FERMENTATION OF DOUGLAS-FIR HYDROLYZATE BY S. *cerevisiae*

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FERMENTATION OF DOUGLAS-FIR HYDROLYZATE BY *S. cerevisiae*

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Introduction

Previous work at the Forest Products Laboratory has demonstrated that the sugars produced by the saccharification of wood could be used to propagate yeast and produce ethyl alcohol by fermentation provided that treatments were given or conditions used which overcame the effect of some of the inhibiting materials in the wood-sugar hydrolyzates. Leonard and Hajny[1] have demonstrated that addition of sodium bisulfite, a heat treatment at neutrality, or the use of large quantities of inoculum aided in overcoming the effect of the inhibiting materials. Peterson and his coworkers[2] found that the use of a rich inoculum was beneficial to the growing of yeast on wood hydrolyzates, Grondal and Berger[3], found that removal of lignin and furfural plus the addition of autolyzed yeast aided in the butanol fermentation of waste sulfite liquor. Sjolander, Langlykke, and Peterson[4], found that clarification of the solutions with activated carbon produced solutions that were easily fermented. Many other notable contributions have been made by others especially in connection with the Scholler process for wood saccharification in Germany[5].

by improvements which affect the ease of fermentation have been made in the process of wood saccharification as developed at the Forest Products Laboratory.[6] Hydrolyzate prepared by these processes were used for study in these experiments.

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[1]Leonard, R. H. and Hajny, G. J.; J. Ind. Eng. Chem. 37, 390 (1945).

[2]Peterson, W. H., Snell, J. F., and Frazer, W. C., Ind. Eng. Chem. 37, 30, (1945).

[3]Grondal, B. and Berger, H. W., Chem. and Met, Eng., 52:6, June 1945.

[4]Sjolandar, N. O., Langlykke, A. F. and Peterson, W. H., J. Ind. Eng. Chem. 30, 1251 (1938).

[5]U. S. Patents: 2,083,347, June 8, 1937; 2,083,348, June 8, 1937; 2,188,192, January 23, 1940, 2,188,193, January 23, 1940.

[6]Harris, E. E., Beglinger, E., Hajny, G. J., and Sherrard, E. C.; J. Ind. Eng. 37, 12 (1945). Saccharification of Wood, FPL Report No. R1475, March 1945.

The need for the removal of furfural from liquors to be fermented has been pointed out by Leonard and Hajny[1] and by Emil Hauser[7]. In recent works at the Forest Products Laboratory a method of flashing the hydrolyzate after neutralization has been used. This procedure removes all but traces of furfural and at the same time permits removal of calcium sulfate at elevated temperatures,

The effect of these changes on the ease of fermentation and a study of the problems connected with pilot-plant-scale fermentations, such as yeast requirements, conditions for growth of yeast, methods for removing yeast from the fermented liquors, nutrient requirements for growing yeast, and for fermentation and other problems are described in this report,

The yeast used was a strain of brewer's yeast S. cerevisiae No. 49 from the University of Wisconsin collection and was selected because of its high alcohol-yielding capacity.

Wood hydrolyzates prepared according to procedures described in earlier reports, which had been neutralized at temperatures of 130° to 135° C. to a pH of 4.7 to 5.0 with lime, filtered within that temperature range, flashed to atmospheric pressure, cooled to 30° C. and then filtered, were treated with sodium carbonate until the hydrolyzate had a pH value between 5.6 and 5.8.

Analytical Methods

Total Yeast Counts Were &de by a Procedure Using a Mold-counting Chamber

The yeast count was made on a sample estimated to contain about 50 million cells per milliliter. For estimating purposes it was found that 1 percent wet volume of yeast was equivalent to about 100 million Torula cells or 80 million brewer's yeast cells per milliliter. Special cam was given to obtain a representative sample. With the concentration of yeast usually used in these fermentations a dilution of one part of sample with two parts of water was satisfactory.

The diluted sample was shaken to provide even distribution of the cells. By means of a capillary pipette a small drop of the diluted sample was placed on a mold-counting chamber (U. S. Dept. of Agr. Bulletin No. 581, 1917) and spread evenly with the pipette. The cover slip was placed on tho counting chamber so that there were no air bubbles or overflow of fluid, and so that Newton's bands appeared at the points of contact between the cover slip and tho edges of the moats,

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[7]Hauser, E., Cellulose Chemie, 1, 41 (1920).

Through the high power objective of a microscope with a micrometer dish inserted in the eyepiece, the cells were counted in A row of 10 large squares. Counting was repeated until 10 fields chosen at random, but scattered over the entire mold chamber, had been counted. Cells touching the left and upper edge of the row were counted while those touching the right and lower edges were disregarded. Buds were counted as separate cells.

Calculations

$$\text{Million cells per milliliter} = \begin{array}{l}\text{Number of cells per large square}\\ \text{times dilution times factor}\end{array} \qquad (1)$$

The factor will vary with each microscope, objective, and eyepiece used. This factor is found by measuring the dimensions represented by a square of the micrometer disk in the eyepiece at a definite setting of draw tube and with the combination of lenses to be used for counting, and then converting the volume represented by a square to 1 milliliter. With the combination of equipment used in this study, the factor was 14.8. When the average number of cells per square are multiplied by this factor and the dilution, the millions of cells per milliliter are obtained.

Wet–yeast volume was determined in a graduated 15–milliliter centrifuge tube by centrifuging 10 milliliters of a representative sample of the suspension of yeast in the fermentation liquor. The wet volume was read directly from the tube; 0.1 milliliter of wet yeast is equivalent to approximately 1 percent wet-yeast volume.

The dry weight of the yeast was determined by the method described by Peterson, Snell, and Frazier.[2]

Reducing sugar, fermentable sugar, and alcohol were determined by methods described by Saeman, Harris, and Kline.[8]

Inoculum Production

In the production of alcohol by the use of large volumes of preformed inoculum, it is desirable to keep the cost of the production of yeast at a minimum. Alcohol being the desired end product, there is no loss if alcohol is produced while propagating the inoculum. It is possible, therefore, to obtain higher utilization of the air and decrease the cost of yeast production by using a lower rate of air to medium than in the usual yeast production. The use of less air when growing yeast on wood hydrolyzates also reduces the amount of organic material which precipitates.

For the growing of yeast, (table 1) inoculum which had been developed from a slant on a glucose and malt–sprout medium was propagated on 2.6 gallons of a

[8] Saeman, J. F., Harris, E. E., Kline, A.; Analyl. Ed. Ind. Eng. Chem. 17, 95 (1945).

sterilized solution containing 2 percent sugar obtained by diluting Louisiana second–crop molasses, 2 grams of ammonium sulfate, 2 grams of disodium phosphate, 8 grams of urea, and the aqueous extract from 200 grams of malt sprouts. This solution was aerated through a cloth sparger for 16 hours and then added to 4.2 gallons of a sterilized solution containing 8 percent sugar from Louisiana second–crop molasses, 12.7 grams disodium phosphate, 12.7 grams ammonium sulfate, and 50 grams of urea. This 6.8 gallons of inoculum was aerated as described for 8 hours and was then placed in a 75–gallon–fermentation tank and, while aerating at a rate of 4 cubic feet of air per minute, neutralized wood hydrolyzate was added at a rate of 2 to 3 gallons per hour for 16 hours. The inoculum thus produced was placed in a 500–gallon fernentor and, while aerating at a rate of 0,2 volume of air to 1 volume of liquid per minute, wood hydrolyzate was added at a rate of 0.75 gallon per minute for 7 to 8 hours.

In general the more concentrated solutions were acted upon more slowly. Fermentation P5 (table 1) did not appear to follow this general observation because the rate of feed of the wood–sugar inoculum was larger, and therefore the amount of yeast added to the large fermentor was greater. The sugar in both fermentations P4 and P5 were utilized in the time allowed while the higher concentrations of fermentations P6 and P7 were used more slowly. The experiment was not continued but it is believed that a longer period of time would have given high yields of yeast in fermentations P6 and P7.

A series of fermentations (P8 to P18 in table 2), were made to study the effect of increasing the time and variations in the ratio of air to medium on the yield of yeast and alcohol from more concentrated sugar solutions. For this series, past was started on 5 gallons of 3 percent sugar from diluted Louisiana second–crop molasses containing 20 grams ammonium sulfate, 20 grams of monosodium phosphate, 40 grams of urea, and the extract from 570 grams of malt sprouts. This medium was sterilized for 30 minutes at 15 pounds per square inch steam pressure, adjusted to pH 4.8, and cooled to 30° C. The yeast was added and then the mixture was aerated at approximately 2.5 air-to–liquid ratio through a 3.5–inch cloth sparger for 16 hours, This molasses inoculum was placed in a 75–gallon fermentor and wood sugar was added while aerating and stirring took place. In fermentations P8 through P13, the sugar was fed in slowly, the addition requiring 6 to 8 hours. Samples were taken at the end of 9 hours and then aeration was continued to the end of 24 hours when samples were again taken. An increase in the rate of aeration decreased the rate of sugar utilization in 9 hours but increased the total amount of yeast produced. The total yeast produced was limited by tho amount of nutrient present which was that required to produce a 20 percent growth of yeast.

