ALCOHOLIC FERMENTATION

Control of Starter Preparation in Fermentation of Molasses

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The first two steps in preparing the starter for alcoholic fermentation of molasses can be easily controlled by measuring the variations in the weight of the fermentation system. Equations and nomograms are presented by means of which this control may be effected.

In alcoholic fermentation with a pure culture of yeast, the preparation of the starter is generally initiated by inoculating a small amount of sterilized mash (about 10 ml.) at a temperature from 25° to 30° C. for a suitable period of time (*2*). This material is then used to inoculate a flask containing approximately 100 to 200 ml. of a sterilized mash. Following incubation, the flask may be used to inoculate larger volumes, and so on. The first steps of this process are not made under control, as it is difficult to check density or sugar concentrations at various times; visual observation is not a good check and should be avoided.

The present work was undertaken to find an easy way to control those two steps by measuring the variation of the weight of the fermentation system.

Variation of Mash Weight

Experiments were made taking 10 ml. of mash in a 15 × 140 mm. test tube and using it to inoculate 100 ml. of mash placed in a 250-ml. Erlenmeyer flask. The following topics were then studied: the variation of the mash weight as a function of time and the percentage decrease of weight in the process of fermentation.

The variation of the mash weight during fermentation was measured by taking five different concentrations of total reducing sugars (100, 120, 140, 160, and 180 grams per liter), and two initial concentrations of yeast (10 and 50 grams of pressed yeast per liter), and combining the five different concentrations of sugars with the two initial concentrations of yeast; for every combination five experiments were made, giving a total of 50 experiments. The temperature was kept at 30° C. The development of the process could be followed by measuring the weight of the mash at various times. The results for one of the test tubes (total reducing sugars 120 grams per liter; initial concentration of pressed yeast 10 grams per liter) are shown in Figure 1. The same type of curve was obtained for the other test tubes.

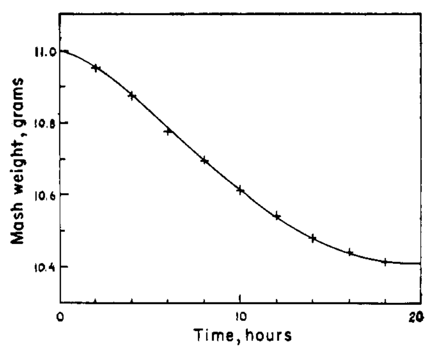

Figure 1. Variation of mash weight with time of fermentation

It was observed, by taking the average of 240 determinations, that the decrease in weight by evaporation was 5 mg. per hour. The actual decrease in weight, as consequence of the fermentation process only, was determined by subtracting from the total weight decrease the decrease due to evaporation (Table I). Each result of Table I is the average of ten determinations made with two initial concentrations of pressed yeast (10 and 50 grams per liter). In order to compare results, the decrease in weight calculated from Gay-Lussac's equation for glucose fermentation is also presented in Table I; it was assumed that 90% of the sugars had been fermented.

If P is the percentage decrease in weight of mash after fermentation and C is the initial concentration of total reducing sugars in the mash, in grams per liter, application of the least squares method to the data of Table I shows that

$$P = 0.043C - 0.4 \qquad (1)$$

As directed by Almeida (*1*), the material should be transferred from the test tube to the Erlenmeyer flask when half of the amount of sugar has been used. At this point, the percentage decrease in weight should be half the values indicated in Table I. If P_0 is taken as equal to $P/2$, from Equation 1 it is evident that

$$P_0 = 0.021C - 0.2 \qquad (2)$$

Equation 2 was verified experimentally with three different concentrations of sugars; the results are shown in Table II.

Table I. Decrease in Weight Due to Fermentation of Mashes with Different Sugar Concentrations

Initial Concn. of Total Reducing Sugars, G./Liter	Decrease in Weight, %: Found	Decrease in Weight, %: Calculated from Gay-Lussac equation
100	3.8	4.2
120	4.8	4.9
140	5.6	5.7
160	6.5	6.5
180	7.2	7.2

Table II. Experimental Check of Equation 2

Initial Concn. of Total Reducing Sugars, G./Liter	Concn. of Total Reducing Sugars (Decrease in Weight = P_0, Eq. 2), G./Liter
105	56
150	72
190	93

A general equation relating the initial mash weight in the test tube, the initial sugar concentration, and the time of fermentation with the total decrease in weight of the fermenting mash can be established. If

m = initial weight (grams) of mash in the test tube
C = initial concentration (grams per liter) of total reducing sugars in the mash
n = time (hours) measured from the instant the mash was placed at the thermostat at 30° C. to the instant in which half of sugars had been used
Δm = decrease in weight (grams) of the material in the test tube after time n; loss of weight due to evaporation is also included in Δm

$$\Delta m = \frac{(0.021\,C - 0.2)m}{100} + 0.005n \qquad (3)$$

In order to show the application of Equation 3, the results of a run are presented in Table III and Figure 2. In this figure the straight line represents the decrease in weight values calculated from Equation 3. At the moment corresponding to point M (Figure 2), the experimental decrease in weight is the same as calculated by Equation 3; consequently, at this moment one should find a concentration of total reducing sugars equal to half the initial concentration. In Figure 3 is presented a nomogram for Equation 3 that permits a rapid evaluation of Δm.

