FOOD-YEAST PRODUCTION FROM WOOD-PROCESSING BYPRODUCTS

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FOOD-YEAST PRODUCTION FROM WOOD-PROCESSING BYPRODUCTS[1,2]

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Food yeast, which may be produced from wood carbohydrates and inorganic salts, is a source of protein, fats, and vitamins. The yeast contains about 50 percent of protein and 2 to 7 percent of fat, and is produced in yields of 40 to 50 percent of the carbohydrate from which it is grown. This yield represents a conversion factor of carbohydrates to protein of 4 or 5 to 1, as compared with a ratio of about 20 to 1 when carbohydrate is fed to pigs. The time of conversion is short and depends on the type of propagator.

Prior to 1943, very little experimentation had been carried out with food yeast in the United States, but the phrase "beefsteak yeast" appeared frequently in press reports originating in Germany. The shortage of protein food and the ease with which food yeast could be produced on various carbohydrate sources were responsible for the development of a food-yeast industry in Germany. Reports on German food-yeast production were prepared by American and other investigators who visited Germany at the end of World War II (2, 16, 21, 23, 25, 39).[4]

Food yeast was produced from several sources in Germany, the most important being sugars obtained by the hydrolysis of wood. Sugars were made by complete hydrolysis, as in the Scholler (33) or Bergius (1) process; from a prehydrolysis of woods such as beech, the residue of which was then used for the production of pulp; and as a byproduct of the hydrolysis that occurs in the sulfite pulping of wood.

At the end of the war, five plants were producing food yeast from sugar obtained by acid hydrolysis of wood (25). They were the Bergius wood-sugar plants at Regensburg and Mannheim, and the Scholler plants at

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[4]Underlined numbers in parentheses refer to Literature Cited.

Dessau, Holzminden, and Tornesch. Their production was about 9,000 tons per year. Hydrolyzate from 1 ton of wood produced 200 to 400 pounds of yeast. A plant for the prehydrolysis of wood or straw at Wittenberg had a rated capacity of 20,000 tons of yeast per year. Four tons of straw treated in this way were expected to yield 1 ton of high alpha pulp and one-half ton of yeast.

The organism used most frequently for food-yeast production was a strain of Torula utilis. After acclimatization, this organism uses hexose and pentose sugars, acetic acid, and possibly other organic compounds. Other strains used in Germany were Torula pulcherima, Monilia candida, and Candida arborea. Considerable advantage was claimed for the latter because the new yeast buds adhere to the parent cell, making the aggregate larger and therefore easier to separate from the spent solutions.

When food yeast is produced on any form of wood hydrolyzate, aeration produces excessive foaming. Two yeast propagators were designed to control the foaming. The Vogelbush propagator was a discontinuous type provided with external tubes extending from the bottom to the top of the yeast container. Air introduced into the tubes at intervals circulated the media and broke the foam. At Holzminden, antifoam was required in amounts equivalent to about 10 percent of the weight of the yeast produced.

The Waldhof continuous propagator made use of a draft tube in the center. Air was introduced through a spinner, which also served as an agitator to circulate the media. In this propagator advantage was taken of the foaming tendency to obtain intimate contact between the air and the media. The contents of the propagator foamed to about three times the actual liquid content. In this condition equilibrium was reached and good foam control achieved without the use of antifoam. Sugar solution with nutrients was introduced continuously and yeast and spent liquor was removed continuously. The yeast was defoamed in a centrifugal foam breaker, separated in a yeast separator, washed, and dried. The Waldhof propagator, thus, appeared to be the more satisfactory of the two types for food-yeast production.

Yeast propagation from wood sugars in the Waldhof-type propagator in Germany required 1.1 kilowatt of electricity, 2.7 pounds of steam, and about 10 gallons of cooling water for each pound of dry yeast produced.

The yeast was used chiefly for humans as a replacement for other protein food.

At the U. S. Forest Products Laboratory, Madison, Wis., research has been conducted on the hydrolysis of wood waste into sugars for fermentation to alcohol (18, 34, 35, 36, 37, 38). In the World War I period, the Laboratory assisted in research for establishing two commercial plants producing alcohol from wood waste. When the price of alcohol fell to a very low value in the 1920s, these investigations and operations were suspended.

In 1943 the Office of Production Research and Development of the War Production Board financed further investigations at the Laboratory on the possibilities of producing alcohol from wood waste (24, 28). In order to get more information on the properties of the sugar, experiments were made concurrently on the growth of yeast on the hydrolyzate.

Preliminary Selection of Organism for Yeast Growth

Peterson and coworkers (22) conducted tests with several organisms to determine those best suited for propagation on wood sugar. The sugar solution was made neutral and 0.05 percent sodium sulfite added and then heated and filtered. Nutrients were added and the solution diluted to about 1.5 percent concentration. Table 1 gives the results of the preliminary study.

The most promising strains were selected for acclimatization. The results of acclimatization in shake flasks for 12 transfers are shown in table 2.

Preparation of the Sugars

Hydrolysis of Wood

Following a study of the kinetics (26, 29) of wood saccharification, several changes were made in the procedure for producing wood sugar (9). These changes made possible higher yields of fermentable sugars and a reduction of the time required for the hydrolysis. The improved process (fig. 1) consists of pumping a stream of 0.5 percent sulfuric acid through a charge of shavings, sawdust, or chipped wood waste at temperatures gradually increasing from 150° to 180° C. The resulting sugars are neutralized to approximately pH 4, cooled, and filtered. The yield of reducing material calculated as glucose averages about 50 percent of the dry, bark-free wood substance. The sugars are present in about 5 percent concentration and are a mixture of pentoses and hexoses. Sugars from softwoods, fermentable to alcohol, are hexoses, while the nonfermentable sugars are designated as pentoses. The yield and type of sugars from various woods are shown in table 3.

Sugars remaining in the hydrolyzate after alcoholic fermentation may also be used for the production of yeast (12). When Douglas-fir hydrolyzate containing 5.0 percent of sugar was subjected to alcoholic fermentation, the remaining reducing material calculated as glucose was about 0.9 percent. If the residual liquors are subjected to yeast propagation, 0.3 percent of reducing material remains. Since these residues after yeast production do not show the presence of sugars by osazone tests, it is concluded that reducing material other than sugar is responsible for this residual reducing material. If the sugars present in the liquor are corrected for nonsugar reducing material, 87 percent of the sugars present are fermentable to alcohol or hexoses and 13 percent to pentoses. Hydrolyzate from southern red oak contains about 16 percent of nonsugar reducing substances. After correcting for these substances, the sugars present are 75 percent hexoses and 25 percent pentoses. Organic acids in the hydrolyzate, such as acetic acid, are utilized for the production of yeast.

Sulfite Waste Liquor

Pulping of wood by the sulfite process yields a pulp representing about 50 percent of the dry wood substance. Softwoods, such as spruce or hemlock, are usually pulped by this process. These woods contain about 67 percent of carbohydrate, 27 percent of lignin, 5.5 percent of extractives, and 0.5 percent of ash. On the basis of carbohydrate about 17 percent of the wood, or 25 percent of the total carbohydrate, is converted into simple sugars or other substances during the process. About half of the hydrolyzed carbohydrate appears as simple sugars in the recovered waste pulping liquor in concentration of 2 to 2.5 percent reducing sugars. These waste pulping liquors are steamed to remove the excess sulfur dioxide and then treated with lime to bring the pH of the solution to 4. Sugars in sulfite waste liquor from spruce or hemlock are about 65 percent fermentable to alcohol. The sulfite waste liquor produced annually contains about 500,000 tons of dissolved sugar.