In fernentations P14 through P18, the sugar was added as a batch and aerated. Fermentation P14 differed from P16 in the rate of agitation, In fermentation P14 the agitation was half the rate used in the series P15 through P18. Yields of yeast in 9 hours in series P14 through P18 were higher than from the series that was fed slowly because the full amount of the sugar was present the entire time and a greater utilization of the air resulted. The yield in 24 hours, however, was about the same, In the series P8 through P18, the alcohol produced in 9 hours was in most fermentations almost equal to that in 24 hours, indicating that only yeast was produced after the first 9 hours, or that alcohol may either have been used in the growing of yeast, or was lost due to evaporation.

Preliminary Pilot-plant-scale Alcoholic Fermentation.

In fermentations shown in table 3, yeast was first grown on 5 gallons Of diluted molasses, then transferred to wood sugar in a 75-gallon fermentor and aerated for 16 hours for further development of yeast, and was then transferred to a 350-gallon batch of wood sugar for anaerobic fermentation. In the series P20, P21, and P22, the yeast, which had increased from about 0.35 pound on a dry basis to more than a pound, was added to about 350 gallons of approximately 5 percent wood-sugar solution, The inoculum in these fermentations did not contain enough yeast to carry the fermentation to completion in 24 hours. Fermentation P22 was allowed to continue for 40 hours. The yield of alcohol was normal for the sugar subjected to alcoholic fermentation, Except for the time required, fermentation P22 was satisfactory.

Fermentations P24, P25, P26, and P29 comprised a study of the rate of fermentation using varying amounts of inoculum. In fernentations P24 and P25 a large amount of inoculum, 1.8 to 2.0 pounds on a dry basis in 76 gallons, was used. Fermentation was complete in 5.5 to 6.0 hours. Half that amount of inoculum, as in fermentation P29, required 14 hours. Fermentation P26 was not complete in 7.5 hours but would have been in 9.5 hours. The sugar subjected to alcoholic fermentation gave good yields, but because the amount used to grow the yeast was large, the over-all yield of alcohol was low,

Growth factors in the form of molasses and extract of malt sprouts were added to fermentations P27 and P28, but the rate of fermentation did not appear to be affected.

Growth factors which may have been carried over in the solution from the molasses inoculum were removed in fermentations P30 through P33 by centrifuging and washing! the yeast before transferring to a wood-sugar solution. The effect of the addition of varying amounts of growth factors was determined by adding molasses to the fermentation. In fermentation P30 molasses was added for the growing of inocula on wood sugars and also for the fermentation. In fermentations P31 and P32, molasses was used in the production of the yeast only, while in fermentation P33 the only sources of growth factors were those in the centrifuged and washed yeast. Fermentations were slower as the amount of molasses was decreased, but all were complete with good conversion of sugar to alcohol in 24 hours. Over-all conversion was low because of the large amount of wood sugar used for growing the yeast.

The past produced in the alcoholic fermentation in these series was usually less than that lost in the beer during separation in a yeast separator. Further experiments were therefore carried out to produce an amount of yeast in each fermentation equal to that lost in the separation of the yeast and through mechanical losses.

The efficiency of the wood-sugar fermentation, where yeast is produced with each fermentation, may be determined from the results in fermentation P29. Since 25 gallons of the 58 gallons of wood–sugar inoculum were used to supply the past for the anaerobic fermentation, the equivalent of 17.9 gallons of 5.23 percent sugar were used to grow the yeast, thus only 24.7 percent of the sugar was converted to alcohol. In the alcoholic fermentation, 45 gallons of 5.28 percent sugar were fermented, producing a yield of alcohol of 40 percent based on the sugar. This gives an over–all efficiency of only 35.8 percent, whereas an average yield of 38 to 39 percent is required to maintain plant output, In order to obtain this efficiency, it would be necessary to recover the past and transfer it to a new batch of sugar solution,

Fermentation of Clarified Wood Hydrolyzate

To doternine the factors that were responsible for the failure to grow sufficient yeast to carry the fermentations through several transfers, variations were introduced.

In a previous report,[1] attention has been called to the precipitate which is deposited on yeast while aerating the wood hydrolyzate during the propagation of yeast. Upon centrifuging, this precipitate collects with the yeast, makes it difficult to determine the amount of yeast actually present, and possibly interferes with the metabolism of the yeast.

Because the precipitate has some of the properties of lignin, it was suggested that its rate of precipitation may be controlled by oxidation, reduction, or coagulation with some multivalent ion. Oxidizing agents such as peroxide, calcium hypochlorite, and bromine, water seemed to have very little affect in quantities that would be permissible. Reducing substances such as sulfur dioxide, hydrogen sulfide, sulfites, and phosphites seemed to have no effect. Polyvalent ions such as the aluminum ion were very effective in clarifying the solution, The amount required was about 2 pounds of aluminum sulfate for each 500 gallons of wood hydrolyzate but quantities as low as 1 pound for 500 gallons were helpful.

Seven–litor fermentation

Wood hydrolyzates which had been clarified by the use of aluminum sulfate were fermented in 7–liter fermentations (table 4) with 0.5 percent, 1 percent, and 1.5 percent yeast by wet volume. At the end of the fermentation the yeast was removed in a centrifuge and was added with 700 milliliters of the fermented liquor to 6,300 milliliters of approximately 5 percent sugar solution containing sufficient urea (0.01 percent concentration) and potassium phosphate (0.003 percent concentration) to permit a 20 percent increase in yeast, The fermentation was carried through four transfers, one each 24 hours, The results are shown in tables 4 and 5.

It appears that an increase in the utilization of sugars and their conversion into alcohol results through continuous transfer of the yeast. There vas no difficulty in maintaining yeast concentrations. All fermentations on clarified solutions were complete in 6 to 12 hours but the yeast was left in the beer to the end of 20 hours to determine any loss in vitality that may have resulted from standing. The yeast was very active and seldom more than half an hour elapsed before vigorous fermentation started, and in all fer–mentors increased beyond that required for maintenance of satisfactory fermentation rate in 20 hours,

Fermentation in 77-gallon Batches by Yeast Transfer

Fermentations of wood hydrolyzates using solutions clarified with aluminum sulfate were also studied in 77–gallon batches. In the first of the fer–mentation series, as shown in table 6, P39 was made on the clarified hydrolyzate, while series P42 was made on liquor that had. been clarified· aid then aerated for 24 hours before using it for the growing of yeast and for fermentation.

The experiments were handicapped because the yeast separator was in poor con–dition and about 20 percent of the yeast was lost with each transfer. The yeast growth during fermentation, however, was sufficient to maintain satisfactory yeast concentrations to bring about fernentat ion in 20 hours.

Yeast inoculum was first grown on a sterilized solution of 825 milliliters of Louisiana second–crop molasses dissolved in 5 gallons of water which contained urea and phosphate sufficient to produce 40 percent of yeast from the sugar. After 16 hours the yeast was removed from this inoculum by centrifuging and transferred to a fermentor containing 10 gallons of water and 36 gallons of approximately 5 percent wood–sugar solution for further yeast growth. This mixture was stirred and aerated slowly with about 0.7 volume of air per volume of liquid per minute for 16 hours. Then 23 gallons of this inoculum was placed in a fermentor and mads up to 77 gallons with wood–sugar solution. At the end of 20 hours the yeast was removed by centrifuging; about 8 gallons of yeast cream were obtained. This yeast cream was added to the next fermentor and made up to 77 gallons with 5 percent wood–sugar solution. The rates of fermentation for fermentations P39 and P42 are shown in figure 1.

The over–all yield of alcohol based on the total sugar used for yeast produc–tion and alcohol for fermentation P39 was 38.2 percent while that for fermentation P42 vas 37.3 percent. The aeration of fermentation P42 produced a precipitate which made the yeast much darker and may have been responsible for the lower alcohol yield.

The clarified wood-sugar solution was used for two other series of fermentations, series C and series D, involving yeast reuse by transfer of the yeast from a completed fermentation to fresh sugar solutions.

Series C was carried out in 55-gallon batches to which 54 milliliters of Louisiana second-crop molasses had been added to supply growth factors. Fermentations were usually complete in 12 to 14 hours, but the yeast was not removed until the end of 20 hours. This series was continued for 38 transfers. The data for these fermentations are given in table 7. The average values for the 38 transfers were fermentability, 83.8 percent; yield of alcohol from total reducing sugar, 39.6 percent; and yield of alcohol from fermentable sugar, 47.4 percent, after correcting for molasses which had been added.

Series D was similar to series C except that the molasses was omitted. The fermentations required 14 to 16 hours and the yeast was removed by means of a yeast centrifuge at the end of 20 hours and transferred to fresh sugar. This series was continued for 25 Cays. The values for the various fermentations are given in table 8. The average values for the 25 transfers were fermentable sugars, 83.9 percent; yield of alcohol on total reducing sugar, 39.4 percent; and on fermentable sugar, 47.0 percent.

In both series C and D, fermentations started readily and yeast sufficient to replace that lost mechanically was grown during the fermentation. Hourly counts of the yeast indicated that the greatest increase in yeast occurred after the alcoholic fermentation was complete. The average values of these fermentations are compared with other fermentations in table 5.

Fermentation of Solutions Produced Under Milder Hydrolysis Conditions

Another variable studied was the use of better control in the production of the sugar. It has been noted that excessive temperatures or long holding periods in the production of the sugar from wood resulted in the formation of large amounts of tar-like material. It was believed that the same reaction which produced tar may also produce substances that affect the fermentability of the hydrolysates.

