the composition of a complex mixture and its vapor. When Raoult's law may be assumed to hold with sufficient accuracy, the methods of computation described in this paper are adequate for the solution of a variety of problems involving vaporization (or condensation) of such mixtures, for which substantial equilibrium between liquid and vapor may be assumed and for which the pressure-temperature conditions are known with sufficient accuracy for the purpose of the computation.

Literature Cited

(1) Brown and Caine, "Studies in Distillation—I," Part 2, *Trans. Am. Inst. Chem. Eng.*, Preprint, August, 1928.
(2) Calingaert and Hitchcock, *J. Am. Chem. Soc.*, **49**, 750 (1927).
(3) Coats and Brown, Univ. Mich. Dept. Eng. Research, *Circ.* **2**.
(4) Leslie and Good, IND. ENG. CHEM., **19**, 453 (1927).
(5) Pircoomov and Beiswenger, *Am. Petroleum Inst. Bull.* **10**, No. 2, p. 52 (January 3, 1929).
(6) Podbielniak, *Oil Gas J.*, No. 35, 38 (1929).
(7) Proceedings 7th Annual Convention of Natural Gasoline Association of America, 1928, p. 73.

Adjustment of Acidity of Cane-Molasses Fermentations for Maximum Alcohol Yields[1]

F. M. Hildebrandt

UNITED STATES INDUSTRIAL ALCOHOL COMPANY, BALTIMORE, MD.

In producing alcohol from cane molasses by fermentation, it is necessary to add acid to the diluted molasses in order to secure the maximum yield. It is known that the quantity of acid necessary differs with various types of molasses and even with successive shipments of a single type. A method of setting cane-molasses fermentations to the point at which maximum alcohol yield is obtained is described. The method involves the use of the potentiometer and quinhydrone electrode and has the advantage that the fermentations themselves give a check on the accuracy of the setting. Experiments show that the maximum yield is obtained when sufficient acid is added so that there is no tendency for the pH of the fermentation solution to drift toward the acid side during the fermentation.

IN THE fermentation of cane molasses for the production of alcohol a number of factors have to be taken into account in order to obtain the maximum yield from the sugar present in the fermentation solution. Such things as concentration of the solution, the temperature of fermentation, and the necessity for an adequate supply of yeast nutrients are known to have a bearing on the process. The regulation of these conditions in the actual procedure of fermentation may be fairly well carried out by combining experience with certain well-known physiological principles. This regulation is, however, a complex problem. An advance is made both in the theoretical and practical aspects of the process when it is found possible to substitute physical and chemical methods for the more empirical type of control.

One of the important ingredients in cane-molasses fermentation solutions is the acid added to bring the molasses to the condition best suited for yeast fermentation (*4*). Sulfuric acid is used ordinarily, and it is generally admitted that the amount of acid should be carefully fixed if the best alcohol yield is to be obtained. The question therefore arises as to how this acid should be regulated in different types of molasses. One method commonly used is to run small-scale fermentations with various quantities of acid (*3*). Determination of the alcohol produced in the presence of these varying amounts of acid makes it possible to pick out the proper acid dose for maximum alcohol production. This method has the disadvantage that it is tedious and requires very careful manipulation if the results are to be a reliable guide, since the absolute differences in yield between the optimum solution and the solutions not optimum are small.

It is known that the growth of microörganisms is dependent on the acidity of the solutions in which they are placed. In the case of yeast, and other organisms as well, this relation stands out when the acidity is expressed in terms of hydrogen-ion concentration rather than as titratable acid (*1*). A control method, therefore, would most logically be based on the setting of the pH of the mash to the point giving the highest alcohol yield. This can be done empirically by means of the small-scale fermentations noted above, but a more rapid method is desirable.

In this paper a method of setting cane-molasses fermentations to the point at which maximum alcohol yield is obtained will be described.

[1] Received March 7, 1929. Presented under the title "Adjustment of Acidity of Cane-Molasses Fermentations" before the Division of Sugar Chemistry at the 77th Meeting of the American Chemical Society, Columbus, Ohio, April 29 to May 3, 1929.

Experimental Procedure

The fermentation solutions were made from cane molasses diluted with water and with no other addition except sulfuric acid. These solutions were not sterilized, but a pure-culture yeast grown in a sterilized molasses solution was used to seed them. The seed yeast constituted 2.5 per cent of the total volume of the mash. Measurements of acidity were made with a quinhydrone electrode and a potentiometer. In carrying out the electrometric determination, 10 cc. of mash were diluted to 100 cc. with distilled water, quinhydrone added, and the reading taken. Since molasses solutions are well buffered, this 1 to 10 dilution does not materially change the pH. Alcohol was determined by distilling an aliquot and weighing the distillate in a vacuum-jacketed pycnometer. In this work it is necessary to adjust all distillate volumes at definite temperature before fermentation and at the same temperature after fermentation in order to get accurate determinations of the alcohol.