Prehydrolysis of Wood or Straw Used for Pulp Production

In alkaline pulping processes carbohydrates are also converted into soluble nonpulp products. In the presence of alkali at pulping temperatures the carbohydrates are converted to reversion products that are nonfermentable and not useful for yeast production. When it is desired to produce a pulp with high alpha cellulose content by an alkaline process from wood or straw, the material may be subjected to a prehydrolysis with 0.3 to 0.5 percent acid at 30 to 50 pounds per square inch of steam pressure for about one-half hour. Hemicelluloses representing 15 to 20 percent of the wood and up to 30 percent of straw are made water-soluble. These are extracted in some type of countercurrent extractor designed to give high recovery of the soluble material in as high a concentration as possible. Frequently the hemicellulose is only partially converted to simple sugars. In order to complete the inversion, acid is added until the solution has a pH of 1.2 to 1.5, and it is then given a secondary hydrolysis at 20 to 30 pounds per square inch of steam pressure for 30 minutes. Lime is then added to give the solutions a pH of 4. Calcium sulfate and other precipitated material is removed by filtration. Sugars representing 15 to 20 percent of wood and 25 percent of straw in concentrations of 6 to 8 percent may be recovered from prehydrolysis. Sugars from prehydrolyzed softwoods, such as pine, are about one-half hexoses and one-half pentoses, and those of hardwoods, such as maple or beech, are 20 percent hexose and 80 percent pentose.

Water Hydrolysis of Wood as in Production of Steamed Fiber

Processing of wood in cold water converts small amounts of the carbohydrates into soluble products. As the temperature and length of treatment are increased, the amount of soluble material is increased. Steaming of chips under pressure to soften them for coarse fiber production converts 15 to 30 percent of the dry weight of the wood into soluble products that are lost in subsequent processing.

In one steamed fiber process using hardwood chips, 15 percent of the wood was converted to soluble products. An analysis of the steamed chips showed that they contained approximately 6 percent of soluble material on the basis of the wet chip. This material was removed by countercurrent extraction with hot water, giving a solution with a concentration of 4.3 percent of total solids of which 3.61 percent was organic material. Before hydrolysis with acid, the solution had a reducing sugar value of only 0.85 percent. After being acidified with 0.5 percent sulfuric acid and heated for 15 to 30 minutes at a steam pressure of 15 pounds per square inch, its reducing sugar was increased to 2.78 percent. The solution was then neutralized with lime and filtered. Only 37 percent of the total sugar was fermentable to alcohol, but 92.3 percent was utilized by yeast growth.

Propagation of Yeast on Wood Sugars

Preparation of Inoculum

The yeast used for all yeast production, except when testing other strains for comparison, was *Torula utilis* No. 3, a hardy strain of food yeast obtained from the University of Wisconsin collection and shown by Peterson and coworkers (22) to be the most suitable strain for yeast production on wood sugars. The inoculum was prepared (13) by transferring a small amount of yeast from a glucose malt-sprout agar slant to an 8-inch test tube containing 30 millimeters of a glucose malt-sprout medium that had 5 percent glucose, 5 percent malt-sprout extract, 0.1 percent urea, and 0.05 percent potassium acid phosphate at pH 5.2. This culture was placed in a shaker and incubated at 30° C. for 24 hours, after which the contents of the tube were placed in 150 millimeters of the same medium in a 500-millimeter Erlenmeyer flask. The cultures were again shaken 24 hours at 30° C. Yeast from this flask was used to inoculate wood-sugar solution.

Methods of Sampling and Analysis

Sugar analysis (13) was made by a modification of the method of Schaffer and Somogyi (27, 31). Yeast-cell volume was obtained by centrifuging a 10-millimeter sample in a graduated centrifuge tube for 5 minutes.

Yeast yield was determined by separating the yeast in 10 millimeters of substrate, washing it, drying it for 24 hours, and weighing it. Nitrogen content was determined by the Kjeldahl method.

Batch Propagation of Yeast

Previous batch propagation of yeast by Fink and Lechner (5, 6) and by Peterson, Snell, and Frazier (22) had shown that it was necessary to dilute the wood sugar to a 1 percent concentration in order to produce satisfactory yields of yeast. That procedure was also used in preliminary tests with the improved wood sugars. Higher concentrations were also tried to determine if they could be used, but yeast yields were low.

The wood-sugar solution obtained by wood hydrolysis is sterile and contains only small amounts of the inorganic salts required for yeast production. Nitrogen in the form of urea, anhydrous ammonia, ammonium sulfate, or ammonium phosphate was added to the feed liquor to produce a 50 percent yield of yeast with a 50 percent protein content. Wood-sugar hydrolyzates contain small amounts of phosphate, but the amount was increased by the addition of phosphate salts. In the German operation it was customary to add potassium and magnesium salts and, therefore, these were added without determining the need for them.

In order to get the yeast growth started it was necessary to adjust the initial pH of the solution to between 4.5 and 5. After the growth had started, because of the utilization of the organic acids in the solution, it was necessary to add acid from time to time to keep the acidity in the pH range of 5 to 5.5.

Standard Fermentor

For one series of tests, a standard type of fermentor was used. The 66-gallon tank was equipped with a pump for circulation at a rate of 15 gallons per minute, with a spray-type water cooler that directed water against the outside of the tank. A bimetallic temperature controller operated an electric heater that maintained a temperature of 30° C. and a sparger of porous tube or cloth was provided for introduction of air.

For the batch propagation of yeast, 25 to 30 gallons of the diluted hydrolyzate, containing 1 to 2 percent of sugar and the necessary nutrient salts, were placed in the tank. The temperature was brought to 30° to 31° C., and yeast sufficient to produce a 1 percent wet-cell volume, or 100 million cells per millimeter, was added. The pumps were started and air introduced at a rate of 1 cubic foot per minute. Foaming was so great at this air rate that it could not be increased and, even so, antifoam had to be added at frequent intervals. After 8 hours, however, foaming decreased so that the air rate could be increased to 2 cubic feet per minute. About 24 hours were required to obtain the maximum utilization of reducing sugar.

Yeast was grown on diluted wood hydrolyzate and on the residues left after alcoholic fermentation of wood hydrolyzates. The high yields, based on reducing sugar obtained with the residues from alcoholic fermentation resulted from the utilization of organic acids in the solution. Each concentration of sugar was used for a series consisting of several experiments. Table 4 shows the average of the results from each of these series.

The low yield of yeast with regard to the fermentor capacity, and the serious foaming difficulty encountered, led to the conclusion yeast growth with the standard type of propagator was impractical.

Special Batch-type Propagator

In order to provide a means of controlling foam, a propagator was patterned after a type of pulp chlorinator that has a draft tube in the center to aid in mixing the air with hydrolyzate. A propeller-type stirrer circulated the solution and aided in the dispersion of the air. Spargers in the bottom introduced the air. A medium similar to that used for the tests shown in table 4 was prepared. Several series of tests were made at each sugar concentration. Table 5 shows the average results of each series. Because considerable amounts of air were pulled in by the action of the stirrer, air consumption could not be calculated.

The special type of propagator had advantages over the standard propagator in that better control of foam was obtained, but it was necessary to add antifoam and the yeast output remained small for the volume. Fifteen to 24 hours were required to utilize the sugar in a batch of the medium.

Continuous Propagation of Yeast

Information became available on a type of propagator used for yeast propagation on wood hydrolyzates in Germany (16, 23, 25). This propagator had been developed by Zellstofffabrik Waldhof for food-yeast production from sulfite pulping waste liquor. With this propagator, advantage was taken of the tendency of the liquid to foam in order to obtain intimate contact of the media with air, and because the process was continuous, the yeast was maintained in a vigorous condition. Active growth took place at all times, and there were no dark-colored products such as are produced by air on spent liquor in the absence of active yeast growth. The yeast was lighter in color and easier to process into a desirable product.

The propagator is designed to disperse air mechanically into the liquid, and simultaneously forces circulation of liquid through the central draft tube. These characteristics prevent the accumulation of nonbreaking foam on the top of the liquid. In German practice the foam was removed from the propagator and broken in a centrifuge. Operation at the Forest Products Laboratory indicates that foam may be broken, after removal from the propagator, by mildly agitating it and adding a small amount of antifoam agent.