Wood hydrolysate, which had been produced under conditions such that the minimum amount of tar was produced as described in Forest Products Laboratory Report No. R1475 (1945) was subjected to 7 liter, (1.85 gals.) 55-gallon and 300-gallon fermentations. The results of these series are shown in table 5.

Tho 7-liter fermentation series shown as series N in table 5 was carried through 14 transfers of the past. (Table 9) Tests were made in duplicate, two starting with 0.5 percent and two with 1.0 percent yeast using 5 percent sugar solutions. Yeast was removed from the fermented solutions at the end of 20 hours by centrifuging and was added with 700 milliliters of fermented liquor to 6,300 milliliters of hydrolyzate for the next fermentation. Urea and phosphate sufficient to permit a 20 percent increase in yeast were added to each fermentation. The change in the sugar utilized and in the alcohol produced by 0.5 percent and 1.0 percent yeast volumes when carried through 14 transfers is shown in figure 2. There was only a slight difference in the utilization of sugar between the fermentors with 0.5 percent volume of yeast and those with 1.0 percent. Sugar utilization increased gradually and reached a maximum at about the thirteenth transfer,

The change in yeast–cell count during the fermentation is shown in figure 3. In the first fermentation the cells retained a considerable amount of the growth rate exhibited during their propagation on molasses and continued to increase rapidly. The inhibiting substances in the wood hydrolyzate decreased this rate of growth in the second transfer and for the next four transfers remained practically constant. After the sixth transfer the yeast became acclimated and began to increase. By the tenth to twelfth transfer the yeast had developed sufficiently to promote a good utilization of the sugar.

During the sixth transfer samples were taken each hour to determine the rate of sugar utilization. Each of the curves in figure 4 shows the averages of 2 fermentations; one curve represents fermentations started with 0.5 percent yeast and the other with 1.0 percent yeast. All four fermentations were complete in 12 to 16 hours but were allowed to stand to the end of 20 hours. This fact may, therefore, account for the small variations in sugar utilization between 0.5 percent and 1.0 percent yeast as shown in figure 2. The fermentability of the sugar and the yield of alcohol for the N series in the first and fourteenth transfers are shown in table 5.

Fermentation in 55–gallon Batches

Yeast propagated as in fermentation P20 was used to start two 55–gallon fermentations on the hydrolyzate produced under milder conditions as follows: "A" series on the hydrolyzate alone, and "B" series on the same hydrolyzate but with 54 milliliters of Louisiana second crop molasses (0.025 percent) per fermentor added to provide growth factors.

The contents of the tanks used for fermenting the A and B series fermentations were agitated by an external centrifugal pump which took liquid at a rate of 4 gallons per minute from the bottom of the fermentor tank and returned it to the tank about 6 inches below the surface of the liquid.

Both fermentations started at about the same rate. The yeast was centrifuged from the beer as 10 percent yeast cream. During each transferring process about 20 percent of the yeast was lost either in the beer or mechanically. The yeast suspension, in these fermentations about 5.5 gallons, was placed in the fermentor and, with the pumps running, wood hydrolyzate containing approximately 5 percent reducing sugar, and urea, and phosphate, sufficient to produce a 20 percent increase in yeast, was pumped into the fermentor. The sugar in the solution dropped immediately, due to the absorption on the large volume of yeast and carbon dioxide began to be evolved in about 2 hours. The fermentations (B series) to which the molasses had been added, was about 90 percent completed in 10 hours. The remaining 10 percent required about 3 hours longer for completion. Tho fermentation (A series), which contained no molasses, was 30 percent complete in 14 hours and did not change after 17 hours. Since all transfers were made after 20 hours, sufficient time had elapsed for complete utilization of the fermentable sugar.

The series A fernentation was continued for 59 days; the values for the first 24 days are given in table 10. The average values for 59 transfers were fermentable sugars, 83.5 percent; yield of alcohol from total reducing sugar, 39.3 percent'; and. from fermentable sugar, 47.2 percent.

The series B fermentation was continued for 25 transfers. The values after correcting for the molasses added are given in table 11.

Yeast growth in the A and B series was adequate to maintain a good rate of fernentation in spite of losses suffered in transferring.

Fermentation in 300-gallon Batches

The hydrolyzate produced under milder conditions was fermented in 300-gallon batches in the L series. Yeast from the A, B, C, and D series fermentations, suspended in 28 gallons of fermented liquor, was placed in a 500-gallon steel tank with a side stirrer and while stirring, 270 gallons of Douglas-fir wood hydrolyzate containing approximately 5.2 percent reducing sugar was added. The quantity of alcohol did not increase after 18 hours although only 78.4 percent of the total reducing sugar was utilized. After 20 hours the yeast was removed as a cream in 30 gallons of liquor in a yeast separator. The yeast cream was returned to the 500-gallon fermentor and 270 gallons of fresh sugar was added. The series was continued for 31 days, transferring the yeast to new hydrolyzate containing 5.0 to 5.6 percent reducing sugar after 20 hours of fermentation. The concentration of alcohol produced ranged from 1.90 to 2.2 grams per 100 milliliters.

The amount of stirring was somewhat less than that used in the 55 and 75 gallon fermentations and the yeast growth during the fermentation was less. Fermentation required 18 hours and did not have time for the yeast to grow after the alcoholic fermentation, a3 was tho case in other series. About 15 percent of the yeast was lost during centrifuging and transferring. Because of the limited growth of yeast, there was no opportunity to increase the yeast except by adding new yeast. The cell count was increased to 120 million per milliliter at the seventeenth transfer, which promoted a more rapid fermentation and a slight increase in the reducing sugar utilized but there was very little increase in the alcohol produced. The average values for tho 31 transfers were fermentable sugar, 81.1 percent; yield of alcohol On total sugar, 39.4 percent; and on fermentable sugar, 48.2 percent.

A summary of the values of the fermentation on clarified hydrolyzate and on the hydrolyzate produced under more carefully controlled hydrolysis conditions is shown in table 5.

It is demonstrated in this study involving 196 fermentations that sugar utilization and alcohol production is improved by acclimitization and continued transfer of the yeast. These 196 fementations have been carried out without bacterial or mold contamination or evidence of contamination with other yeasts.

In the pilot plant work involving the 55–gallon and 300–gallon fermentations, the amount of fermented liquor to be centrifuged was too small to obtain satisfactory results with the yeast separator available. It is believed that with larger quantities and larger separators better adapted for the work that higher yeast counts can be maintained and that 8- to 16–hour fermentations in large batches can be expected,

Using 2 percent yeast (dry basis), or approximately 4 percent wet yeast volume, fermentations have been completed in 5 to 6 hours (forumtations P24 and P25, table 3). The ability to separate and return high quantities of yeast has been difficult in pilot plant work. Further work on higher rates of fermentation is in progress.

Distillation of Fermented Solutions

Fermented liquors containing approximately 2 grams of alcohol per 100 milliliters were distilled in a 50–gallon still equipped with a 4-inch bubble–cap–plate column. Ninety–eight percent of the alcohol was obtained in a liquor containing approximately 70 percent alcohol. This 70 percent alcohol was further fractionated by distilling in a 5–foot, 25 millimeter glass laboratory fractionating column packed with glass helices. In this distillation a 20 to 1 reflux ratio was used. A volume equivalent to 0.3 percent of the alcohol present in the still distilled as a yellow liquid before the temperature of 78° C. was reached. Samples of this low–boiling material are being collected for further examination.

The alcohol had a strong odor but this was reduced by the addition of a small amount of alkali. Ten millimeters of alcohol, which had been treated with alkali, did not reduce 1 cubic centimeter of 0.1 percent potassium permanganate in 20 minutes.

The fusel oil remaining in the flask after distilling off the alcohol was separated by salting out and extraction, and was equivalent to 0.31 percent of the alcohol produced. Further work on the composition of this material is in progress.

Pilot–Plant Distillation

In order to obtain information concerning the behavior of alcohol solutions when subjected to distillation in a pressure still, 1,200 gallons of fermented liquor containing 1.99 grams of alcohol per 100 milliliters was shipped to the Vulcan Copper and Supply Co., Cincinnati, Ohio and distilled in their pilot plant 61 plate, bubble-cap column which is 4 inches in diameter. The following information is from their report on this distillation.

The same still was used for both stripping and refining operations. The stripping operation was carried out with a steam pressure of 17.8 pounds per square inch gage pressure at the bottom, Tho feed was preheated to 107° C. and fed into the still at an average rate of 121 pounds per hour. The steam rate was 17.6 pounds per hour. Alkali was fed in at the rate of 0.13 pounds per hour. Alcohol distilled over at the rate of 2.38 pounds per hour. Reflux rate was 18.6 pounds per hour and the spent beer rate was 135 pounds per hour. Ethanol content of the spent beer was 0,022 percent. The alcohol obtained was 189.5 proof.

Some losses resulted because of variations in making adjustments in the distillation equipment but a satisfactory balance between input and yield was found. No appreciable amounts of fusel oil were obtained.

The same still was set up for the purification of the crude 189.5 proof alcohol. The distillation was made at atmospheric pressure. The crude alcohol was preheated to 71° C. and fed in at a rate of 8.9 pounds per hour at the thirtieth plate. Steam was supplied to an external calandria at the bottom of the column at a rate of 8.3 pounds per hour. Reflux was at a rate of 14.44 pounds per hour. The amount of heads was so small that it was not possible to draw off any until the still had been in operation for 7 hours, after which a quantity of heads equivalent to about 0.5 percent of the alcohol was removed.

