

# THE ANAEROBIC ASSIMILATION OF GLUCOSE BY YEAST CELLS

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For many years it was generally assumed that yeast cells could convert sugars into cellular polysaccharides only in the presence of molecular oxygen. This assumption was based on the fact that sugar is converted quantitatively into fermentation products by yeast cells under strictly anaerobic conditions if the reaction is allowed to continue for an extended length of time. In industrial fermentations, Joslyn (1) has pointed out that 95 per cent of the sugar present is converted to carbon dioxide and alcohol, while the remaining 5 per cent can be accounted for by other products such as glycerol, fusel oil, succinic acid, and lactic acid. Kluyver (2) showed that glucose can be determined with a fair degree of accuracy by measuring the amount of carbon dioxide produced when a small amount of sugar is fermented by a relatively large quantity of yeast. Evidence has accumulated, however, showing that assimilation actually does occur during fermentation and that the quantitative results noted above may be due to the utilization of the stored carbohydrate after the exhaustion of the sugar.

Winzler and Baumberger (3) showed that the heat produced during the fermentation of glucose by a strain of bakers' yeast was only 63.2 per cent of the theoretical value, although the glucose had completely disappeared. From this they concluded that 29.5 per cent of the sugar was assimilated and 70.5 per cent was fermented. Van Niel and Anderson (4) found that only 70 per cent of the theoretical amount of carbon dioxide had been produced when the glucose had completely disappeared. They also showed that much of the remaining 30 per cent could be accounted for by an increased dry weight of the cells. Stier (5) came to the surprising conclusion that a larger fraction of the added glucose was assimilated under anaerobic than under aerobic conditions, since the total hydrolyzable polysaccharide content of the cells increased to a slightly greater extent in the absence of oxygen. Assimilation also occurs during the fermentation brought about by brewers' yeast, since Meyerhof and Schulz (6) found that only 74 per cent of the theoretical amount of carbon dioxide was produced, while the total carbon content of the cells increased to an extent equivalent to the assimilation of about 18 per cent of the added sugar.

The purpose of this paper is to describe experiments in which the carbon dioxide production, the total polysaccharide content, and the glucose concentration were measured at intervals during the anaerobic fermentation of glucose by a suspension of *Saccharomyces cerevisiae*, thereby giving the relationships between the amount of glucose consumed, fermented,<sup>1</sup> and assimilated during the entire course of the fermentations.

## EXPERIMENTAL

### *Methods*

Fresh Fleischmann's bakers' yeast (*Saccharomyces cerevisiae*) was washed three times and suspended in a 0.1 M  $\text{NaH}_2\text{PO}_4$  solution. The fermentations were carried out in a liter Erlenmeyer flask with 500 ml. of the yeast suspension. The flask was in a water bath and the suspension was very rapidly stirred by a large glass-covered, motor-driven, magnetic stirrer for the duration of the experiments. The flask had two outlets, one below the surface of the suspension for removing samples and the other above the surface of the suspension. The latter outlet was connected to a 2-way stop-cock, one branch of which led to a gas burette for measuring the carbon dioxide production, while the other branch led to a gas burette filled with nitrogen for replacing the samples that were removed. Before adding glucose, the atmosphere was displaced by nitrogen and the system was allowed to come to equilibrium. After the glucose was added, the carbon dioxide production was measured in the gas burette, care being taken that the system was at atmospheric pressure at all times. The temperature of the water bath, the temperature of the gas burette, and the atmospheric pressure were also recorded. It was assumed that equilibrium was maintained between the gaseous and dissolved carbon dioxide. Thus the total carbon dioxide production was taken as the sum of the observed burette reading and the calculated carbon dioxide in solution. Since the suspension medium was acidic, any variance in the carbon dioxide retention was reduced to a minimum.

Samples of the suspension were withdrawn at intervals during the fermentations and appropriate corrections were made for the resulting changes of volume. The following determinations were made on the samples: (1) the reducing value of the cell suspension; (2) the reducing value of the super-

<sup>1</sup> Assuming equal molar concentrations of carbon dioxide and ethanol throughout the fermentations, the carbon dioxide determination becomes a measure of the formation of both products. This assumption seems justifiable, since it was found that little or no acetaldehyde accumulates during the fermentations, all the acetaldehyde formed by the decarboxylation of pyruvic acid being converted to ethanol. Also other investigators (4, 6) found that equal molar quantities were formed in their fermentation studies.

natant fluid after centrifugation; (3) the reducing value of the cells after hydrolyzing for 2 hours at  $100^{\circ}$  with 1 N HCl and neutralizing with NaOH.