Laboratory Propagator

A small laboratory propagator, somewhat modified from those used for industrial-scale equipment in Germany, was designed and built at the Forest Products Laboratory (30). This propagator has given satisfactory results for several years and a slightly larger one has been constructed in order to produce small amounts of yeast for livestock feeding tests. A drawing of the Laboratory propagator is shown in figure 2.

The tank has a 34-liter operating capacity and is constructed of stainless steel. The central draft tube is supported from the sides; these supports also act as a baffle to aid in stopping swirling of the contents. The diameter of the tank is 16 inches and that of the central draft tube 6 inches. The aeration-wheel disc is slightly larger than the draft tube. The air ducts are attached to this disc and extend beyond it to insure distribution of the air outside the draft tube. The air ducts which also turn with the aeration wheel lead from a 3/4-inch hollow central shaft. The hub of the disc fits a vulcanite sleeve bearing in the bottom of the propagator. A brass cap for introduction of air fits the top of the shaft and was bored to a tight running fit.

Temperature control is obtained with a single 1/2-inch tube coiled around the draft tube for cooling purposes, and two 150-watt contact strip heaters controlled with a bimetallic thermoswitch.

In the German propagator a cock in the bottom provided the means of removing the product. This was replaced in the Laboratory design with a vertical overflow tube of adjustable height, which keeps the contents of the propagator at constant level during continuous operation.

In operation, the height of the overflow tube is adjusted to give a workable balance between the height of the liquid in the tank and that in the overflow tube. A free-flowing foam flows down the draft tube, loading the aeration wheel and aiding the dispersion of air.

Because the foaming characteristics of the wood-sugar solutions change as the sugar is used, the equipment has not proven satisfactory for batch operation. The maximum amount of air that can be used is limited by the amount of liquid moving down the draft tube. When too much air is introduced, foam collects on the surface and rises above the top of the propagator. Maximum air input has been 1 cubic foot per minute. Air pressures of 3 to 5 pounds per square inch are sufficient for air supply.

Foam properties and the amount of air that may be used vary with the acidity of the solution. In experiments at the Forest Products Laboratory, the lower limit at which yeast has been grown satisfactorily is pH 4. As the pH rises, yeast growth and sugar utilization become more rapid, the tendency to foam increases, and the yeast becomes darker. At about pH 6, it becomes more difficult to keep the propagation free from infection.

Starting the Propagator

Approximately 2 liters of yeast inoculum were prepared in shake flasks by dividing 150 millimeters of the inoculum into four parts and using it to inoculate 4-liter flasks containing 500 millimeters each of glucose malt-sprout media. The inoculum was placed in the propagator and the aerator, air, and media feed pump started.

Wood-sugar solution of approximately 2 percent concentration was fed at a rate of 2 liters per hour until the quantity of yeast had been built up. When about 9 liters of media had been added, making a total of 11 liters in the propagator, foamed-up yeast and spent liquor began to flow from the propagator. When 4 liters had overflowed, the foam in the discharged sample was broken by adding a few drops of antifoam and stirring. The yeast was removed in a yeast separator and returned to the propagator. After yeast was collected and returned for 5 hours, the concentration of the wood-sugar solution was changed to that of the full-strength hydrolyzate, which contained about 5 percent of reducing sugar, and the rate of pumping was increased from 3 to 5 liters per hour.

Operating Conditions

Under equilibrium conditions, the apparent average density of the foamed contents was about 0.33, and therefore the working capacity of the 34-liter propagator was about 11 liters. A sugar-solution feed containing 5 percent of reducing sugar produced yeast sufficient for about a 12 percent wet-cell volume. Yields of dry Torula were normally 39 to 50 percent of the sugar in the feed. The throughput time for the propagator was 2 to 5 hours, depending upon the pH of the solution. Air requirement for best operation was about 200 cubic feet per pound of dry yeast or 50 cubic feet per pound of wet-centrifuged yeast cells. The yields of yeast obtained with different conditions are shown in table 6.

It was customary to remove the entire contents of the continuous propagator once a month in order to inspect working parts, clean out any accumulation of dried yeast, and do any other servicing required. In most cases, the whole charge was returned and continued as before. Operation has been continued without growing up new yeast for as long as 6 months. Sterile conditions were not used. When propagation was carried out at pH 5 to 5.5, there was no evidence of contamination. Because organic acids were used by the yeast, it was necessary to add acid from time to time to maintain the propagator at the desired pH. A pH controller was found satisfactory for recording the pH and for adding the acid.

More recently a propagator with 40-gallon capacity has been constructed at the Forest Products Laboratory and put into use. Equipment in use with this propagator is shown in figure 3. In this equipment, the media feed rate is 16 to 20 liters per hour and the air rate 2-1/2 to 3 cubic feet per minute. Maximum yeast production is 15 to 20 pounds of dried yeast per 24-hour day.

A 24-hour supply of feed is prepared in a storage tank and held at room temperature. This is pumped by a metering pump through a filter to remove any sediment that may have precipitated, and then to a disc on the rotating shaft just above the draft tube. This device sprays the solution evenly over the surface of the yeast suspension, where the sugar is rapidly absorbed. Air is introduced through a filter to the spinning aeration wheel, which also acts as a pump to force the foamed yeast suspension over the sides of the draft tube, where carbon dioxide is given up. Constant temperature is maintained in the propagator by means of a bimetallic switch that operates a valve controlling the cold water flowing in the jacket of the propagator. The heat generated in yeast production is sufficient to maintain the needed temperature. The feed is usually held at a low pH to aid in the control of contamination and also to make it easier to control the pH of the propagator. Under normal growing conditions, a small amount of acid (10 percent sulfuric acid) is added by the pH controller once each 4 to 6 hours to maintain a pH of 5.4 to 5.6 in the propagator. Yeast overflows continuously into a 10-gallon receiver equipped with a stirrer and an oiling device that supplies a small amount of antifoam from time to time. The defoamed yeast suspension flows through a connecting tube to another receiver equipped with a float-operated switch that operates a motor-driven pump for pumping the yeast suspension to storage tanks where it is cooled and stored until separated. Once each day the yeast is separated.

In large-scale operation, the yeast would be delivered continuously as a cream containing 10 to 15 percent of dry solids from the separator. Water would be added to the cream to wash the yeast, and then it would be reseparated. In small-scale operation, it is not possible to have sufficient material for continuous operation, and the yeast is therefore kept as a paste in the separator bowl and run in batches. The paste is removed from the bowl, resuspended in water, and separated again for washing. The washed yeast paste, containing about 25 percent of dry solids, is autolysed by heating and stirring it in a jacketed kettle at 85° to 95° C. for about 30 minutes. This heating converts the paste to a thin fluid, which is fed to a drum drier equipped with operating rolls heated to about 120° C. The yeast is removed in a continuous sheet. It is then ground in a mill to flake or powder form and sent out for feeding tests.

Nutrient Requirements for Yeast Production

In early work at the Forest Products Laboratory, nutrients were supplied in the quantities used in Germany without determining the actual need. The amounts of nitrogen, phosphate, potash magnesium, and air needed for yeast production were determined by holding all factors except one constant and varying this one above and below the recommended value. For example, the recommended value for nitrogen is 3.5 pounds of nitrogen for each 100 pounds of sugar. In table 7 the results of varying the nitrogen from 2.5 to 12 pounds per 100 pounds of sugar are shown (22). Reducing-sugar utilization was almost constant at all levels of nitrogen above 3.2 pounds per 100 pounds of sugar. Below that value, sugar utilization decreased. Also, recovery of nitrogen in the yeast was low at nitrogen levels above 3.2 pounds of nitrogen per 100 pounds of sugar.

In the same manner, the practical level of each variable was measured. The amounts of potassium and phosphate required for maximum sugar utilization and maximum yeast yields are shown in tables 8 and 9. There appeared to be very little difference in the yield of yeast or utilization of sugar when magnesium was added. It was therefore concluded that sufficient magnesium was present. In some reports (4, 5, 20) it has been suggested that the presence of sulfite aids in yeast growth. This was confirmed in this work (15). When 1 pound of sodium sulfite, or 0.1 pound of sulfur dioxide per 200 pounds of sugar in the feed, was used, yields of yeast were improved.