In the tables showing the results of these experiments, the acid concentration is given as cubic centimeters of 60 Bé. sulfuric per 1000 cc. of the fermentation solution. Brix readings were taken with the hydrometer and corrected to 17.5° C. The acidity was determined electrometrically at the beginning and end of the fermentation. The efficiency represents percentage of theoretical yield on the basis of the total sugar. To obtain the theoretical yield, the weight of molasses used per 100 cc. of the fermentation solution is multiplied by the sugar percentage to give grams sugar per 100 cc. The sugar percentage used is sucrose by Clerget method × 1.0528 plus the invert sugar as determined by the Munson and Walker method. This gives total as invert.

Multiplying grams invert sugar per 100 cc. by 0.61 (number of cubic centimeters of absolute alcohol to be expected from the chemical reaction of transformation of invert sugar to alcohol, corrected for the sugar used in the formation of substances other than alcohol) gives the theoretical volume percentage of alcohol, and this divided into the alcohol actually obtained gives the efficiency figure.

First Series of Experiments

Table I gives the results of an experiment made on Porto Rican molasses.

Table I—Results with Porto Rican Molasses
(Average of duplicate experiments)

Flask	Acid Concn.	Sp. Gr. at Start	Acidity at Start	Acidity at End	Change in Acidity	Alcohol	Efficiency
	Cc.	*° Brix*	*pH*	*pH*	*pH*	%	%
1, 2	None	23.7	5.75	5.48	−0.27	8.5	83.7
3, 4	0.5	23.7	5.39	5.30	−0.09	8.6	84.8
5, 6	1.0	23.7	5.20	5.14	−0.06	8.7	85.9
7, 8	1.5	23.7	5.00	5.00	0	8.8	86.1
9, 10	2.0	23.7	4.80	4.80	0	8.8	86.1

It will be noted that if sufficient acid is added to make the pH 5.0 or lower, the fermentations do not drift toward the acid side from the beginning to the end, and that when the pH value does not drift toward the acid side, the maximum alcohol yield is obtained.

Table II shows further experiments on Cuban molasses.

Table II—Results with Cuban Molasses

Flask	Acid Concn.	Sp. Gr. at Start	Acidity at Start	Acidity at End	Change in Acidity	Alcohol	Efficiency
	Cc.	*° Brix*	*pH*	*pH*	*pH*	%	%
1	None	21.05	5.65	5.40	−0.25	7.9	89.0
2	1.3	21.05	5.05	5.00	−0.05	8.1	91.5
3	2.0	21.05	4.70	4.90	+0.20	8.1	91.5
1a	None	21.2	5.65	5.30	−0.35	7.7	87.4
2a	1.0	21.2	5.05	5.03	−0.02	7.9	90.2
3a	1.5	21.2	4.70	4.90	+0.20	7.8	88.7

These data were secured from two different lots of molasses, experiments 1, 2, and 3 having been performed on one lot and experiments 1a, 2a, and 3a, on a second lot. It will be observed that if the fermentations are set on the alkaline side of 5.0 pH they become more acid during fermentation, drifting toward 5.0 pH, and this drift is associated with a lowered yield of alcohol. Runs set on the acid side of 5.0 pH sometimes show a drift and sometimes do not. If they do change, the tendency is to move toward the alkaline side—that is, toward a point approximating 5.0 pH (*2*).

A set of experiments on another lot of Cuban molasses gave the results shown in Table III.

Table III—Results with Cuban Molasses

Flask	Acid Concn.	Sp. Gr. at Start	Acidity at Start	Acidity at End	Change in Acidity	Alcohol	Efficiency
	Cc.	*° Brix*	*pH*	*pH*	*pH*	%	%
1	None	21.2	5.60	5.40	−0.20	7.6	86.3
2	1.4	21.2	5.05	4.97	−0.08	7.9	89.8
3	2.0	21.2	4.80	4.80	0	7.9	89.8

In this series again, the drift from the alkaline side toward a pH value of 5.0 is associated with a lowered yield of alcohol in flask 1. In the case of flask 2, where the change is in the same direction but is not so large, the alcohol yield is higher. In flask 3 there is no pH change during fermentation and the yield is the same as in flask 2.

Second Series of Experiments

Several months after the experiments noted above were carried out, a second series was run using a somewhat modified technic which gave greater accuracy. Three types of molasses were used and each type was set without acid, with the optimum amount, and with twice the optimum amount. A pH of 5.0 is considered the optimum, since molasses at this acidity shows little or no tendency to become more acid during fermentation. This setting point of 5.0 pH is determined by noting the behavior of the fermentations under the particular conditions of the experiments. It should be borne in mind that under a different set of conditions this point might be at some other acidity.