These heads were distilled in a glass still and found to contain about 50 percent ethanol, 25 percent methanol, 10 percent acetaldehyde, and 15 percent a yellow-colored material having come of the properties of diacetyl but which boiled just below ethanol. It must have been a constant-boiling mixture of diacetyl with ethanol. Treatment with a small amount of permanganate or strong caustic removed the material.

Alcohol removed from the base of the purifying column was treated with varying amounts of 5 percent potassium permanganate for further purification. Treatment of the rectified alcohol with 0.2 milliliter of 5 percent potassium permanganate per 100 milliliters of alcohol and distilling gave a product that remained pink for 30 minutes when 10 milliliters of alcohol was tested with 1 milliliter of 0.1 percent potassium permanganate according to the Army Specification F-4f. This material easily meets specifications which require that the color remain for 20 minutes.

Conclusions

Brewer's yeast, S. cerevisiae, No. 49 University of Wisconsin collection, has been propagated on 1 percent wood sugar giving yeast yields of 16 to 28 percent in 14 to 18 hours using a 2 to 1 air-to-liquid ratio.

Yeast produced on wood-sugar solutions converted 5 percent wood sugar into ethyl alcohol. When 2 percent of yeast by dry weight was used, fermentation was complete in 5.5 hours whereas 0.5 percent yeast required 24 hours.

Continuous transfer of yeast from an 18-to-20 hour fermentation to fresh 5 percent sugar solution has been continued through as many as 59 transfers without loss of alcohol yield and without contamination from bacteria or other yeast.

The addition of 0.025 percent of Louisiana second-crop molasses to the fermentation increases the rate of fermentation but does not effect the yield after correction has been made for molasses when transfers are made after 18 to 20 hours.

The use of wood-sugar solutions clarified by the addition of aluminum sulfate or of solutions prepared under operating conditions which produced less tar improved the rate of growth of yeast.

Yeast growth during fermentation was sufficient to overcome a loss of about 20 percent during each transfer.

Alcohol yields in six series of continuous transfers ranged from 39.2 to 40 percent of the total sugar and from 47.0 to 47.9 percent of the fermented sugar. When 5 percent solutions were used, yields of 95 percent alcohol were 2.64 gallons per 100 gallons of hydrolyzate and 64.5 gallons per ton of dry, bark free wood.

Large samples of the fermented sugar solutions have been distilled in pilot-plant equipment. The alcohol produced was of high quality. Quantities Of heads and fusel oil were unusually low.

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Table 1.--Growing of yeast on wood sugar using slow-feed addition of sugar solution

Fermentation No.	Wood-sugar inoculum: Sugar solution added to 6.8 gallons: Amount	Concentration	Air	Alcohol concentration	Yeast concentration	Fermentation No.	Growing yeast: Sugar solution added to inoculum: Amount	Concentration	Air-liquid ratio	Sugar used	Yield: Alcohol	Yield: Yeast	Time allowed
	Gallons	Percent	Cu. ft. per min.	Percent	Percent		Gallons	Percent		Percent	Percent	Percent	Hours
P4	33.2	1.19	4.0	0.00	0.68	P4	340	1.19	0.2:1	76	26	5.9	7.5
P5	45.2	1.69	4.0	.35	.62	P5	313	1.69	.2:1	82	25	8.3	7.0
P6	28.2	2.34	4.0	.34	.90	P6	325	2.34	.2:1	72	24	6.4	8.0
P7	28.2	3.54	4.0	.74	.66	P7	240	3.54	.2:1	59	16	3.4	8.0

Table 2.--Growing of yeast on higher concentration of wood sugar in 48-gallon batches

Fermentation No.	Inoculum: Yeast	Sugar solution added: Concentration	Sugar solution added: Method	Air-liquid ratio	9 hours: Sugar used	9 hours: Yield[1]: Alcohol	9 hours: Yield[1]: Yeast	24 hours: Sugar used	24 hours: Yield[1]: Alcohol	24 hours: Yield[1]: Yeast	Calculated time for completion
	Pound	Percent			Percent	Percent	Percent	Percent	Percent	Percent	Hours
P11	0.2	3.49	Slow feed	0.2:1	60.0	28.8	2.85	83.5	32.0	8.2	11
P8	.17	3.39	....do.....	.5:1	44.5	17.3	4.2	84.5	25.7	12.7	12
P9	.20	3.41	....do.....	1.0:1	47.5	16.8	2.9	84.0	12.6	16.2	14
P12	.19	3.42	....do.....	2.0:1	28.0	9.3	4.2	79.5	17.1	28.0	15
P13	.17	3.39	Slow feed and agitation......	.5:1	61.8	23.1	7.2	86.2	24.6	17.9	18
P14	.22	3.26	Batch.....	.5:1	38.0	17.6	4.4	86.0	30.5	15.25	15
P15	.26	3.33	....do.....	.2:1	25.0	5.7	4.5	82.7	29.0	11.5	Incomplete
P16	.31	3.31	....do.....	.5:1	52.4	18.7	6.3	83.3	23.0	13.3	12
P17	.31	3.33	....do.....	.8:1	78.5	25.5	8.6	85.0	6.6	19.2	10
P18	.36	3.18	....do.....	1.4:1	66.5	21.6	8.3	83.5	22.2	20.8	11

[1]Yield calculated on total sugar.

Table 3.--Growing of yeast and alcoholic fermentation

Aerobic growing of yeast

Fermentation No.	Inoculum: Yeast	Water added	Sugar solution added: Amount	Sugar solution added: Concentration	Sugar solution added: Method	Air-liquid ratio	16 hours: Sugar used	16 hours: Alcohol[1]	16 hours: Yeast[1]
	Pound	Gallons	Gallons	Percent			Percent	Percent	Percent
P20	0.37	....	48	4.90	Batch...	0.5:1	26.5	6.6	3.0
P21	.31	10	38	5.2	...do....	.5:1	77.0	25.4	7.4
P22	.40	10	38	5.28	...do....	.5:1	57.5	18.8	4.8
P24	.37	6	42	5.37	...do....	.8:1	80.0	21.0	9.7
P25	.31	11	38	5.37	...do....	.8:1	78.0	23.8	9.4
P26	.27	10	38	5.28	...do....	.8:1	78.0	23.0	12.8
P29	.25	11	38	5.23	...do....	.8:1	77.0	24.7	10.7
P27	.34	11	37	[2]5.22	...do....	.8:1	79.5	23.8	11.5
P28	.34	11	37	[3]5.18	...do....	.8:1	77.0	23.9	12.0
P30	[4].20	10	38	4.80	...do[5]...	.8:1	83.0	16.8	10.0
P31	[4].28	10	38	4.80	...do[6]...	.8:1	80.0	24.6	12.1
P32	[4].32	13	35	4.80	...do[7]...	.8:1	80.0	23.6	10.2
P33	[4].24	10	38	4.80	...do....	.8:1	78.0	23.4	7.0

Alcoholic fermentation (batch)

Fermentation No.	Wood-sugar inoculum: Amount	Wood-sugar inoculum: Yeast	Sugar solution added: Amount	Sugar solution added: Sugar concentration	Solution fermented: Volume	Yield for varied time periods: Time allowed	Yield for varied time periods: Sugar fermented	Yield for varied time periods: Alcohol[1]	Yield for 24-hour period: Sugar fermented	Yield for 24-hour period: Alcohol[1]	Calculated time for completion
	Gallons	Pounds	Gallons	Percent	Gallons	Hours	Percent	Percent	Percent	Percent	Hours
P20	53	1.0	350	4.98	403	24	17.0	8.9			
P21	53	1.5	347	5.2	400	24	46.5	21.2			
P22	53	1.2	347	5.2	400	40	79.0	38.0			
P24	53	2.0	23	5.37	76	5.5	80.0	39.0	....	....	5.5
P25	53	1.8	23	5.37	76	6.0	81.0	39.5	....	....	6.0
P26	33	1.5	42	5.28	75	7.5	65.0	32.5	....	....	9.5
P29	25	.9	45	5.28	70	8.5	54.5	26.4	82.5	40.0	14.0
P27	33	1.6	42	5.22	75	7.0	71.0	34.4	....	....	8.5
P28	38	1.6	38	5.18	76	6.5	78.0	38.0	....	....	8.0
P30	15	.54	[8]35	4.80	50	6.5	71.5	33.7	82.0	38.0	
P31	15	.65	35	4.80	50	6.5	65.0	29.4	83.0	36.5	
P32	15	.57	35	4.80	50	6.0	61.0	29.0	80.0	39.0	
P33	15	.40	35	4.85	50	6.5	63.7	28.5	83.0	39.6	

[1]Yield based on total reducing sugar.

[2]Molasses added: 1.3 pound.

[3]Malt sprouts added: 1 percent.

[4]Washed yeast used.

[5]Molasses added: 1.6 pound.

[6]Molasses added: 0.63 pound.

[7]Molasses added: 52 milliliters.

[8]Molasses added: 1.4 pound.