Varying the air from 0.2 cubic foot to 1 cubic foot per minute per cubic foot of media in the propagator, when the feed rate was one-third the liquid content per hour and the sugar concentration was 4.6 percent, showed that about 0.5 cubic foot of air per minute per cubic foot of media gave maximum yields of yeast and sugar utilization. Air requirement per pound of dry yeast produced was about 200 cubic feet. Increasing the depth of the yeast propagator by 25 percent increased the capacity but did not change the ratio of air to yeast produced.

Effect of Sugar Concentration on Yeast Growth

Much of the previous work on yeast growth had been with dilute solutions. Peterson, Snell, and Frazier (22) used solutions containing 1 percent reducing sugar and German yeast production (21, 23, 25, 39) was with solutions containing 2.5 to 3 percent of sugar. Table 10 gives the results of a series of experiments in which the sugar concentration ranged from 1 to 8 percent. In order to produce solutions that would differ only by the effect of dilution, wood hydrolyzate from Douglas-fir was evaporated to 20 percent sugar concentration by the procedure described for molasses production (11) and then diluted to the various concentrations used in the experiments. The air rate was the same in all experiments except the last and, therefore, the ratio of air to yeast produced was greater with dilute solutions. When 8 percent sugar solutions were fed, air supplied was insufficient to grow yeast, and alcohol was produced also. Increasing the air rate to 0.75 cubic foot per minute increased the yield to 38 percent. The high sugar utilization, and also the high yield of yeast when 6 percent sugar solutions were introduced, represented what appeared to be the maximum yeast growth for the air supplied.

Effect of Wood Species on Yeast Growth

Various hydrolyzates were compared for their ability to grow yeast because species may vary in respect to inhibiting properties and in the amount of nonsugar-reducing material. Table 11 shows the results.

The hydrolyzate from these species of wood was evaporated to 20 percent concentration and later diluted to 5 percent for yeast growth.

The air rate was 0.5 cubic foot per minute, and nutrients in the feed, per 100 pounds of sugar, were 3.4 pounds of nitrogen, 1.6 pounds of phosphorous pentoxide and 1.1 pounds of potassium chloride.

Growth of Baker's Yeast (*Cereviseae*) on Wood Hydrolyzate

The success attained in growing *Torula* yeast in the Waldhof-type propagator has evoked interest in the possibility of growing a commercial baker's yeast on wood hydrolyzate. A sample of baker's yeast was used to inoculate a sample of wood sugar. There was a delay of a few hours before the yeast began to develop, but after the initial period, acclimatization was complete and the yeast grew readily on Douglas-fir hydrolyzate. When the sugar feed rate was 3 liters of 5 percent sugar solution per hour, the air rate 0.5 cubic foot per minute, and nutrients present in the quantities used in other tests, yields with Douglas-fir hydrolyzate were 44.6 to 49.9 percent utilized, as shown in table 12. Oak hydrolyzate appeared to contain inhibiting substances that did not permit the growth of baker's yeast.

Acclimatization of Various Yeasts to Wood Sugar

Twenty yeasts belonging to the genera *Torula candida*, *Willia*, *Mycotorula*, and *Saccharomyces* were chosen at random from the stock collection of the Laboratory and tested for their ability to produce high-protein yeast (17).

Measurements were made in shake flasks with media prepared in the usual manner. The sugar concentration was 5 percent in all cases. To allow for admission of large amounts of air, cotton plugs were inserted in the flasks.

After 24 hours all of the yeast from each flask was removed from the sugar solution by centrifuging. Each day yeast sufficient to produce 1 percent cell volume was resuspended in fresh wood-sugar medium. This procedure was repeated for 30 transfers. Yields of sugar utilized and yeast yield were determined on each sample.

The length of time required for acclimatization varied with the strain of yeast; the shortest was 5 days for two strains, and the longest was 27 days. Most of the yeasts had reached their maximum acclimatization by the twelfth to fifteenth transfer, and further transfers did not result in increased conversion of the sugar. Table 13 shows the strains of yeast and their acclimatization. Figure 4 illustrates the yeast growth and sugar utilization during acclimatization.

Yeast as an Agent for Utilization of Sugars in Wood-processing Wastes

Many chemical processes for utilization of wood have sugars as a byproduct. Thousands of tons of sugar are present annually in the United States in pulping processes. The disposal of these byproducts presents problems because of their biological oxygen demand.

In Germany, processes were developed in the period 1936 to 1944 for the conversion of these wood-processing wastes into high-protein yeast (2, 25, 39) for human food. Production during the war reached 20,000 tons per year, and further expansion was proposed.

Sulfite waste pulping liquors contain 2 to 4 percent of reducing sugars in addition to other organic substances. These sugars represent 40 to 50 percent of the biochemical oxygen demand, and their utilization decreases stream pollution to that extent. The yield of dry yeast is equivalent to 100 to 200 pounds per ton of pulp produced.

In the production of coarse fiber, 15 to 30 percent of the wood is converted into soluble products, which are largely sugars. These sugars were utilized in experiments at the Forest Products Laboratory (14) by first subjecting them to hydrolysis and then using them to grow yeast. Seventy-five to eighty-five pounds of yeast may be produced from the soluble byproducts of a ton of coarse fiber with biological oxygen demand reduced about 55 percent.

Pentose sugars and organic acids remaining in sulfite waste liquors or wood hydrolyzate after alcohol fermentation may be utilized for yeast production. It is estimated that the pentoses present in the spent liquors from one plant producing alcohol in the United States from sulfite waste liquor would be sufficient to produce 15 to 20 tons of food yeast daily. Utilization of these pentoses would reduce biological oxygen demand.

Composition of Yeast

Yeast produced from wood hydrolyzate and other wood sugars is a high-quality food containing, as shown by Forest Products Laboratory studies, 8.5 to 9.5 percent of total nitrogen, 46 to 56 percent of protein, 2 to 7 percent of fat, 22 to 35 percent of nitrogen-free extract, and 8 to 12 percent of ash. These values agree with those given by Fink (5). Amino acids and vitamin content have been determined by Fink and Just (6) and by Scheunert (32). It was shown that yeast produced on wood sugar contained less vitamin B_1, but more vitamin B_2 than brewer's yeast. Yeast is not a complete protein food as determined by feeding tests, but can supply a large portion of protein requirement. Fink and Hock (7) showed that cystine must be fed with yeast. Some of the protein of *Torula* produced on wood sugar may not be as readily assimilated as milk proteins.

Economics of Yeast Production

Information on the production of yeast for food purposes from wood sugars in the United States has not progressed to the stage of making estimates of cost of production. Assuming no value for the sugar, except preparing the solution for yeast production, cost of manufacture from the sugars in sulfite waste liquor should be the lowest of any source because the sugars are a byproduct from another operation. A study of nutrient requirements shows that 8.5 pounds of nitrogen as ammonia or urea, 4 pounds of phosphoric anhydride as sodium or calcium phosphate, and 3.75 pounds of potassium chloride are required to produce 100 pounds of dry yeast with about 50 percent protein. Using the German values for power and steam, 110 kilowatts of electricity and about 600 pounds of steam at 15 pounds per square inch pressure are needed for each 100 pounds of yeast. The cost of chemicals and power is about $1.50 per 100 pounds of yeast produced. Plant, labor, and overhead costs will differ according to the type of installation.

Summary

A food yeast high in protein and vitamins may be produced rapidly and in good yields from the carbohydrate contained in waste wood or from carbohydrates contained in byproducts from the chemical processing of wood. Wood waste may be converted into sugar that is suitable for yeast production in yields of 45 to 50 percent of the wood substance. Sugars present in sulfite pulping waste liquor represent about 11 percent of the wood substance. These sugars are suitable for food-yeast production. Carbohydrates, which are now converted into useless products during alkaline pulping, could be removed before pulping and used for the production of food yeast.