It can be seen that determination (2) = glucose concentration;  $(1-2)$  = reducing substances in the cells;  $(3-(1-2))$  = "total hydrolyzable polysaccharides."<sup>2</sup>

All of the reducing values were determined as glucose by the Shaffer (8) ferricyanide electrode method. The effect of the electrolyte differences in the samples was overcome by using corresponding concentrations of electrolytes in the reference electrode.

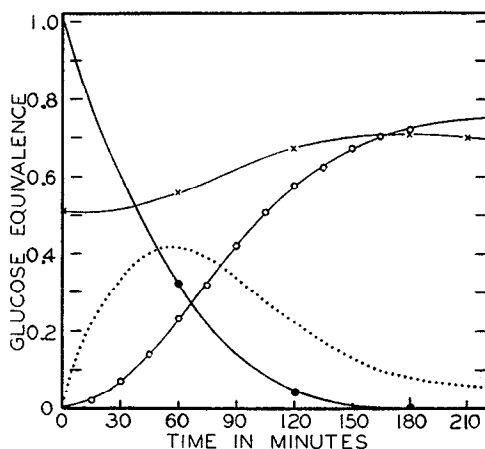


FIG. 1. The anaerobic fermentation of 500 mg. of anhydrous glucose by a fresh suspension of bakers' yeast. The suspension contained 2.5 gm. (wet weight) of yeast made up to 500 ml. with 0.1 M  $\text{NaH}_2\text{PO}_4$  solution. ●, glucose concentration, mg. per ml.; ○, glucose fermented to carbon dioxide and ethanol, mg. per ml.; ×, total hydrolyzable polysaccharide content of the yeast cells as glucose, mg. per ml.; the dotted curve represents the intermediary products, estimated by difference.

A stained preparation of the suspension was examined at the conclusion of each experiment. No gross bacterial contamination was ever found.

### Results

The results obtained by the fermentation of 500 mg. of anhydrous D-glucose with a freshly prepared yeast suspension are shown in Fig. 1.

<sup>2</sup> It should be noted that the values obtained for the "total hydrolyzable polysaccharide" by this method do not actually represent the total polysaccharides of the cells, since there is an insoluble carbohydrate fraction which is not hydrolyzed by hot hydrochloric acid (7), and ferricyanide is reduced to some extent by other substances present besides the sugars. However, if we assume that these factors remain constant during the fermentation, the increase in the "total hydrolyzable polysaccharide" should give a good approximation of the carbohydrate assimilated.

For convenience, the products are plotted on the basis of their glucose equivalence; *i.e.*, the glucose utilized in their formation. The glucose consumption curve shows that the sugar had completely disappeared after 180 minutes.<sup>3</sup> At that time 0.72 of a glucose equivalent had been converted into carbon dioxide and ethanol, and the hydrolyzable polysaccharide content of the cells had increased from 0.51 to 0.71 of a glucose equivalent, while 1.00 equivalent of glucose had been utilized. Therefore, 72 per cent of the sugar was fermented to carbon dioxide and ethanol, 20 per cent was assimilated, and 8 per cent formed other products (probably glycerol, succinic acid, etc.). Results of similar experiments are shown in Table I. The extent of fermentation found by the method used in these experiments

TABLE I  
*Anaerobic Fermentation and Assimilation of Glucose by Commercial Strain of Bakers' Yeast*

Experiment No.	Fate of added sugar		
	Carbon dioxide and ethanol	Synthesized polysaccharides	Other products
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	72	20	8
2	69	20	11
3	71	21	8
4	69	24	7
*	70.5		
†	68.9-72.3		

\* Calorimetric determination by Winzler and Baumberger (3).

† Warburg determinations by Van Niel and Anderson (4).

is in excellent agreement with the results obtained by other methods (see Table I).