M 66091 F

Table 4.--Permentation by continuous transfer of yeast in 7-liter batches using 5 percent reducing sugar in series CT

Transfer No.	Yeast concentration by volume	Fermentable sugar	Alcohol from Total sugar	Alcohol from Fermented sugar
	Percent	Percent	Percent	Percent
1	0.5	75	35.0	47.0
1	.5	77	35.4	46.0
1	1.0	77	34.6	45.0
1	1.0	77	34.0	44.0
1	1.5	77	34.0	44.1
1	1.5	75.5	33.6	44.5
2	.8	80	35.2	44.0
2	.8	80	35.2	44.0
2	1.1	81	35.6	44.1
2	1.1	81	35.4	43.5
2	1.4	82	34.6	43.3
2	1.2	82	35.5	44.0
3	1.0	80	35.6	45.0
3	1.0	80	35.6	45.0
3	1.2	82	35.6	43.5
3	1.2	82	33.5	41.0
3	2.0	84	35.0	41.5
3	2.0	84	35.0	41.5
4	1.0	83	38.6	46.5
4	1.0	83	38.5	46.5
4	1.5	83	39.0	47.0
4	1.5	84	39.0	47.0
4	1.8	84	39.2	46.9
4	2.0	84	39.2	47.0

Table 5.--Fermentation of wood sugars using yeast transfer

Series	Hydrolyzate	Transfer No.	Quantity fermented	Yeast count (cells per milliliter) Start	Yeast count (cells per milliliter) Finish	Sugar used	Alcohol produced based on Total reducing sugar	Alcohol produced based on Fermented reducing sugar
			Gallons	Million	Million	Percent	Percent	Percent
CT	Clarified	1	1.85	30	70	76.0	35.2	44.6
CT	Clarified	1	1.85	120	130	76.5	33.8	44.4
CT	"	4	1.85	72	95	83.0	38.5	46.5
CT	"	4	1.85	130	150	84.0	39.2	47.0
D	Clarified	1	55	44	50	81.3	37.9	46.6
D	"	Average of 25	55	85	110	83.9	39.4	47.0
C		[1]1	55	60	127	83.3	38.3	46.0
C		Average of 38[1]	55	80	100	83.8	39.6	47.4
N	Unclarified	1	1.85	35	80	76.9	34.1	44.0
N	"	1	1.85	72	115	47.3	34.0	44.0
N	"	14	1.85	91	92	81.2	39.7	49.6
N	"	14	1.85	120	130	82.1	40.3	49.8
A	Unclarified	1	55	35	68	79.5	37.0	46.6
A	"	Average of 59	55	85	120	83.5	39.3	47.2
B		[1]1	55	35	68	79.5	37.0	46.6
B		Average of 25[1]	55	85	120	83.8	40.0	47.9
L	Unclarified	Average of 31	300	85	100	81.1	39.4	48.2

[1] 54 milliliters molasses added per fermentor.

Table 6.--Fermentation of clarified wood sugars using yeast transfer (77-gallon batches)

Fermentation No.	Amount fermented	Yeast count (cells per milliliter): Start	Yeast count (cells per milliliter): Final	Sugar used	Alcohol from: Total sugar	Alcohol from: Fermented sugar
	Gallon	Million	Million	Percent	Percent	Percent
P39 Inoculum	38	....	....	79.0	25.8	....
1st Transfer	54	50	101	83.5	40.4	48.4
2nd "	65	71	87	79.3	39.4	49.7
3rd "	69	65	100	84.0	39.0	46.4
P42 Inoculum	38.0	....	....	72.0	23.0	....
1st Transfer	54.0	50	92	85.8	40.7	47.5
2nd "	68.5	74	86	77.4	37.4	48.4
3rd "	60.5	57	93	83.5	38.8	46.4

Table 7.--Fermentation of wood sugars. Series C. (Clarified, 54 milliliters of molasses per fermentation added.)

Transfer No.	Feed	Fermented liquor fed back	Reducing sugar feed	Calculated initial reducing sugar	Final sugar	Unfermented reducing sugar carried forward	Alcohol carried forward	Final alcohol	Yeast count (cells per milliliter) Initial	Yeast count (cells per milliliter) Final	Fermentability	Alcohol on total reducing sugar	Alcohol on fermented reducing sugar
	Gallons	Gallons	Percent	Percent	Percent	Percent	Gm./100	Gm./100	Million	Million	Percent	Percent	Percent
1	54	23	5.28	3.88	0.80	0.26	0.31	1.78	43	84	85.0	40.6	47.7
2	64	12	5.19	4.52	.87	.14	.30	1.98	60	127	83.3	38.3	46.0
3	67	10	5.15	4.75	.89	.11	.26	2.05	78	94	83.3	38.5	46.3
4	68	9	5.25	4.74	.87	.10	.24	2.01	67	175	83.4	38.2	45.8
5	68	9	5.25	4.74	.98	.10	.24	1.99	96	98	81.0	37.7	46.5
6	68	9	4.87	4.41	.84	.11	.23	1.98	77	100	83.0	40.7	49.0
7	66	11	4.78	4.23	.79	.12	.28	1.89	92	80	83.6	39.1	46.8
8	69.5	7.5	4.82	4.43	.89	.08	.18	1.92	63	102	81.4	40.0	49.1
9	71.5	5.5	4.89	4.61	.81	.06	.14	1.90	72	151	83.5	38.7	46.3
10	66.5	10.5	4.85	4.24	.80	.11	.26	1.94	94	141	81.1	40.7	48.8
11	70	7	4.86	4.48	.80	.07	.18	1.92	99	125	83.4	39.4	47.3
12	71	6	4.75	4.43	.82	.06	.15	1.88	89	139	82.5	39.6	47.9
13	48.5	6.5	4.90	4.42	.80	.07	.22	1.88	107	177	83.3	38.2	45.8
14	48	7.0	4.79	4.28	.78	.10	.24	1.89	68	107	83.6	39.4	47.1
15	50	5	4.79	4.43	.81	.07	.17	1.89	78	92	83.0	39.4	47.4
16	49	6	4.87	4.43	.76	.09	.21	1.91	86	111	84.7	39.2	46.3
17	50	5	4.78	4.42	.76	.07	.17	1.91	83	116	84.2	40.0	47.5
18	51	4	4.84	4.54	.78	.06	.14	1.90	93	....	84.0	39.3	46.8
19	51	4	4.37	4.11	.73	.06	.14	1.78	....	80	83.5	40.5	48.5
20	44	11	4.49	4.01	.70	.15	.17	1.76	89	89	85.6	41.2	48.0
21	50.5	4.5	4.35	4.06	.68	.06	.14	1.78	97	97	84.5	41.0	48.5
22	51	4	4.23	3.98	.58	.05	.13	1.74	86	86	86.5	40.9	47.4
23	49.5	5.5	4.23	3.85	.57	.06	.17	1.73	75	89	86.3	41.2	47.7
24	50	5	4.21	3.88	.56	.05	.16	1.67	....	98	86.6	39.4	45.5
25	51	4	4.27	4.01	.62	.04	.12	1.69	72	....	85.4	39.5	47.7
26	50.5	4.5	4.16	3.87	.63	.05	.14	1.67	....	106	84.7	40.0	47.2
27	50.5	4.5	4.18	3.89	.64	.05	.14	1.65	86	104	85.4	39.5	46.4
28	50.5	4.5	4.14	3.86	.64	.05	.13	1.67	68	76	84.5	40.4	47.8
29	50	5	4.18	3.85	.65	.06	.15	1.67	66	68	84.4	40.1	47.5
30	50.5	4.5	4.61	4.28	.80	.05	.14	1.76	51	63	82.2	38.3	46.6
31	50	5	4.56	4.22	.85	.07	.16	1.74	52	71	81.3	38.3	47.2
32	51	4	4.55	4.27	.75	.06	.13	1.78	56	141	83.5	39.2	46.9
33	50.5	4.5	4.61	4.29	.81	.06	.15	1.75	88	109	82.5	37.9	46.0
34	50.5	4.5	4.39	4.11	.74	.07	.14	1.80	77	92	83.6	41.1	49.4
35	50	5	4.54	4.20	.75	.07	.16	1.78	85	111	83.7	39.2	46.9
36	49	6	4.56	4.15	.75	.08	.19	1.79	93	139	83.5	39.3	47.1
37	49.5	5.5	4.49	4.11	.76	.07	.16	1.79	96	115	83.1	40.4	48.6
38	49.0	6.0	4.78	4.34	.71	.08	.20	1.96	96	166	82.7	41.3	48.5
										Average	83.8	39.6	47.4

M 66092 F

Table 8.--Fermentation of wood sugars. Series D (Clarified)