Food yeast is produced in about equal yields from either the pentose or hexose sugars obtained from wood. For high yields and rapid propagation, yeast must be acclimatized to wood sugars. Because wood sugars are produced under conditions that produce sterile solutions, continuous propagation of yeast is possible. Foaming is a serious problem in the propagation of yeast from wood sugars. A propagator has been developed that makes use of this foam to provide intimate contact of air with liquid and at the same time controls the type of foam. Food-yeast propagation on wood sugar requires 3.4 pounds of nitrogen in the form of ammonia, ammonium sulfate, or urea, 1.6 pounds of phosphoric anhydride as phosphate salts, and 1.1 pounds of potassium chloride or potassium sulfate for each 100 pounds of wood sugar to give maximum yields of yeast. Food-yeast production on wood sugars may be used as a means of lowering the biochemical oxygen demand of wood-processing wastes. Food yeast produced on wood sugar contains about 50 percent of protein, 4 percent of fat, small amounts of vitamin B_1, and large amounts of vitamin B_2.

Acknowledgement

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Table 1.--Growth of various yeasts in wood hydrolyzate

Organism	Time	Sugar	Dry yeast on total reducing sugar
	Hours	Percent	Percent
Series I:			
Saccharomyces cereviseae	24	57	4.1
	48	67	4.7
S. cereviseae ATCC 764	24	67	7.3
	48	71	17.3
Torula utilis No. 3	24	67	24.3
	48	86	39.1
Series II:			
Candida guilliermondi	24	92	23.5
C. tropicalis	24	91	32.9
C. kruseii	24	74	22.5
Endomyces magnusii	24	86	15.0
Hansenula anomala	24	90	30.4
P-13	24	91	40.4
Rhodotorula Sp.	24	43	10.3
T. utilis No. 3	24	81	33.2
Series III:			
S. cereviseae	24	75	15.3
	48	83	20.1
S. cereviseae ATCC 764	24	63	17.2
	48	73	27.9
T. utilis No. 3	24	70	29.8
	48	89	39.9

Table 2.--Effect of acclimatization on sugar utilization and yeast yield[1]

Organism	Sugar used		Yeast yield based on total sugar	
	First transfer	Twelfth transfer	First transfer	Twelfth transfer
	Percent	Percent	Percent	Percent
Hansenula anomala	75.1	88.5	27.0	40.5
Candida tropicalis	75.1	90.1	29.3	41.3
P-13	77.4	92.3	20.5	43.7
Torula utilis No. 3	70.2	91.8	22.9	38.1

[1]Initial reducing sugar content about 1 percent.

Table 3.--Yield of sugar from various woods

Species of wood	Yield		
	Total sugar	Hexose	Pentose
	Percent[1]	Percent	Percent
Spruce	51.5	81	14
Douglas-fir	51.5	83	13
Southern yellow pine	50.5	79	14
Ponderosa pine	51.0	80	15
White fir	49.6	78	18
Western white pine	49.9	82	14
Eastern white pine	50.9	82	15
Sugar pine	52.0	80	16
Western hemlock	52.8	82	12
Western larch	56.3	84	12
Redwood	41.0	71	15
Western redcedar	49.0	75	16
Southern red oak	46.0	63	25
Sugar maple	48.0	70	25
Yellow birch	50.0	67	26
Beech	46.8	74	26
Sweetgum	47.0	72	26
Pecan	45.0	64	25
Mesquite	29.2	63	24
Elm, American	47.0	64	25
Yellow-poplar	49.0	73	20
Aspen	51.0	75	20
Hickory	47.2	63	25

[1]Based on weight of dry wood.

Table 4.--Batch production of yeast on wood sugars in a standard yeast propagator

Series No.	Type of hydrolyzate	Sugar concentration	Air per minute	Air per pound dry yeast	Sugar used[1]	Dry yeast yield on total sugar
		Percent	Cu. ft.	Cu. ft.	Percent	Percent
1	Diluted hydrolyzate	0.94	1.20	1,500	94.0	45.0
2	Diluted hydrolyzate	1.20	1.20	1,200	92.0	43.0
3	Diluted hydrolyzate	1.50	1.25	1,100	89.0	39.0
4	Diluted hydrolyzate	2.00	1.20	1,250	84.0	25.0
5	Alcohol fermentation residue	.71	2.00	1,550	75.0	125.0
6	Alcohol fermentation residue	.81	2.50	1,830	76.0	116.0
7	Alcohol fermentation residue	.67	2.50	1,950	76.0	130.0
8	Alcohol fermentation residue	1.10	2.50	1,820	65.0	100.0

[1]Sugar utilization was based on the loss from the solution of reducing material calculated as glucose, and does not account for material precipitated by aeration or for nonsugar organic material used by the yeast.

Table 5.--Batch growth of yeast in special propagator

Series	Type of medium	Sugar concentration	Air per minute	Sugar used	Dry yeast yield on total sugar
		Percent	Cu. ft.	Percent	Percent
X-1	Diluted hydrolyzate	2.19	0.0	50	25
X-2	Diluted hydrolyzate	1.4	.5	86	35
X-3	Fermentation residue	.77	.0	80	100
X-4	Fermentation residue	.93	1.5	80	110

Table 6.--Yeast production with continuous propagation of *Torula utilis*

Series No.	Rate of feed	pH of propagator	Air per minute	Air per pound of dry yeast	Reducing sugar used	Dry yeast on total sugar	Protein content of yeast
	Liters per hr.		Cu. ft.	Cu. ft.	Percent	Percent	Percent
			Wood hydrolyzate 4.6 percent sugar, pH 4				
9	2.0	5.0	0.75	630	92.0	35.9	52.3
39	2.0	5.5	.50	450	90.0	38.0	52.8
10	3.0	4.7	.75	415	88.4	36.4	52.0
40	3.0	5.0	.50	240	94.1	40.0	53.7
14	3.0	6.6	.75	295	93.8	49.8	50.9
41	3.0	6.0	.50	233	94.3	43.5	52.4
11	4.0	5.0	.75	313	86.5	36.0	52.7
15	4.0	6.0	.75	210	93.4	52.0	57.3
42	4.0	5.2	.50	210	91.8	38.1	54.0
12	4.5	5.0	.75	275	85.3	36.5	54.4
16	4.5	6.0	.75	212	93.2	46.5	56.5
13	5.0	5.0	.75	296	83.1	30.5	52.5
43	5.0	5.2	.50	249	89.0	26.4	56.0
17	5.0	6.0	.75	203	91.1	43.4	52.5
44	6.0	5.2	.50	218	77.4	29.0	51.1
			Fermentation residues 0.94 percent sugar, pH 4				
19	3.0	5.4	.60	1,100	60.0	52.0	50.0
20	4.0	5.5	.60	950	55.0	47.0	50.0
			Sulfite waste liquor from blow pit neutralized with lime sugar content 1.66 percent, pH 4				
21	2.0	5.5	.50	1,040	79.0	49.5	51.6
22	3.0	5.5	.50	695	78.0	49.0	51.5
23	4.0	5.5	.50	540	75.0	50.0	51.0
24	5.0	5.5	.50	550	59.0	50.2	50.0
			Sulfite waste liquor steam stripped to 2.56 percent sugar, pH 4				
748	2.0	5.5	.50	890	77.0	49.5	42.6
752	2.0	5.5	.50	850	74.3	52.0	43.2
765	3.0	5.5	.50	850	75.0	46.1	43.6
770	3.0	5.5	.50	850	73.9	47.4	51.4
			Magnesia-base sulfite waste liquor, 2.50 percent sugar, pH 4				
L-1	2.0	5.5	.50	1,150	75.4	52.5	----
L-6	4.0	5.5	.50	645	71.5	49.0	----

Table 7.--Nitrogen requirements for yeast growth[1]

Nitrogen[2] per 100 pounds of reducing sugar in feed[3]	Nitrogen content of yeast	Nitrogen recovered in yeast	pH		Reducing sugar utilized
			Feed	Propagator	
Pounds	Percent	Percent			Percent
12.0	8.70	32.7	4.2	5.5	93.8
9.0	8.50	40.6	4.2	5.5	93.6
6.0	8.54	59.7	4.2	5.5	93.7
4.0	8.44	88.5	4.2	5.5	93.0
3.5	7.82	91.5	4.2	5.5	93.1
3.2	7.72	94.6	4.2	5.5	93.0
3.0	7.69	96.5	4.2	5.5	82.0
2.5	7.43	96.0	4.2	5.5	70.0

[1]Other nutrients were held constant as follows: Phosphoric pentoxide (as $(NH_4)_2HPO_4$), 2 pounds; potassium chloride, 1.6 pounds; and magnesium sulfate as 1 pound per 100 pounds of sugar in feed.