Table III. Decrease in Weight of a Fermentation System

(Test tube with approximately 10 ml. of mash, at different times, experimentally determined and calculated by Equation 3. Observe coincidence after about 8 hours of fermentation, point M in Figure 2)

Time, Hours	Decrease in Weight, Gram: Exptl.	Decrease in Weight, Gram: Calcd. from Eq. 3
0	0.00	0.26
2	0.04	0.27
4	0.12	0.28
6	0.22	0.29
8	0.31	0.30
10	0.40	0.31
12	0.46	0.32
14	0.52	0.33
16	0.56	0.34
18	0.59	0.35
20	0.60	0.36

Table IV. Decrease in Weight Due to Fermentation of 100 Ml. of Mash

Initial Concn. of Total Reducing Sugars, G./Liter	Decrease in Weight, %: Exptl.	Decrease in Weight, %: Calcd. from Eq. 1
97	3.5	3.8
135	5.0	5.4
175	6.9	7.1

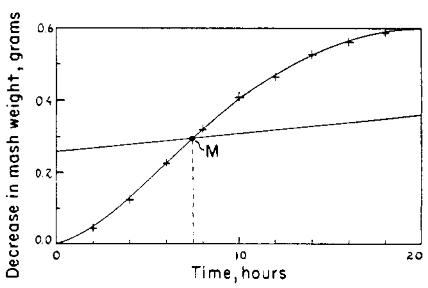

Figure 2. Graphical determination of inoculation moment

At point M experimental decrease in weight (+) agrees with that calculated by Equation 3 (straight line). That moment is the optimum inoculation moment.

Twelve other experiments were made in Erlenmeyer flasks of 250-ml. capacity with 100 ml. of mash at various concentrations. The concentrations of total reducing sugars were 97, 135, and 175 grams per liter, and the initial concentrations of pressed yeast were 10 and 50 grams per liter. The temperature was maintained between 28° and 29° C. The following conclusions were reached: The average decrease in weight due to fermentation is 21 mg. per hour; and the per cent decrease in weight due to evaporation only is in accordance with the one given by Equation 1, as shown in Table IV.

In this case, when 100 ml. of mash is used, Equation 3 becomes:

$$\Delta m = \frac{(0.021C - 0.2)m}{100} + 0.021n \qquad (4)$$

Figure 4 is a nomogram for this equation analogous to Figure 3.

Conclusions

The first two steps of the starter preparation for alcoholic fermentation of molasses may be easily controlled by measuring the variation of weight of the system. This control is realized with Equations 3 and 4 or with the corresponding nomograms. The use of an initial concentration of 10 or 50 grams of pressed yeast per liter of mash gives approximately the same weight decrease.

Acknowledgment

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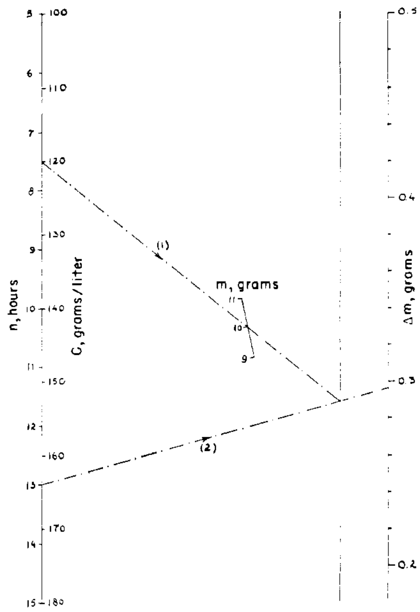

Figure 3. Nomogram for equation $\Delta m = \dfrac{(0.021C - 0.2)m}{100} + 0.005n$

Applies to fermentations carried on in 15 × 140 mm. test tubes at 30° C. It permits rapid evaluation of Δm from the equation, in order to compare this value with the experimental weight decrease.

Literature Cited

(1) Almeida, J. R., "Alcool e Destilaria," p. 60, Livraria e Papelaria Brasil, Piracicaba (São Paulo, Brasil), 1940.
(2) Prescott, S. C., and Dunn, C. G., "Industrial Microbiology," 2nd ed., p. 116, New York, McGraw-Hill Book Co., 1949.

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Figure 4. Nomogram for equation $\Delta m = \dfrac{(0.021C - 0.2)m}{100} + 0.021n$

Applies to fermentations in Erlenmeyer flasks of 250-ml. capacity at 28–29° C. Permits easy evaluation of Δm from equation in order to compare this value with experimental weight decrease.