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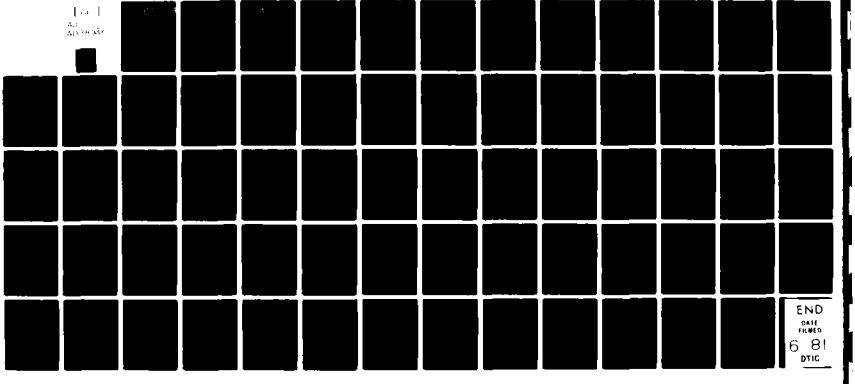

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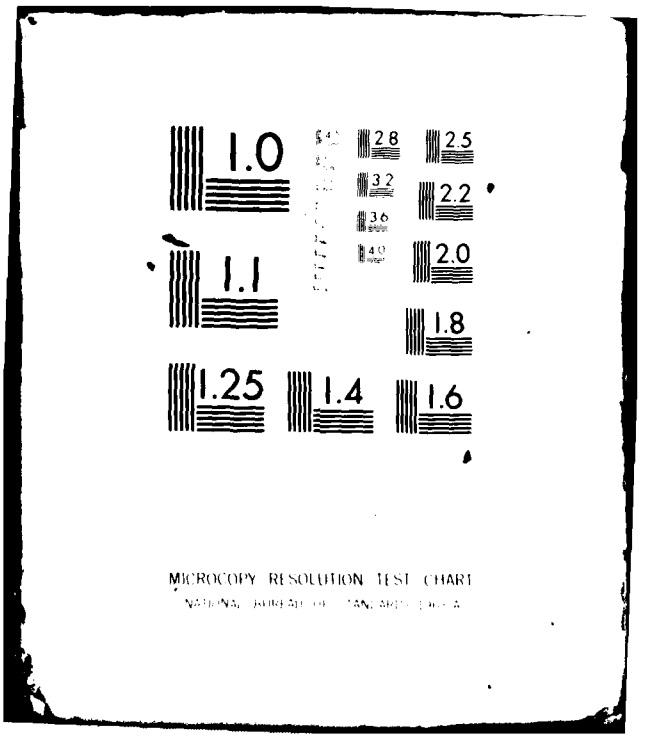
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United States
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Forest Service

**Forest
Products
Laboratory**

Research
Paper
FPL 385

March 1981

Biological Utilization of Wood for Production of Chemicals and Foodstuffs

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ABSTRACT

In the long term, mankind will have to depend on solar energy and photosynthetic processes rather than on fossil materials for energy and material needs. Cellulose and the hemicelluloses, which make up about 70 percent of the dry matter of trees and shrubs, are the most abundant, renewable, raw materials on earth. At present, the highest uses of wood are for structural material and as a source of fiber. There are, however, large quantities of wood residues produced during harvesting and manufacture that might be used. Intensive silviculture can greatly increase the supply of wood for all purposes. This paper reviews the work the U.S. Forest Products Laboratory has done over nearly 70 years to produce chemicals and feedstuffs from wood residues. Wood has been converted successfully to fermentation chemicals such as ethyl alcohol, glycerol, arabitol, erythritol, butanol, acetone, and 2,3-butylene glycol as well as to feedstuffs such as molasses and yeast, and to wood modified to make the polysaccharides digestible by ruminants. At present such use of wood is economically marginal, but is potentially economic in the future.

ACKNOWLEDGMENT

Most of the work reported here was done by scientists and technicians at the U.S. Forest Products Laboratory. The work, however, was greatly facilitated by the generous help and cooperation of Professors W. H. Peterson, Larry D. Satter, Elizabeth McCoy, and J. C. Garver of the University of Wisconsin; Prof. B. R. Baumgardt of Pennsylvania State University; Prof. W. H. Pfander of the University of Missouri; Dr. L. J. Wickerham of Agricultural Research Services, Peoria, Ill.; Dr. J.F.T. Spencer of the Prairie Regional Laboratories, Saskatchewan, Canada; and R. Katzen of Katzen Associates. To these and others whose names we may have inadvertently omitted, we extend our heartfelt thanks and gratitude.

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Forest Service

**Forest
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Laboratory**[1]

Research
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FPL 385

Biological Utilization of Wood for Production of Chemicals and Foodstuffs.

By
GEORGE J. HAJNY, Chemist

BACKGROUND

From prehistoric time to the late 19th century, wood was mankind's principal source of energy for the production of heat and power. Even today, on a worldwide basis, one half of all wood harvested is used for fuel. In the developed countries it makes only a small contribution to the energy budget, but in many developing countries it is still an important source of energy.

In the United States, in colonial times and for many years after, wood was the energy source even though coal deposits were known at the time of the Revolution. It was not until about 1850 that the use of coal as an industrial fuel grew rapidly in the United States, but by 1885 it had surpassed wood.

