143). "Ebensowenig wie Hefeauszüge vermag sterile Trockenhefe (Acetondauerpräparat oder durch Alter sterilisierte Hefe) verdünnte Zuckerlösungen zu vergären, weil allen diesen Stoffen die Fähigkeit der Konzentration, die nur lebenden Zellen eigen ist, fehlt." The statement appears to be founded on the fact that Buchner [1903, p. 260] used 40 % cane sugar in his fermentation experiments with zymin, and only in one instance published results obtained with another concentration. Buchner, moreover, used 4 g. cane sugar and 10 cc. of water to 2 g. zymin (Dauerhefe), a proportion which gives almost immediate fermentation.

Fig. 3. Autofermentation of 2 g. zymin + 0.2 cc. toluene.

A. + 10 cc. H_2O } added direct.
 B. + 25 cc. H_2O }

Actually, more dilute solutions of glucose are fermented by zymin more rapidly than 40 % solutions, provided that only a small volume of the solution is used.

In Table I are given figures for 10 cc. and 25 cc. respectively of each of the concentrations 2.5, 5, 10, 20 and 40 g. of glucose per 100 cc. with 2 g. zymin.

Abderhalden and Fodor made experiments using 10 g. dried yeast, 10 g. sugar and 250 cc. of H_2O and it is therefore not a matter for surprise that they obtained long induction periods, followed by fermentation due to the growth of yeast cells.

Table I.

2 g. zymin with (a) 10 cc. glucose solution + 0.2 cc. toluene } at 25°.
 (b) 25 cc. glucose solution + 0.2 cc. toluene }
 cc. of CO₂ evolved.

Time of incubation (mins.)	(a) 10 cc. Concentration, g. per 100 cc.					Time of incubation (hrs.)	(b) 25 cc. Concentration, g. per 100 cc.				
	2.5	5	10	20	40		2.5	5	10	20	40
10	1.8	3.3	2.1	2.5	2.5	1	2.7	2.2	3.6	3.6	4.5
20	4.4	8.1	5.1	5.2	4.5	2	3.0	2.7	7.8	7.7	6.4
30	8.4	12.5	8.7	7.9	5.9	3	15.0	11.1	17.8	16.2	8.7
40	12.8	16.0	11.6	10.6	7.2	8	71.4	58.1	64.5	47.8	30.0
50	16.5	20.0	14.0	13.7	8.4						
60	19.9	24.6	16.5	15.9	9.9						
130	43.8	50.1	38.1	32.8	19.8						

Sobotka, who has more recently taken up the study of fermentation by dried yeast [1924] has carried out further work on the same lines as Abderhalden and Fodor and has come to the conclusion (which he also ascribes to them, erroneously as it seems to me) that the effect produced by dried yeast is to be attributed exclusively to living cells; "die Leistung der Trockenhefe ausschliesslich auf lebende Zellen zurückzuführen ist" (p. 9).

Sobotka used 1 g. of dried yeast with 1 g. glucose and 20 cc. of water and observed periods of induction of 80–260 minutes with one group of yeasts and 4–19 hours with another group. Only one experiment on the effect of yeast concentration is recorded. In this the quantity of yeast per 20 cc. of 5 % glucose was varied and the resulting very marked decrease in the incubation period explained as being due to the shorter time required for the production of the necessary number of cells capable of fermenting.

There seems to be little doubt that Sobotka's results were actually due to the growth of living yeast cells contained in his preparations but his general conclusion is too sweeping. The "Dauerhefe" prepared by Buchner and Albert was carefully tested by them and found to be free from yeast cells capable of growth and yet they attained good and immediate fermentation by its use. Three successive samples of zymin prepared in this laboratory were also found to be free from yeast cells capable of growth, so that the undoubted fermentative powers of these samples must be attributed to the enzyme contained in the dead cells. Now ordinary dried yeast, which admittedly contains living yeast cells, behaves in an analogous manner to zymin when brought into different volumes of sugar solution and it is therefore only reasonable to attribute part at least of the fermentation produced by dried yeast to the enzyme in the dead cells.

No entirely satisfactory explanation of the phenomenon has as yet been found. The facts suggest that the problem, as suggested by Abderhalden and Fodor [1921], is concerned with the dispersion of the enzyme. If we suppose that this occurs rapidly in a more concentrated solution of the extractives of zymin, less rapidly in a more dilute solution, the long induction period in a large volume of sugar solution would be explained. In favour of

this view is the fact that the onset of fermentation can be greatly hastened in two different ways, each of which is consistent with the foregoing suggestion, viz. by

- (1) The addition of a boiled extract of the dried yeast.
- (2) The addition of certain salts, *e.g.* sodium acetate or sodium bicarbonate.