From the typical experiment shown in Fig. 1, it can be seen that during the earlier period of the fermentation the glucose was being utilized at a faster rate than the products were appearing, while in the later period the reverse was occurring. In other words the fractions we analyzed failed to account for all of the glucose used by the cells and therefore some of the glucose must be considered to be in an unanalyzed form. This unanalyzed form is called an intermediate in the rest of the discussion. In order to account for the rapid initial decrease in glucose we assume an initially high

<sup>3</sup> A correction was made for a small residue of reducing material remaining in the supernatant fluid at the conclusion of the fermentations. This material was not glucose or accumulated aldehydes, but was made up of other substance exuded from the cells. Yeast cells were unable to ferment any part of this reducing material, even after it had been concentrated 50 times by evaporation.

rate of conversion to intermediates, and similarly the later more complete accountability of the sugar in our analyses we ascribe to a diminution in the quantity of intermediates. The course of the accumulation of intermediary products can be seen in Fig. 1, the dotted curve representing the glucose that had disappeared but was not accounted for by the formation of the two main fermentation products or by assimilation. It can be seen that as much as 40 per cent of the added sugar appeared in this curve at one stage of the fermentation.

The heat production during fermentation as measured by the micro calorimeter of this laboratory (3) is shown in Fig. 2. Since the heat production curve shows an induction period entirely similar to the carbon dioxide production curve (Fig. 1), it is apparent that little or no heat is liberated in the

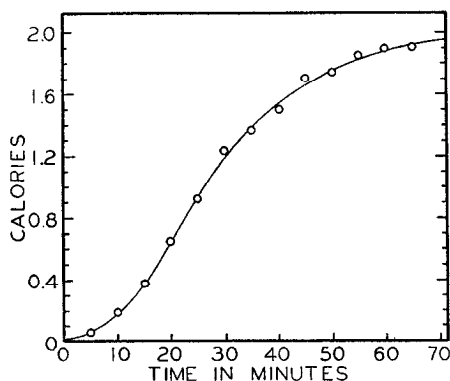


FIG. 2. The heat production during the anaerobic fermentation of 25 mg. of anhydrous glucose by a fresh yeast suspension. The suspension contained 0.30 gm. (wet weight) of yeast, obtained from a 24 hour pure culture of *Saccharomyces cerevisiae*, race FF-17, washed and made up to 20 ml. with phosphate buffer (pH 6.8).

formation of the intermediary products during the rapid initial disappearance of glucose. No conclusion can be drawn as to the steps involved in the formation of intermediary products from the calorimetric data at hand, however, since the conversion of acetaldehyde to ethanol (the last step in the fermentation) is the exothermic reaction largely responsible for the heat produced.

It should be noted that one cannot account for the very large accumulation of intermediary products noted above on the basis of fructose-1,6-diphosphate alone, since Macfarlane (9) has shown by analysis that the extent of the accumulation of this intermediate is relatively small and because much of the hexose diphosphate would not appear in the "intermediary fraction" in the present investigation since it is easily hydrolyzed to fructose-6-phosphate, a sugar derivative having a high reducing value.

Several experiments were carried out with yeast cells that had been starved by continuous aeration for 6 days. In the typical experiment shown in Fig. 3, it can be seen that 21 per cent of the added glucose was assimilated, thus agreeing with the extent of assimilation in normal yeast cells. The only noteworthy difference from the normal yeast suspension is that the induction period of assimilation was shortened. This can be seen in Fig. 4, which shows the assimilation curves of two normal and two starved yeast suspensions. The shortening of the induction period of assimilation

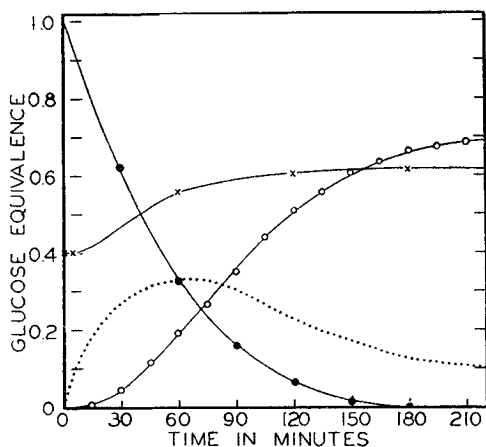


FIG. 3. The anaerobic fermentation of 500 mg. of anhydrous glucose by a yeast suspension starved for 6 days by continuous aeration. The same yeast suspension was used as in the experiment shown in Fig. 1. ●, glucose concentration; ○, glucose fermented to carbon dioxide and ethanol; ×, total hydrolyzable polysaccharide content of the cells as glucose; the dotted line represents the intermediary products, estimated by difference.

was not brought about by differences in the rates of the over-all fermentations, since the glucose consumption was almost identical in the four experiments.