The results of the experiments are given in Table IV.

Table IV—Experiments with No Acid, Optimum Acid, and Twice Optimum Acid[a]
(Specific gravity 20.0° Brix)

Type of Molasses	Acid Concn.	Acidity at Start	Acidity at End	Change in Acidity	Total Alcohol	Efficiency
	Cc.	*pH*	*pH*	*pH*	*Cc.*	%
San Dominican, Flask 1	None	5.70	5.30	−0.40	34.56	82.46
Flask 2	1.4	4.98	5.02	+0.04	35.10	83.79
Flask 3	2.8	4.40	4.48	+0.08	35.12	83.77
Cuban, Flask 1	None	5.85	5.40	−0.45	33.87	85.46
Flask 2	1.75	5.00	5.01	+0.01	34.10	85.99
Flask 3	3.50	4.48	4.53	+0.05	34.06	85.96
Porto Rican, Flask 1	None	5.80	5.25	−0.55	36.35	84.31
Flask 2	1.16	5.00	4.83	−0.17	36.80	85.40
Flask 3	2.32	4.48	4.45	−0.03	36.92	85.76

[a] The data given in Table IV may be compared with those shown in previous tables except that the total cc. of alcohol in 500 cc. of fermentation solution is given in place of the volume per cent.

It will be noted that in every case a drift toward the acid side is associated with a lowered efficiency of fermentation. The Porto Rican molasses used needed a large dose of acid to give its best yield.

Table V gives results of experiments on two types of molasses, Cuban and Porto Rican. The experiment on Cuban molasses requires a relatively large amount of acid, and in the case of No. 3 there is a change toward the acid side even when 3 cc. of 60° Bé. sulfuric were used.

Table V—Comparison of Cuban and Porto Rican Molasses

Type of Molasses	Acid Concn.	Acidity at Start	Acidity at End	Change in Acidity	Total Alcohol	Efficiency
	Cc.	*pH*	*pH*	*pH*	*Cc.*	%
Cuban, Flask 1	None	6.00	5.50	−0.50	34.02	85.93
Flask 2	1.5	5.28	5.02	−0.26	34.37	86.79
Flask 3	3.0	4.73	4.60	−0.13	34.40	86.73
Porto Rican, Flask 1	None	5.80	5.25	−0.55	36.58	84.82
Flask 2	1.0	5.08	4.90	−0.18	36.83	85.37
Flask 3	2.0	4.58	4.57	−0.01	37.31	86.54

Third Series of Experiments

A series of experiments, run in duplicate, was conducted as follows: (1 and 2) Cuban cane molasses solution adjusted to pH 6.25 by use of sodium hydroxide. This is well off the optimum on the alkaline side. (3 and 4) Same solution but with no addition of either acid or alkali. (5 and 6) Same solution adjusted to 5.00 pH. (7 and 8) Same solution adjusted to 4.55 pH. The results are given in Table VI.

Table VI—Experiments with Cuban Molasses of Various Acidities

Expt.	Acidity at Start	Acidity at Finish	Change	Efficiency
	pH	*pH*	*pH*	%
1, 2	6.25	5.50	−0.75	85.74
3, 4	5.63	5.26	−0.37	85.90
5, 6	5.00	5.02	+0.02	87.18
7, 8	4.53	4.63	+0.10	86.73

Completeness of Fermentation

In connection with the results here reported, the question naturally arises as to whether the fermentations were complete in each case. This factor was followed in all the runs by weighing the flasks at intervals. Knowing the characteristics of the fermentation curve, it is thus possible to establish the fact that the fermentations are complete when the solution is distilled for determination of the percentage of alcohol.

Table VII gives the weight losses on the experiment whose results are shown in Table VI. Examination of this table shows a total weight loss of from 29 to 30 grams, which is

normal for complete fermentation of the molasses under investigation. The loss in the last 12 hours was 1.5 grams or less. This very slight decrease in weight shows that the evolution of gas had practically ceased and that continuation of the experiment would result in loss of alcohol by evaporation. Data of this sort were used in each experimental run as a criterion of the completion of fermentation.

Table VII—Weight Loss from Experimental Flasks of Table VI

Flask	At 48 Hours	At 60 Hours	In Last 12 Hours	Acid Treatment
1	28.4	29.6	1.2	Received enough NaOH to bring starting pH to 6.25
2	29.3	29.8	0.5	
3	28.4	29.5	1.1	Neither alkali nor acid added, starting pH 5.63
4	28.2	29.3	1.1	
5	28.2	29.5	1.3	0.55 cc. 60° Bé. H_2SO_4 added to bring starting pH to 5.0
6	28.2	29.7	1.5	
7	28.9	30.2	1.3	1.10 cc. of 60 Bé. acid added to bring starting pH to 4.53
8	28.9	30.2	1.3	

The fermentations reported were examined under the microscope at the end of the experiments and there was no evidence that infection had played any part in determining the results.