On the other hand the action of these salts is to some extent specific and experiments still in progress indicate that washing zymin with sodium acetate solution does not remove the enzyme any more rapidly than does washing with distilled water, as would be expected if it caused rapid dispersion of the enzyme. Various other possible explanations suggest themselves and these are now being investigated.

The effect of an extract of the dried yeast and of sodium acetate or bicarbonate solution is exhibited in the following experiments.

A. *Extract of zymin or of dried yeast.*

(1) *Zymin.*

2 g. of zymin + 0.2 cc. toluene were treated at 25° in each case with (1) 1 g. glucose + 10 cc. H₂O; (2) 1 g. glucose + 10 cc. of an extract of 4 g. zymin in 20 cc. of boiling water; (3) 2.5 g. glucose + 25 cc. H₂O; (4) 2.5 g. glucose + 25 cc. of an extract of 4 g. zymin in 50 cc. boiling water. The resulting solutions of yeast extractives in (2) and (4) were therefore approximately twice the concentrations of those in (1) and (3). As seen from the figures showing the course of fermentation, the addition of extract hastened the onset of fermentation slightly in the case of 10 cc., very considerably in that of 25 cc. and further, as would be expected, increased the rate of fermentation owing to the additional phosphate and co-enzyme added.

Time (hours)	cc. CO ₂ evolved.			
	2 g. zymin + 1 g. glucose		2 g. zymin + 2.5 g. glucose	
	10 cc. H ₂ O	10 cc. extract	25 cc. H ₂ O	25 cc. extract
0.25	1.4	7.7	0	2.8
0.5	5.6	17.6	0	5.2
1.0	16.5	31.8	1.5	11.2
2.0	36.4	52.5	10.5	23.9
3.0	—	—	28.8	46.8

(2) *Dried yeast.*

(a) 2 g. dried yeast + 2.5 g. glucose + 0.2 cc. toluene in each case: (1) + 25 cc. H₂O; (2) + 25 cc. extract made by heating 4 g. dried yeast with 50 cc. H₂O in a boiling water-bath for 17 minutes. No fermentation was produced in 20 hours in the absence of extract; whereas in its presence fermentation commenced after an hour and a half.

(b) 2 g. dried yeast + 1 g. glucose + 0.2 cc. toluene in each case: (1) + 10 cc. H₂O; (2) + 10 cc. extract made by heating 4 g. of dried yeast with 20 cc. H₂O in a boiling water-bath.

Time (hours)	cc. CO ₂ evolved.			
	2 g. dried yeast + 2.5 g. glucose		2 g. dried yeast + 1 g. glucose	
	25 cc. H ₂ O	25 cc. extract	10 cc. H ₂ O	10 cc. extract
0.25	0	0.2	0	1.3
0.5	0	0.7	0.2	3.2
1.0	0	1.5	0.7	8.6
1.5	0	3.6	3.8	16.2
2.0	0	6.9	10.2	23.2
3.0	0	17.0		

B. *Sodium acetate.*

Preliminary experiments showed that the substitution of 0.2 *M* NaC₂H₃O₂ for water caused a great diminution of the "period of induction" without serious change of *p*_H and further investigation showed that the effect was a maximum at about 0.2–0.3 *M* concentration of the acetate. No analogous effect could be produced with sodium chloride. Some irregularity was at first obtained in the results with different concentrations, but it was found that this could be obviated by adding to the 2 g. of zymin 5 cc. of water, shaking for 1 minute and then adding 15 cc. of a solution containing 2.5 g. of glucose and 5 cc. of acetate of the requisite concentration.

Typical fermentation curves for zymin with a series of concentrations of NaC₂H₃O₂ are given in Fig. 1, *C*, *D*, *E*, *F*. Similar results were obtained with dried yeast.

Fig. 4. 2 g. zymin + 0.2 cc. toluene.

A. +25 cc. of 10 % glucose; 0.05 *M* NaHCO₃ } Solutions added after preliminary moistening for
 B. " " ; 0.1 *M* NaHCO₃ } 1 minute.
 C. " " " }

C. Sodium bicarbonate.

Sodium bicarbonate produces a similar effect, the most favourable concentration, under the conditions employed above, being 0.1 *M*. Regular results were only obtained by adopting the same method of adding the solutions as was used for the acetate (Curves *A* and *B*, Fig. 4). An interesting point of difference between the curves obtained with acetate and bicarbonate is that the latter show a pronounced "phosphate effect" whereas the former do not.

SUMMARY.

1. Air-dried yeast and zymin (yeast dehydrated by acetone) produce fermentation rapidly in a small volume of sugar solution, only after considerable delay in a large volume.
2. The addition of yeast extract or solutions of certain salts such as acetates or bicarbonates greatly accelerates the onset of fermentation in presence of a large volume of sugar solution.
3. Air-dried yeast possesses a definite fermenting power which is not dependent on the presence of living cells.

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