Van Niel and Anderson (4) assumed that the assimilated "products represent stored, non-reducing sugar polymers, such as glycogen, capable of being fermented at a considerably slower rate," in order to account for the nearly quantitative conversion of glucose to fermentation products upon extended anaerobiosis. This anaerobic utilization of reserve material by yeast cells is so slow that Winzler and Baumberger (3) found that little or no heat was produced. Spiegelman and Nozawa (10) conclude that intact yeast cells were unable to ferment their carbohydrate reserves, although the data showed a slow but definite carbon dioxide production. The results of an experiment in which 50 mg. of glucose rather than the usual 500 mg. were

fermented by a fresh yeast suspension are shown in Fig. 5. There was a slow but definite carbon dioxide production both before the glucose had been

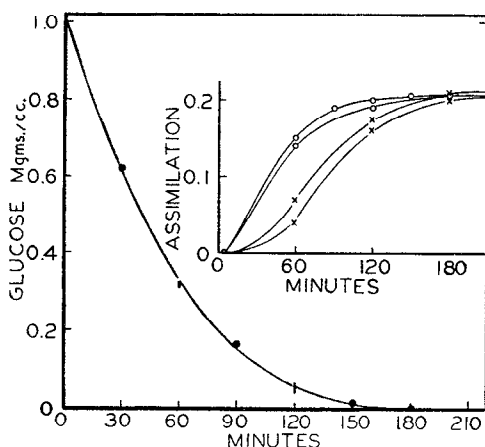


FIG. 4. The assimilation of glucose during the anaerobic fermentation of 500 mg. of glucose by starved and fresh yeast. Data from the experiments shown in Figs. 1 and 3 and from a duplicate experiment of each are presented. The assimilation equals the increase in total hydrolyzable polysaccharide content.  $\times$ , fresh yeast;  $\circ$ , starved yeast; represents four points (two for the fresh and two for the starved suspensions) all falling within the bar.

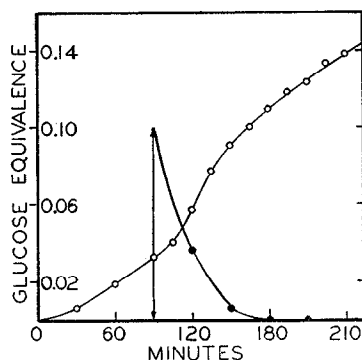


FIG. 5. The anaerobic fermentation of 50 mg. of glucose by a fresh yeast suspension. The sugar was added at the arrow.  $\circ$ , carbon dioxide;  $\bullet$ , glucose concentration.

added and after the fermentation was complete. In order to make this carbon dioxide production obvious, the scale used in Fig. 5 is much larger than that in the preceding figures. Upon inspection of the results shown in Fig. 5, it can be seen that even more than the theoretical amount of carbon

dioxide may be produced if the concentration of reserve material is high in comparison to the concentration of the added glucose. This is in agreement with Guillemet (11) who found that more than the theoretical amount of carbon dioxide was produced by yeast rich in carbohydrate reserves.

#### DISCUSSION

From the experimental evidence presented, it would appear that in the over-all fermentation process there is a definite pattern of progressive changes in the rate of fermentation, in the rate of assimilation, and in the concentration of intermediary products, as if these were all integrated by the equilibria involved. Starvation modifies this pattern, as if the concentration of reserve material present influences the progress of fermentation.

#### SUMMARY

The anaerobic fermentation of glucose as carried out by living yeast cells was followed by a series of quantitative analyses of carbon dioxide, glucose, and stored carbohydrate during the course of the process. The following conclusions are drawn.

1. In the fermentation brought about by a commercial strain of bakers' yeast, 20 per cent of the added sugar is assimilated and 70 per cent is converted to carbon dioxide and ethanol.
2. Starved yeast cells synthesize the same proportion of the added sugar into cellular carbohydrate material as do cells which contain a normal amount of glycogen, but the synthesis is accomplished earlier in the process.
3. A surprisingly large quantity of intermediary products accumulates during the early phases of the fermentation. It was found by the difference between the disappearance of the glucose and the formation of the final products that as much as 40 per cent of the added sugar appeared as intermediary products at one stage of the fermentation studied.
4. The nearly quantitative yield of fermentation products obtained after long periods of anaerobiosis is due to the utilization of stored material after the complete disappearance of the substrate.

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