Transfer No.	Feed	Fermented liquor fed back	Reducing sugar feed	Calculated initial reducing sugar	Final sugar	Unfermented reducing sugar carried forward	Alcohol carried forward	Final alcohol	Yeast count (cells per milliliter) Initial	Yeast count (cells per milliliter) Final	Fermentability	Alcohol on total reducing sugar	Alcohol on fermented reducing sugar
	Gallons	Gallons	Percent	Percent	Percent	Percent	Gm./100	Gm./100	Million	Million	Percent	Percent	Percent
1	37	18	4.79	3.95	0.95	0.26	0.18	1.58	44	50	81.3	37.9	46.6
2	49.5	5.5	4.76	4.38	.85	.10	.16	1.80	56	103	82.5	38.3	46.4
3	49.5	5.5	4.92	4.49	1.19	.09	.18	1.68	80	92	75.0	34.1	45.4
4	50	5	4.80	4.46	.77	.11	.15	1.89	110	118	84.8	40.0	47.1
5	50	5	4.77	4.42	.78	.07	.17	1.90	103	....	83.7	39.8	47.5
6	50	5	4.37	4.03	.76	.07	.17	1.77	....	141	82.5	40.5	48.8
7	48.5	6.5	4.48	4.23	.70	.08	.08	1.75	89	119	85.1	40.2	47.3
8	49.5	5.5	4.30	3.95	.66	.07	.18	1.76	100	115	84.8	40.7	48.1
9	49	6	4.15	3.77	.59	.07	.19	1.72	83	93	86.0	41.4	48.2
10	50.5	4.5	4.20	3.91	.55	.05	.14	1.71	69	89	87.0	40.7	46.7
11	49	6	4.24	3.84	.59	.06	.19	1.68	....	96	86.0	39.4	45.8
12	47	8	4.24	3.73	.60	.09	.24	1.66	87	....	86.0	39.0	45.4
13	50.5	4.5	4.17	3.87	.62	.05	.14	1.64	....	112	85.2	39.3	46.2
14	50	5	4.18	3.85	.62	.06	.15	1.62	88	115	85.4	38.3	45.5
15	49	6	4.12	3.75	.66	.07	.17	1.66	86	95	84.0	40.5	48.2
16	49.5	5.5	4.13	3.81	.65	.07	.17	1.66	78	108	84.5	39.8	47.1
17	48	7	4.59	4.10	.76	.08	.21	1.75	71	93	82.9	38.3	46.1
18	50.5	4.5	4.56	4.26	.77	.06	.14	1.77	76	112	83.1	38.8	46.6
19	49.5	5.5	4.61	4.22	.68	.08	.18	1.78	100	128	85.6	38.7	45.2
20	50.5	4.5	4.61	4.29	.80	.06	.15	1.74	97	100	82.6	37.7	45.5
21	50.5	4.5	4.42	4.13	.73	.07	.14	1.79	77	88	83.6	40.6	48.5
22	52	3	4.53	4.30	.74	.04	.10	1.78	92	100	83.2	39.4	47.2
23	50.5	4.5	4.54	4.22	.76	.06	.15	1.76	86	91	83.1	38.7	46.6
24	50.5	4.5	4.46	4.14	.75	.06	.14	1.78	109	104	83.1	40.2	48.4
25	50	5	4.75	4.38	.70	.07	.16	1.94	88	71	85.4	41.3	48.4
										Average	83.9	39.4	47.0

M 66093 F

Table 9.--Fermentation of 5.0 percent wood sugar in 7-liter batches (6.300 Milliliters wood sugar, 700 milliliters fermented liquor fed back)

Transfer No.	Fermentor No. 1[1]			Fermentor No. 2[1]			Fermentor No. 3[2]			Fermentor No. 4[2]		
	Fermented sugar	Yield alcohol		Fermented sugar	Yield alcohol		Fermented sugar	Yield alcohol		Fermented sugar	Yield alcohol	
		(3)	(4)		(3)	(4)		(3)	(4)		(3)	(4)
	Percent											
1	76.9	33.8	44.0	76.9	34.3	44.5	77.1	33.6	43.5	77.4	33.6	43.5
2	77.0	34.4	44.5	77.5	34.2	44.1	77.8	34.4	44.3	77.8	33.6	43.2
3	77.0	33.7	42.9	77.2	35.3	45.2	77.7	34.0	43.8	78.0	34.6	44.4
4	77.7	35.2	46.6	78.5	35.7	45.7	77.2	35.2	45.5	77.1	35.6	46.2
5	78.0	35.4	45.4	78.0	35.4	45.5	77.8	34.6	43.9	78.6	34.6	43.6
6	78.5	35.4	45.1	78.5	35.0	44.6	80.6	36.5	45.3	82.1	37.5	45.6
7	74.9	33.2	44.4	75.1	33.3	44.6	79.3	35.4	44.6	81.2	36.7	45.2
8	78.4	35.0	44.5	79.5	35.3	44.4	82.5	36.5	44.4	82.6	36.1	43.7
9	83.9	34.8	42.5	82.3	36.1	43.9	84.8	37.8	44.6	82.0	36.9	44.9
10	84.5	37.0	43.7	84.2	36.9	43.8	81.0	36.4	46.3	81.3	37.1	45.7
11	86.0	36.2	45.1	82.0	36.5	44.4	84.4	38.0	45.1	83.9	37.9	45.3
12	83.6	39.1	47.0	79.6	39.2	49.1	85.2	39.5	46.4	83.4	39.4	47.3
13	83.5	39.6	47.3	82.1	40.0	48.6	81.6	40.0	49.0	82.6	39.5	47.7
14	80.2	39.2	49.6	81.2	40.3	49.6	82.1	40.3	49.0	82.1	40.0	48.7

[1] Fermentation started with 0.5 percent yeast.

[2] Fermentation started with 1.0 percent yeast.

[3] Yield of alcohol on total reducing sugar.

[4] Yield of alcohol on fermentable sugar.

Table 10.--Fermentation of wood sugars. Series A

Transfer No.	Feed	Fermented liquor fed back	Reducing sugar feed	Calculated initial reducing sugar	Final sugar	Unfermented reducing sugar carried forward	Alcohol carried forward	Final alcohol	Yeast count (cells per milliliter) Initial	Yeast count (cells per milliliter) Final	Ferment-ability	Alcohol on total reducing sugar	Alcohol on fermented reducing sugar
	Gallons	Gallons	Percent	Percent	Percent	Percent	Gm./100	Gm./100	Million	Million	Percent	Percent	Percent
1	37	18	4.75	3.93	1.01	0.26	0.18	1.54	35	68	79.5	37.0	46.6
2	50.5	4.5	4.79	4.48	.95	.08	.13	1.78	43	77	80.2	37.5	46.7
3	50	5	4.81	4.46	.81	.09	.16	1.86	59	74	83.5	38.9	46.6
4	49	6	4.73	4.31	.80	.09	.20	1.88	74	93	83.1	39.8	47.8
5	50.5	4.5	4.73	4.42	.79	.07	.15	1.87	88	....	83.5	39.5	47.4
6	50.5	4.5	4.27	3.99	.76	.06	.15	1.74	....	112	82.2	40.4	49.3
7	50	5	4.19	3.88	.62	.07	.16	1.75	93	76	85.5	41.7	48.7
8	51	4	4.24	3.97	.72	.05	.13	1.61	83	121	82.9	37.7	45.6
9	51.5	3.5	4.12	3.91	.59	.05	.10	1.68	80	98	86.0	40.9	47.6
10	49.5	5.5	4.18	3.82	.56	.06	.17	1.69	....	92	86.7	40.4	46.6
11	49	6	4.15	3.80	.65	.06	.18	1.69	96	....	84.2	40.4	47.9
12	49.5	5.5	4.21	3.84	.66	.07	.17	1.68	....	96	84.3	40.0	47.4
13	50	5	4.15	3.85	.67	.05	.14	1.64	76	109	83.7	39.5	47.2
14	49.5	5.5	4.16	3.82	.64	.07	.16	1.67	89	117	85.0	40.3	47.5
15	49.5	5.5	4.16	3.82	.66	.06	.17	1.67	112	109	84.1	39.9	47.4
16	49	6.0	4.09	3.73	.70	.07	.18	1.62	97	115	82.8	39.3	47.5
17	51	4.0	4.08	3.84	.72	.05	.12	1.57	100	129	82.3	38.2	46.4
18	52	5.0	4.06	3.77	.62	.06	.14	1.59	100	124	85.0	39.1	46.0
19	51.5	4.5	4.06	3.78	.69	.05	.13	1.55	84	121	83.0	38.1	45.9
20	50	5	4.62	4.25	.82	.06	.14	1.84	99	115	82.0	40.6	49.6
21	51	4	4.79	4.50	.85	.06	.13	1.85	87	99	83.5	38.8	47.1
22	52	3	4.83	4.62	.84	.05	.10	1.77	76	99	82.6	36.5	44.2
23	49	6	4.68	4.25	1.02	.09	.19	1.77	100	92	77.5	38.0	48.9
[1]24	56.5	3.5	4.80	4.59	.72	.06	.10	1.98	67	77	85.5	41.6	48.6
Average of 24 transfers											83.3	39.34	47.3
Average of 59 transfers											83.5	39.3	47.2

[1] 25 additional transfers are not tabulated.

ZM 66094 F

Table 11.--Fermentation of rood sugars. Series B. (54 milliliters of molasses per fermentation added.)