[2]Nitrogen was introduced as urea.

[3]Douglas-fir hydrolyzate reducing-sugar content, 4.6 percent.

Table 8.--Phosphate requirement for yeast growth[1]

Phosphate[2] (P_2O_5) per 100 pounds of reducing sugar in feed[3]	Duration of test	Yeast yield based on sugar used	Protein content of yeast	Reducing sugar utilized
Pounds	Hours	Percent	Percent	Percent
2.0	48	44.1	51.9	93.7
1.5	24	43.0	52.8	93.8
1.0	24	36.4	50.9	92.5
.5	25	35.5	49.8	76.9
0.0	25	27.8	38.3	33.5

[1]Other nutrients were held constant as follows: Nitrogen, 3.4 pounds; potassium chloride, 1.6 pounds; magnesium sulfate, 1.5 pounds per 100 pounds of sugar in feed.

[2]Phosphate (P_2O_5) was added as $(NH_2)_2HPO_4$.

[3]Douglas-fir hydrolyzate with reducing sugar content 4.5 percent. Feed rate was 3 liters per hour into a tank with 11.2 liters operating capacity.

Table 9.--Potassium requirements for yeast growth[1]

Potassium chloride per 100 pounds of reducing sugar[2] in feed	Duration of test	Yeast yield based on sugar used	Protein content of yeast	Reducing sugar utilized
Pounds	Hours	Percent	Percent	Percent
2.0	24	42.0	52.0	93.8
1.5	24	42.1	53.0	93.0
1.0	22	40.2	50.4	90.0
.5	22	41.0	54.0	80.7
0.0	33	37.2	49.9	42.2
1.1	24	42.0	50.9	93.0

[1]Other nutrients were held at: Nitrogen, 3.4 pounds; magnesium sulfate, 1.5 pounds; phosphoric pentoxide, 1.6 pounds per 100 pounds of sugar in feed.

[2]Douglas-fir hydrolyzate with reducing-sugar content 4.5 percent. The feed rate was 3 liters per hour into a tank with 11.2 liters operating capacity. Air was introduced at 0.5 cubic foot per minute.

Table 10.--Effect of sugar concentration on yeast growth

Concentration of sugar in feed[1]	pH	Dry yeast on sugar used	Protein content	Sugar utilized
Percent		Percent	Percent	Percent
1	5.3	63.3	39.1	95.1
2	5.2	49.9	42.1	94.5
4	5.1	42.6	45.3	95.1
6	5.3	40.6	55.3	93.4
8	5.0	26.6	55.1	90.0
[2]8	5.5	38.0	52.5	92.0

[1]The sugar in the feed was Douglas-fir hydrolyzate, which had been concentrated to 20 percent and then diluted for use and fed at a rate of 3 liters per hour. Air was introduced at a rate of 0.5 cubic foot per minute.

[2]Air rate 0.75 cubic foot per minute.

Table 11.--Growth of yeast on various hydrolyzates

Hydrolyzate	Rate of feed per hour	Acidity	Yeast yield	Protein content	Sugar used
	Liters	pH	Percent	Percent	Percent
Western larch	3	5.3	45.9	49.0	91.2
Western larch	3	6.3	47.4	47.4	91.7
Douglas-fir	3	5.0	42.6	45.3	95.1
Lodgepole pine	3	5.9	51.7	53.8	94.0
Southern yellow pine	3	5.5	45.0	52.1	92.1
Aspen	3	5.5	45.2	52.7	94.8
Southern red oak	3	5.5	49.3	54.0	83.8

Table 12.--Growth of yeast on various hydrolyzates[1]

Hydrolyzate	Rate of feed per hour	pH	Dry yeast on sugar used	Protein content	Sugar utilized
	Liters		Percent	Percent	Percent
Larch.............	3	5.3	45.9	49.0	91.2
Do..............	3	6.3	47.4	47.4	91.7
Do..............	5	4.5	33.0	52.4	49.3
Do..............	5	6.3	37.6	42.4	50.6
Douglas-fir........	3	5.0	42.6	45.3	95.1
Lodgepole pine....	3	5.9	51.7	53.8	94.0
Southern red oak..	3	5.5	49.3	54.0	83.8

[1]The hydrolyzates from these species of wood were concentrated to 20 percent sugar concentration and later diluted to 5 percent for yeast growth.

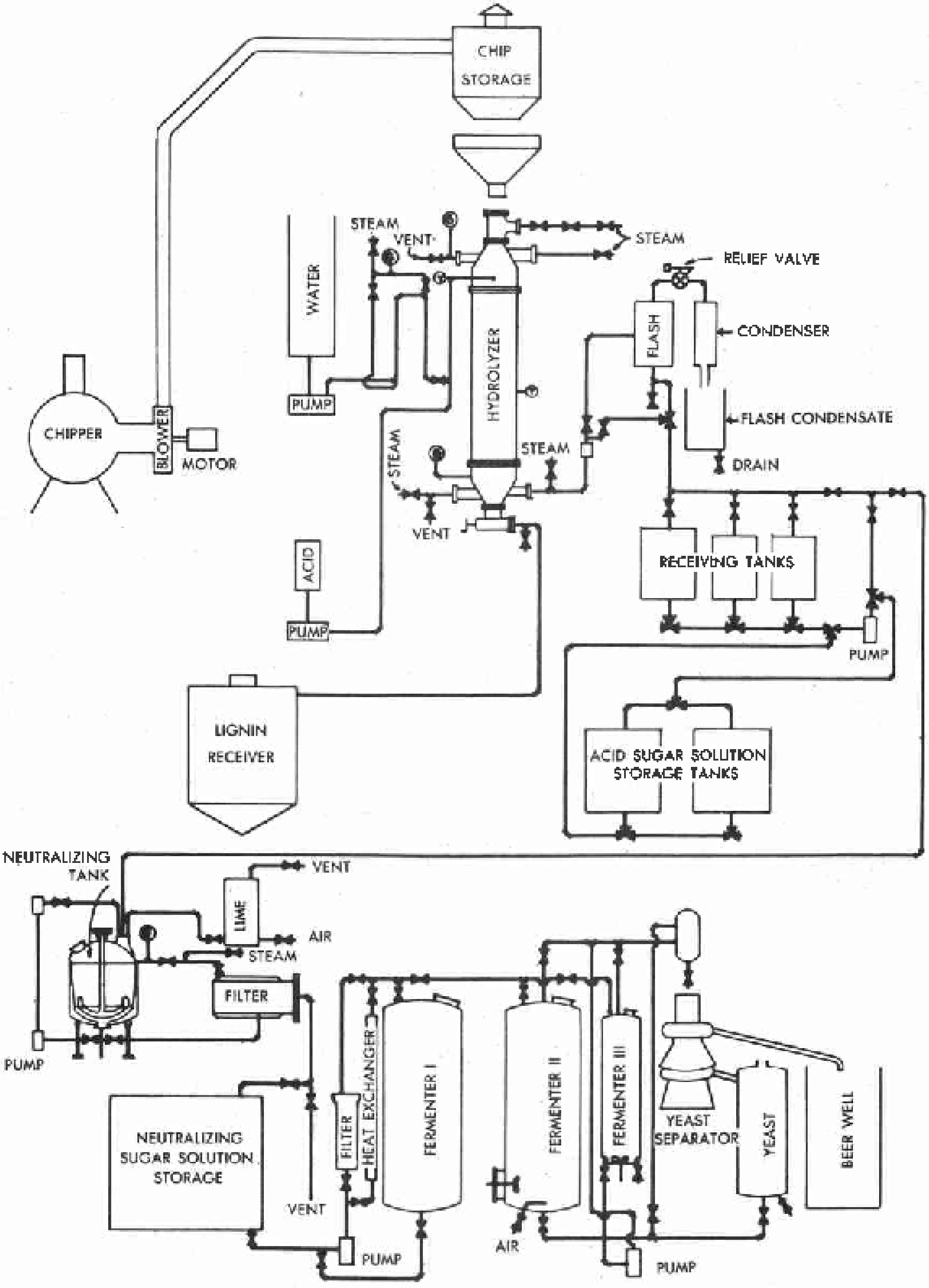
Table 13.--Growth of various strains of yeast in wood hydrolyzate in 24 hours