Conclusion

The results here reported throw light on one of the factors affecting the complex process of fermentation of molasses by living yeast—namely, the acidity of the fermentation solution. They indicate that a molasses fermentation solution so adjusted that it does not become more acid during the fermentation process will give the maximum alcohol yield if other factors are kept constant. The acid concentration is measured with the potentiometer and the optimum concentration is about 5.0 pH. This is not, however, to be considered a fixed point. The real criterion for a solution optimum with respect to H ions is that there is no tendency for the solution to show a lower pH value at the end than at the beginning of the fermentation.

Literature Cited

(1) Houssiau, *Chimie et industrie*, Spec. No. 663–7, May, 1927.
(2) Nelson and Bloomfield, *J. Am. Chem. Soc.*, **46**, 1025 (1924).
(3) Owen and Bond, Ind. Eng. Chem., **16**, 387 (1924).
(4) References to former practice in regard to acid addition to molasses fermentation: Hawaiian Sugar Planters' Assocn., *Bull.* **28**; *Louisiana Planter*, **68**, 126, 206 (1922); Henneberg, "Gärungsbacteriologisches Praktikum," p. 188; Effront-Prescott, "Enzymes and Their Application," p. 89; *Bull. assocn. chim. sucr. dist.*, **31**, 936 (1916). In these references amounts of acid varying all the way from one gallon of acid per 100 of mash to one gallon of acid per 10,000 of mash are recommended. Also a number of authors have reported on the optimum pH for fermentation, but there is considerable disagreement as to what is the optimum point, the recommendations varying from 4.5 to 7.0. If the results here given are generally valid, it can be readily seen why these discordant figures are obtained.

X-Ray Study of the Zonal Structure of Silica Brick from the Roof of a Basic Open-Hearth Furnace[1]

G. L. Clark and H. V. Anderson

University of Illinois, Urbana, Ill.

X-ray diffraction patterns by the "powder method" have been obtained for pure quartz, ganister, burned silica brick (unused), and the various zones of silica brick after the usual service in an open-hearth furnace.

The interplanar distances obtained for these materials are given, together with diffraction data on low quartz (Harrington) and high cristobalite (Wyckoff). The various lattice constants, as obtained from the literature, associated with these two forms of silica are given.

The presence of both quartz and cristobalite in burned silica brick (unused) is definitely established. Also after service in an open-hearth furnace the silica brick still retains the quartz formation, although there is a greater tendency towards the cristobalite state in the hotter zones than in the cooler zones of the brick. The probable cristobalite interplanar distances are indicated.

The x-ray diffraction method suggests itself as another procedure for the detection of the various forms of silica in silica refractories.

IT IS of interest to those directly connected with open-hearth furnace refractories and users and manufacturers of silica brick to know just what transformations take place in a silica brick during or after service at high temperatures.

The recent researches of Greig (*3*) on the immiscibility in silicate melts establishes the temperature range at which cristobalite is in equilibrium with two liquid phases, thus determining points on the liquidus of cristobalite. This investigation has contributed a vast amount of valuable information to this field of endeavor which is also directly applicable to the changes occurring in silica brick under actual working conditions.

The usual methods for identifying the various transformations of quartz are based on density, optical methods, or the dilatometer method for volume changes at the inversions. It is known that measurements of density are unreliable, as these transformations of quartz have a low density on account of fine pores. Likewise, optical methods fail in the identification of very fine grained and porous cristobalite (*18c*), as it may appear isotropic and cannot be distinguished from amorphous silica. The dilatometer method (*20*) is the most dependable, but requires great care in the hands of a skilled operator.

In the diffraction of x-rays by fine powders and with subsequent registration on a photographic film of the different orders of reflections from the various atomic planes of a crystalline aggregate, a method is available whereby one can positively identify the constituents of ceramic and other materials, and to a much greater degree of certainty than by any optical procedure known at present. Obviously, the powder method of x-ray analysis is a very sensitive, convenient, and modern analytical procedure and one which is daily finding a wider application in the endeavor to establish with greater accuracy the real nature and constitution of matter.

There have been various applications of x-ray methods in the field of ceramics and unique data yielding entirely new information or settling doubtful and controversial interpretations of other types of data have recently been published (*1*, *4*, *5*, *8* to *16*, *19*). This investigation was undertaken to determine just what x-ray analysis would reveal in studies of burned silica brick (unused) and the zonal structure of

[1] Received February 18, 1929.