Transfer No.	Feed	Fermented liquor fed back	Reducing sugar feed	Calculated initial reducing sugar	Final sugar	Unfermented reducing sugar carried forward	Alcohol carried forward	Final alcohol	Yeast count (cells per milliliter) Initial	Final	Fermentability	Alcohol on total reducing sugar	Alcohol on fermented reducing sugar
	Gallons	Gallons	Percent	Percent	Percent	Percent	Gm./100	Gm./100	Million	Million	Percent	Percent	Percent
1	37	18	4.84	3.98	0.94	0.26	0.18	1.58	29	44	81.6	37.6	46.0
2	50	5	4.78	4.43	.84	.09	.14	1.82	59	89	82.9	38.7	46.8
3	50.5	4.5	4.80	4.46	.82	.07	.15	1.89	81	80	82.9	39.6	47.8
4	51	4	4.73	4.46	.83	.06	.14	1.90	86	116	82.5	40.0	48.5
5	50	5	4.73	4.38	.83	.08	.17	1.90	82	....	82.5	40.2	48.7
6	51	4	4.27	3.96	.74	.06	.14	1.79	....	87	82.6	42.3	51.2
7	50.5	4.5	4.15	3.88	.71	.06	.15	1.77	85	85	83.0	42.4	51.1
8	50.5	4.5	4.09	3.80	.55	.06	.14	1.72	86	97	86.9	42.3	48.6
9	50	5	4.18	3.85	.55	.05	.16	1.70	91	90	86.9	40.6	46.6
10	49	6	4.23	3.82	.55	.06	.17	1.72	77	89	87.0	41.3	47.4
11	50	5	4.23	3.88	.56	.05	.16	1.72	....	86	86.6	40.7	47.0
12	51.5	5.5	4.15	3.80	.64	.06	.17	1.70	73	....	84.5	40.9	48.4
13	51	4	4.19	3.94	.63	.05	.12	1.68	....	86	85.2	40.1	47.2
14	50.5	4.5	4.10	3.82	.68	.05	.14	1.65	77	97	83.4	40.1	48.1
15	50.5	4.5	4.10	3.82	.67	.06	.13	1.68	74	89	83.9	41.2	49.1
16	50.5	4.5	4.15	3.87	.63	.05	.14	1.66	80	83	84.8	39.8	46.9
17	50.5	4.5	4.11	3.83	.71	.05	.14	1.59	71	82	82.5	38.3	46.5
18	50.5	4.5	4.08	3.81	.72	.06	.13	1.57	60	67	82.0	38.4	46.5
19	51	4	4.08	3.84	.68	.05	.11	1.61	41	119	83.4	39.5	47.4
20	49.5	5.5	4.06	3.73	.73	.07	.16	1.56	81	91	82.0	38.3	46.6
21	50	5	4.63	4.29	.91	.07	.14	1.79	74	106	80.0	39.1	48.9
22	51	4	4.79	4.47	.87	.07	.13	1.87	96	111	81.9	39.5	48.4
23	49	6	4.78	4.34	.83	.07	.20	1.89	76	117	82.6	39.8	48.2
24	50	5	4.77	4.42	.84	.08	.17	1.88	74	100	82.6	39.2	47.8
25	51	4	4.80	4.51	.72	.06	.14	1.99	97	110	85.1	41.6	48.8
										Average	83.8	40.06	47.9

Z M 66095 F

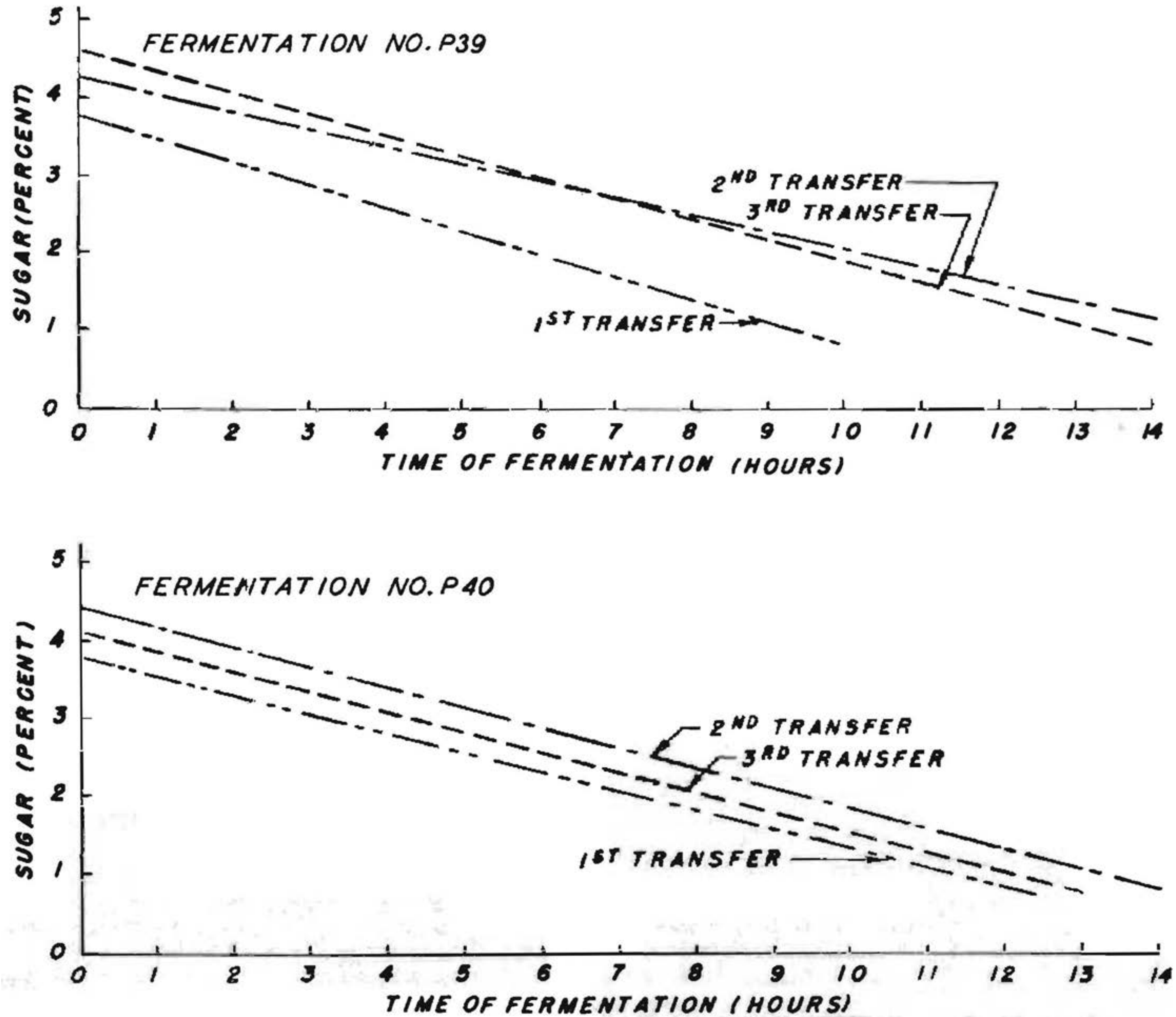

Figure 1.--Rate of utilization of wood sugar by fermentation.

Z M 65753 F

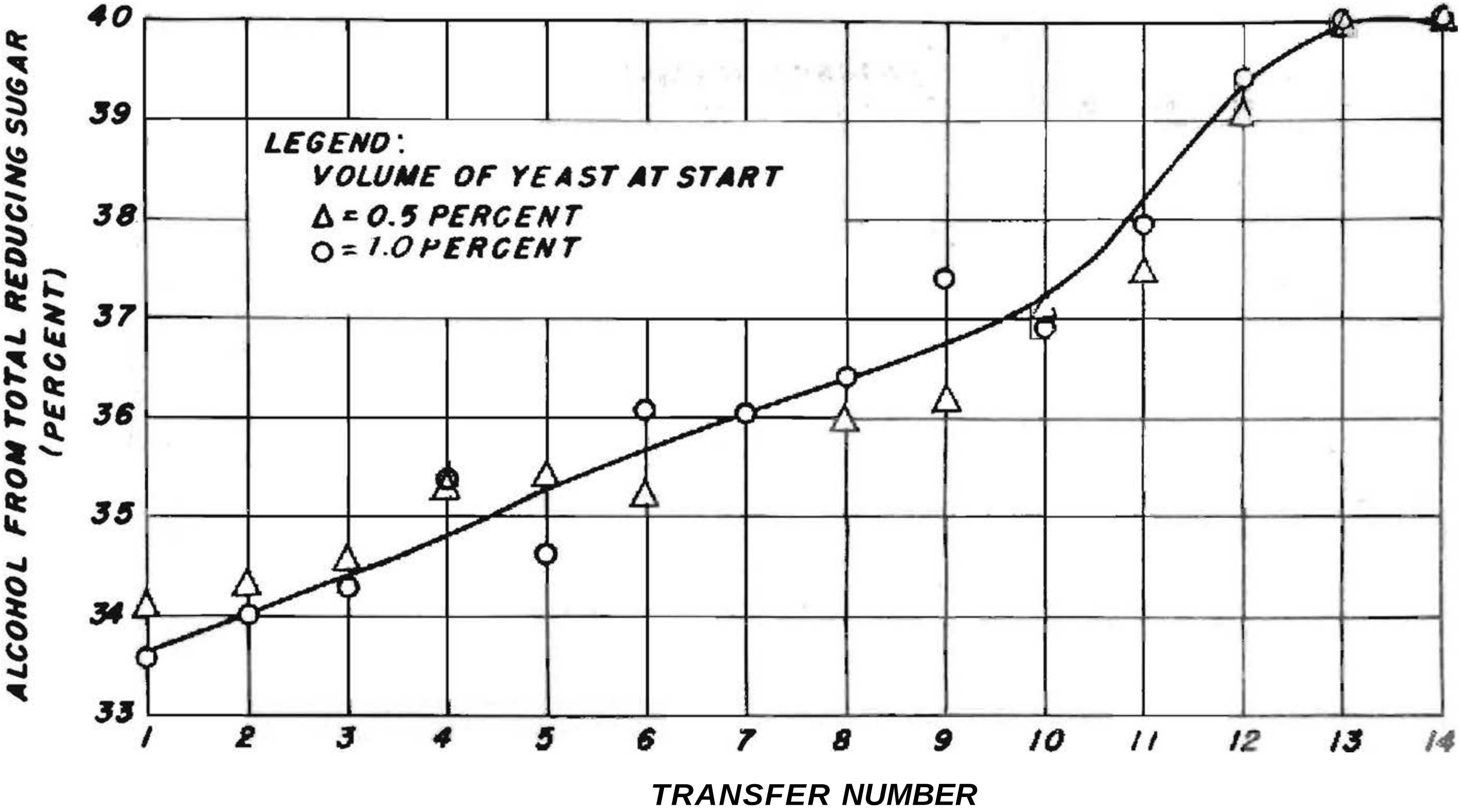

Figure 2.--Alcohol production in the series N fermentations of Douglas-fir wood hydrolyzate by continuous transfer using 6,300 milliliters of 5 percent reducing sugar and 700 milliliters of fermented liquor.