Strain	Initial sugar utilized[1]		Yield of yeast[1]	
	First transfer	Twelfth to fifteenth transfer	First transfer	Twelfth transfer
	Percent	Percent	Percent	Percent
Torula utilis major	78	82	25	42
Torula utilis thermophilus	78	80	33	38
Torula utilis No. 2	78	82	25	38
Torula utilis No. 900	47	79	20	38
Torula utilis No. 3	50	88	22	37
Torula utilis No. 660	80	83	32	35
Torula utilis No. 793	80	85	31	38
Torula utilis No. 957	80	82	30	37
Candida albicans	81	94	32	41
Candida arborae	45	86	21	35
Candida arborae No. 197	30	83	15	37
Candida arborae No. 198	25	84	12	37
P-13	10	78	6	35
Mycotorula lipolytion	10	84	5	36
Unidentified (x)	75	83	30	35
Hansenula anamala	50	80	21	33
Hansenula suaveolens	25	82	11	34
Saccharomyces anonensis	75	84	30	36
Saccharomyces cerevisiae No. 46	50	83	22	30
Saccharomyces ellipsoideus	60	80	27	35
Best yeast No. 2	33	80	15	38

[1]Based on total sugar in solution.

Figure 1.--Equipment for saccharification of wood and fermentation of wood-sugar solutions.

ZM 68309 F


CHIP STORAGE
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VENT
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WATER
HYDROLYZER
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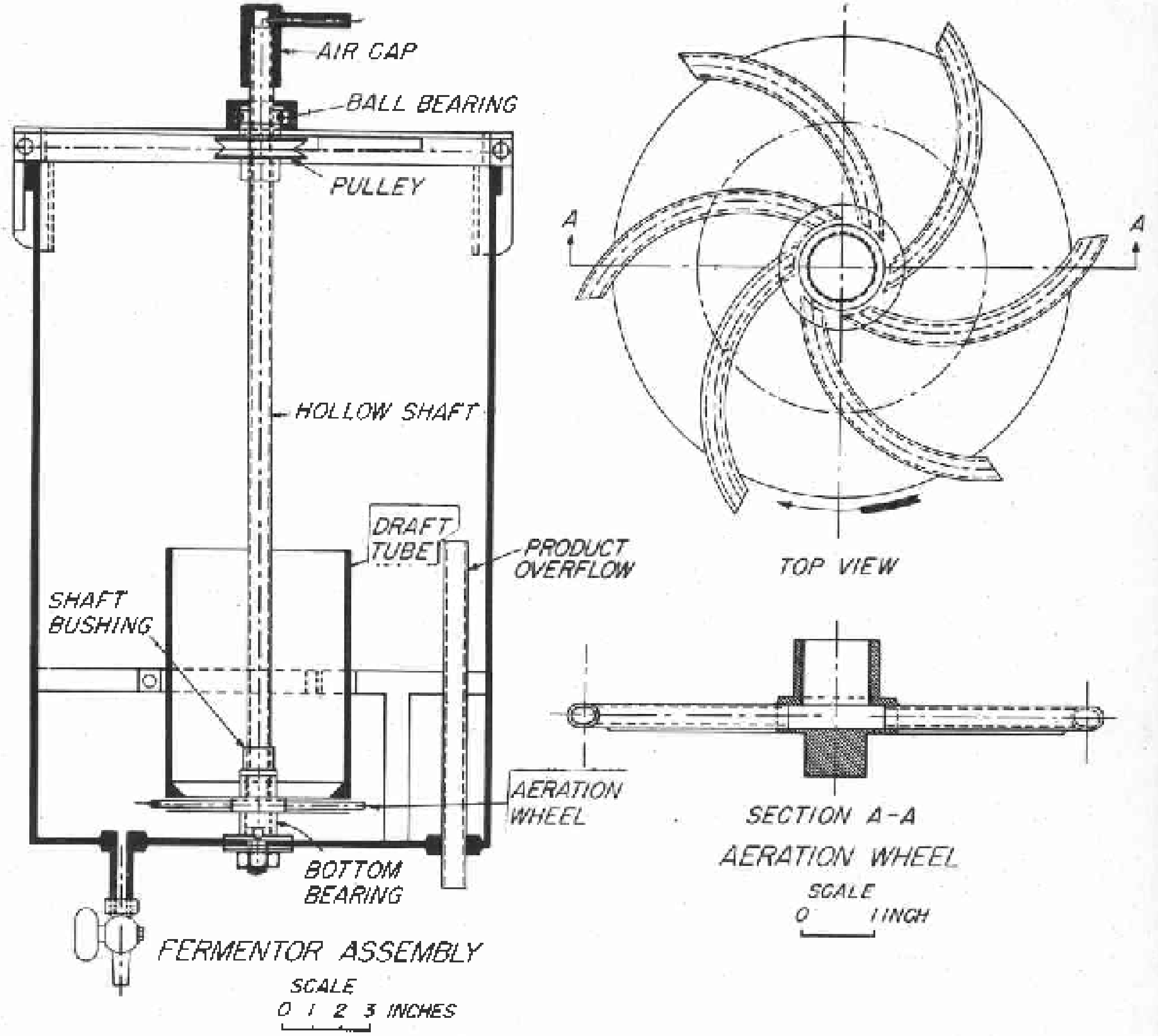
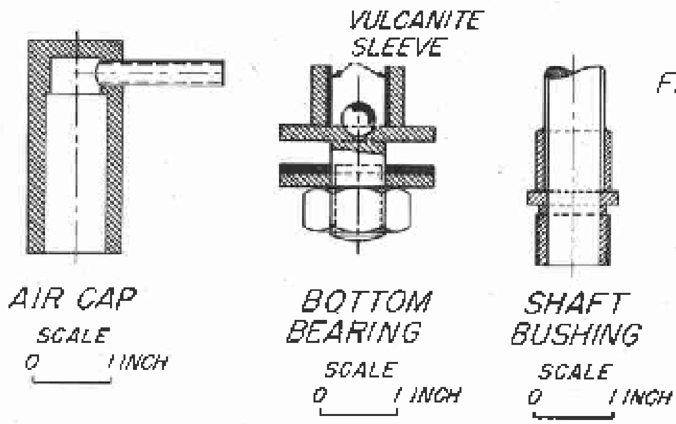
BLOWER
MOTOR
PUMP
STEAM
STEAM
DRAIN
VENT
ACID
PUMP
RECEIVING TANKS
PUMP
LIGNIN RECEIVER
ACID SUGAR SOLUTION STORAGE TANKS
NEUTRALIZING TANK
VENT
LIME
AIR
STEAM
FILTER
PUMP
HEAT EXCHANGER
FILTER
FERMENTER I
FERMENTER II
FERMENTER III
YEAST SEPARATOR
YEAST
BEER WELL
NEUTRALIZING SUGAR SOLUTION STORAGE
VENT
PUMP
AIR
PUMP

Z M 68509 F

Figure 2.--Laboratory-scale experimental fermentor with mechanical aerater built at the Forest Products Laboratory with design altered somewhat from industrial-scale equipment used in Germany.