Z M 65754 F

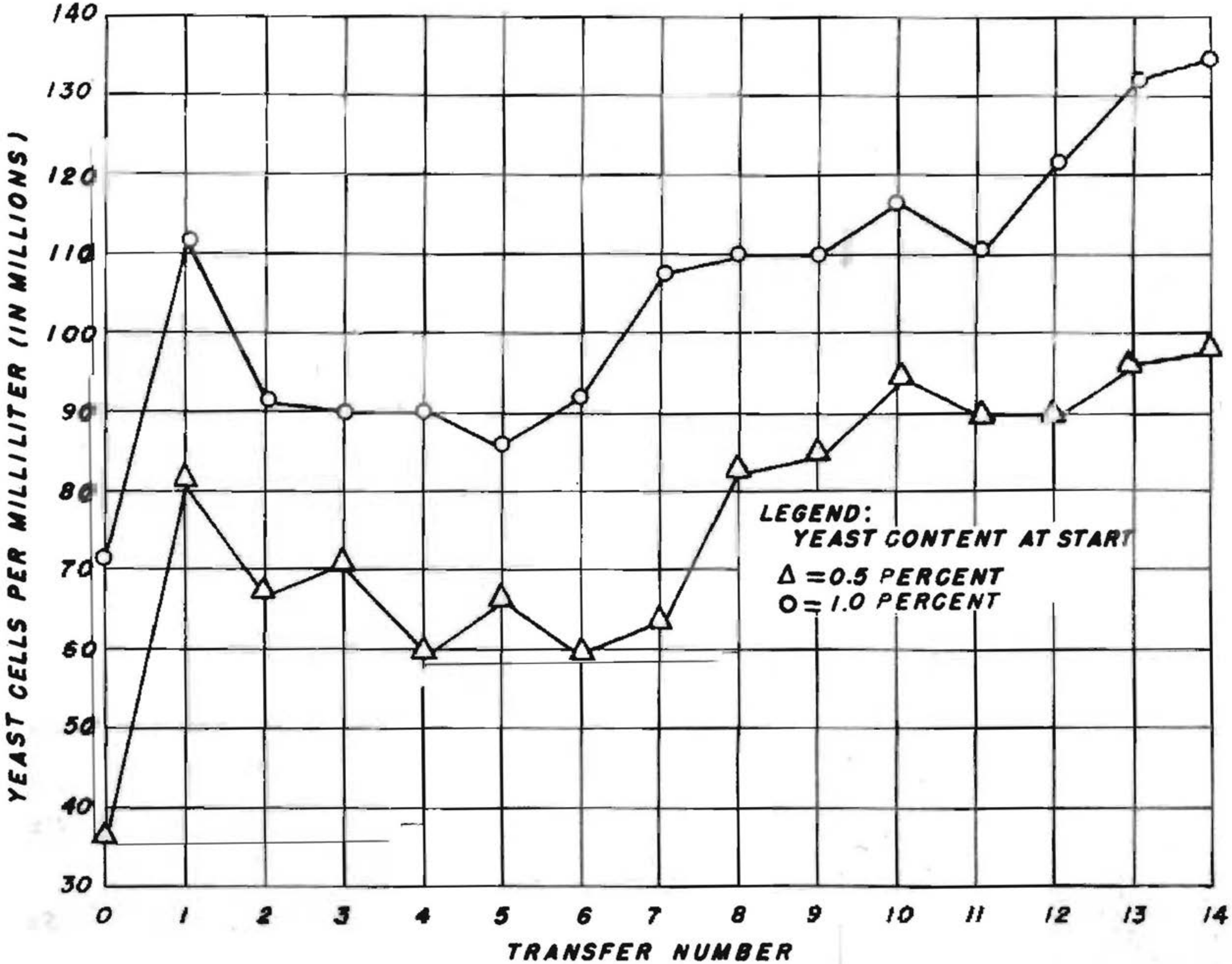

Figure 3.--Yeast cells per milliliter in the series N fermentations of Douglas-fir wood hydrolyzate by continuous transfer in 7-liter fermentation.

Z M 65755 F

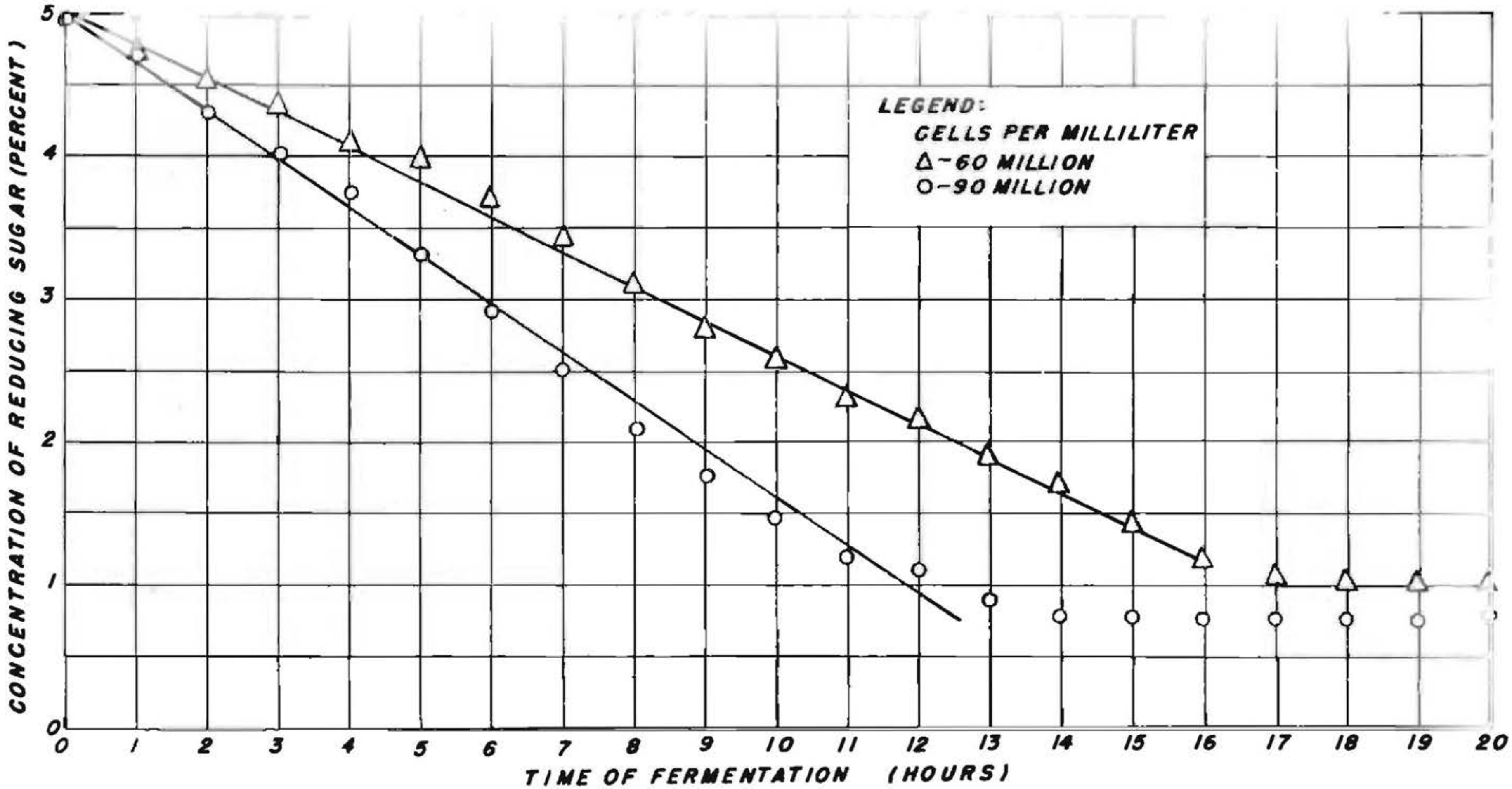

Figure 4.--Rate of fermentation in the sixth transfer in the series N fermentations of Douglas-fir wood hydrolyzate by continuous transfer in 7-liter fermentation.

Z M 65756 F