(ZM 70406 F)





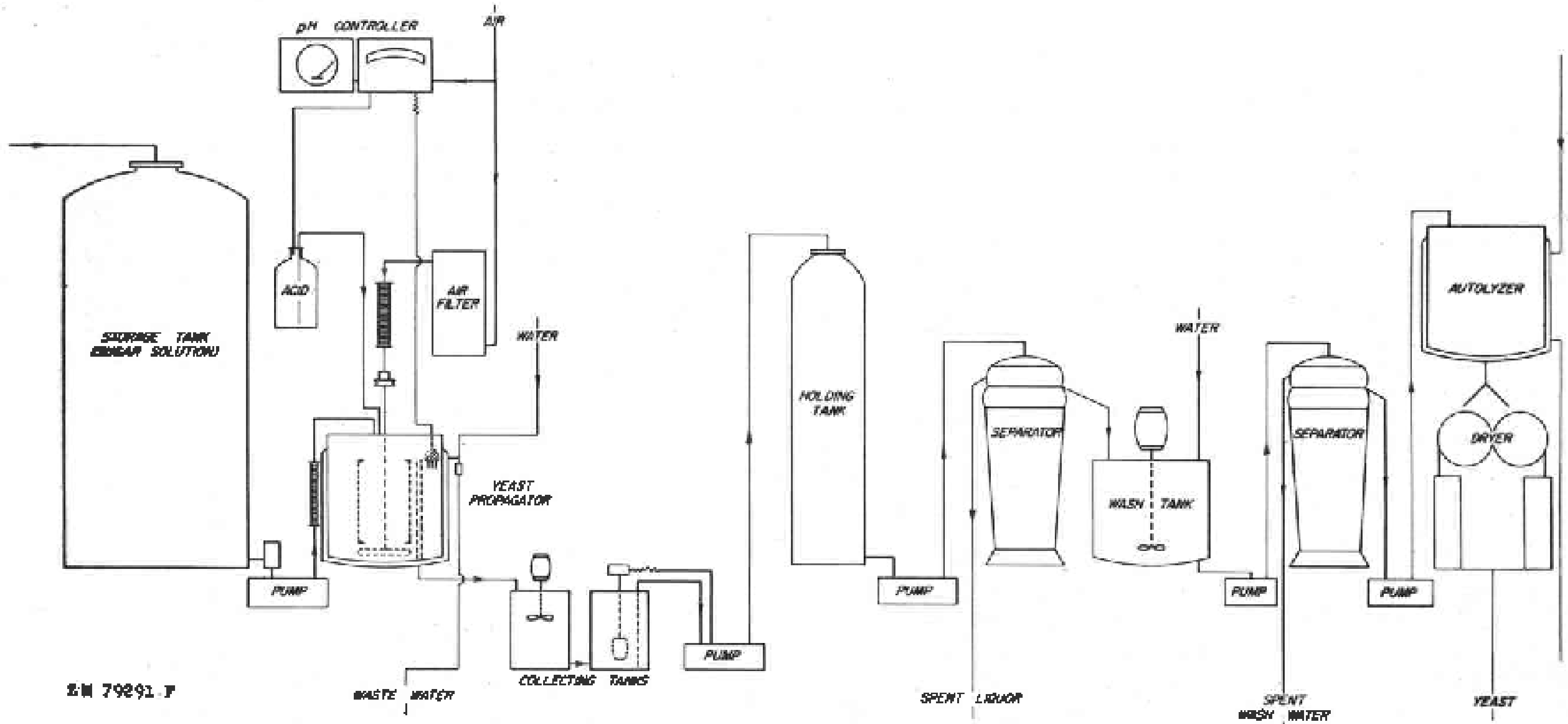
LABORATORY SCALE EXPERIMENTAL FERMENTOR WITH MECHANICAL AERATOR

FOREST PRODUCTS LABORATORY
MADISON, WISCONSIN

Z M 70405 F

Figure 3.--Flow diagram for yeast production from wood sugars.

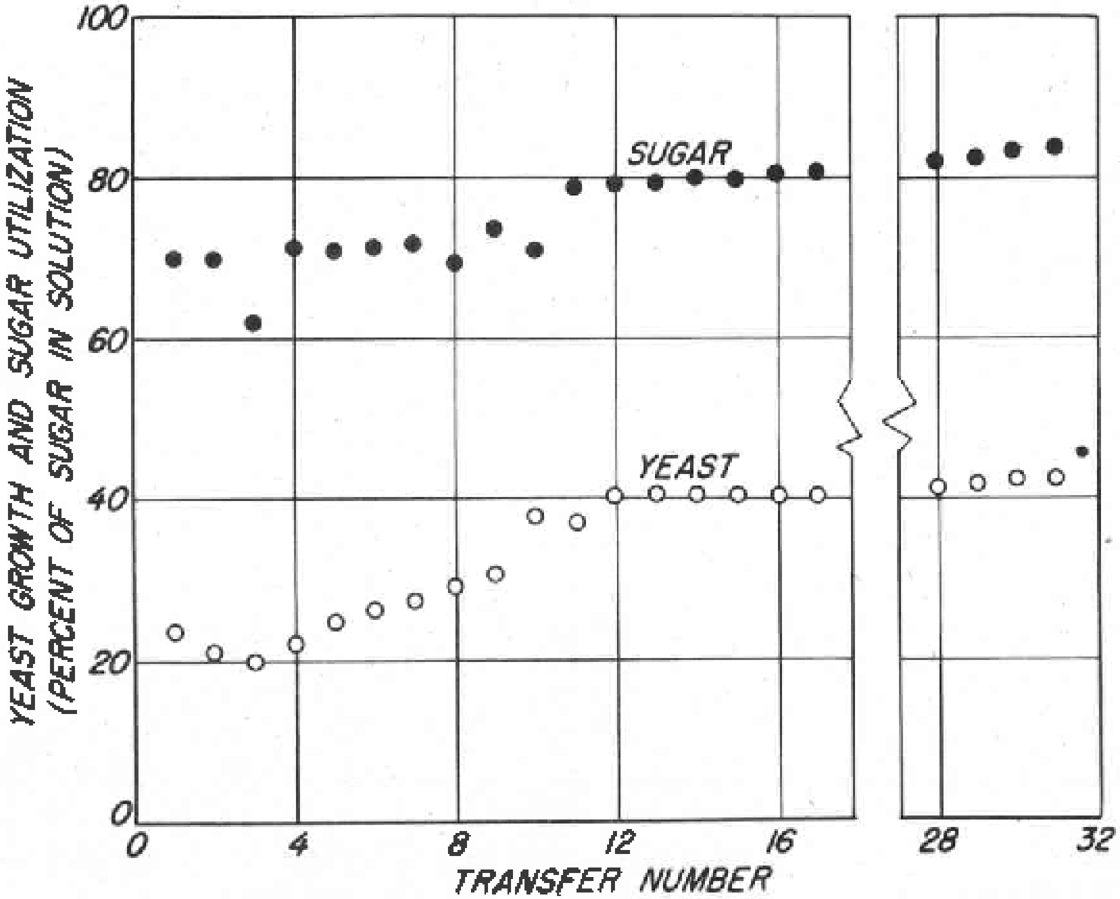
(ZM 79291 F)



STORAGE TANK
(SUGAR SOLUTION)
ACID
pH CONTROLLER
AIR
AIR FILTER
WATER
YEAST PROPAGATOR
PUMP
WASTE WATER
COLLECTING TANKS
PUMP
HOLDING TANK
PUMP
SEPARATOR
SPENT LIQUOR
WATER
WASH TANK
PUMP
SEPARATOR
SPENT WASH WATER
PUMP
AUTOLYZER
DRYER
YEAST
Z-M 79291 F

Figure 4.--Growth of yeast and utilization of sugars in yeast production with *Torula*.

(ZM 76393 F)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(PERCENT OF SUGAR IN SOLUTION)
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60
40
20
0
SUGAR
YEAST
0
4
8
12
16
28
32
TRANSFER NUMBER

Z N 76993 F