In the early 19th century, there was no synthetic organic chemical industry. Organic chemicals were natural products either extracted from the raw material, obtained as byproducts, or produced by fermentation.

The production of charcoal and tars is the oldest of all chemical wood-processing methods. Charcoal was probably discovered by an early caveman when he found that the black remains of a previous fire burned with intense heat and little smoke. In all early civilizations charcoal braziers were used for heating and cooking. In the days of the wood sailing ships, naval stores—tar, pitch, and turpentine—were obtained by the destructive distillation of the pines. The softwoods were destructively distilled to obtain the volatiles, with charcoal being a byproduct; with hardwoods, the charcoal was the principal product.

In colonial times hardwood charcoal was produced as a fuel for blast furnaces for the production of pig iron, and as an ingredient in gunpowder. Collection of volatiles from hardwood distillation began in the early part of the 19th century. Pyroligneous acid was refined to produce methanol, acetone, acetic acid, and tars. These byproducts of the hardwood distillation industry were important commercial chemicals until the market was lost to the petroleum industry. In 1920 approximately 100 plants in the United States were recovering these materials from charcoal production; the last of these plants closed in 1969.

Intense and widespread study of coal chemistry began in 1856, when W. H. Perkins discovered mauve, the first coal tar dye. This discovery created the basis for the growth of industrial organic chemistry. A half century later, industrial chemists turned their attention to petroleum chemistry with results that need no recounting. Wood chemistry has never received that kind of attention, although wood at one time was an important source of chemicals.

The soaring cost of petroleum products, shortages of natural gas in some areas during severe winters, long lines of cars at gasoline stations on days when they are open, have shown, as no amount of lecturing could do, that supplies of fossil fuels are indeed finite. Other incidents raise doubts about nuclear energy. Solar energy, in its broadest sense, offers a safe and dependable alternative, especially in regard to plant growth.

An estimate of the productivity of photosynthetic processes on the earth is provided in table 1. The total of 155 billion tons of dry matter per year could, by direct combustion, provide an amount of energy many times the present world consumption. Forests are by far the largest producers of biomass, accounting for 41.6 percent of the total. In the United States it is estimated that the aboveground wood, bark, and foliage on commercial forest land amounts to about 25 billion tons and the net annual excess production over product output amounts to 375 million tons *(171)*.[2] Stone estimated that the

[1] Maintained at Madison, Wis. in cooperation with the University of Wisconsin.

[2] Italicized numbers in parentheses refer to Literature Cited at the end of this report.

lignocellulosic wastes from the forest products and pulp industries amount to 200 million tons annually *(162)*. The total amount of annual residues from agricultural and forest industries in the United States has been estimated at about 1 billion tons.

The use of solar energy can take many forms, but one of particular interest that is being evaluated is renewable photosynthetic materials or biomass for the production of chemicals. At the present time, there is great research activity throughout the country on biomass for the production of organic chemicals, especially ethyl alcohol. Wood is probably the most promising form of biomass. This paper will discuss the work of the U.S. Forest Products Laboratory in research on production of chemicals by fermentation of sugars derived from wood, and some other biological studies directed at increased utilization of wood.

RAW MATERIAL

The world's most abundant renewable organic raw materials are the carbohydrates, represented by the sugars, starches, hemicelluloses, and cellulose. Of these plant materials, cellulose is by far the most abundant. In 1819, Braconnot showed that cellulose or vegetable fiber could be converted into fermentable sugars by concentrated acids *(13)*. From Braconnot's time to the present, much work on the hydrolysis of cellulose has been directed primarily to production of sugars that could be fermented to ethyl alcohol. For example in 1854, two French patents, Nos. 1246 and 2281, had for their respective titles "Manufacture of Ligneous Alcohol" and "Obtaining Alcohol from Organic Substances" *(74)*. One hundred and twenty-five years later, the literature abounds in articles on the production of ethyl alcohol from biomass.

Chemically, wood consists of two groups of components. The major group comprises the structural components of wood and consists of cellulose, hemicellulose, and lignin. The minor group, designated as extraneous and extractive substances, varies greatly in the nature of the compounds and the quantities in the various species. In this group are found tannins, coloring matter, resins, essential oils, fats, waxes, sterols, pectins, gums, starch, organic acids, nitrogen compounds, and mineral matter. Wood contains 40 to 50 percent of cellulose, 20 to 30 percent hemicellulose, 15 to 30 percent lignin, and 2 to 15 percent of extraneous material *(161)*.

The total carbohydrates of the cell wall of extractive-free wood have been named holocellulose by Ritter *(124)*. Holocellulose consists of alpha-cellulose and a heterogeneous mixture of polysaccharides called hemicellulose. Analyses of some representative woods are shown in table 2 *(169)*.

The pentosans, uronic acid anhydride, and acetyl components listed in table 2 are contained in the hemicellulose fraction.

Cellulose is a linear polymer of β-D-glucopyranose units linked by β-(1-4)-glycosidic bonds. The degree of polymerization or number of glucose units per molecule can range from 500 to 10,000 or more depending on the source and processing history of the sample.

In contrast to cellulose, the hemicelluloses of wood are heteropolymers of glucose, mannose, xylose, galactose, and arabinose as well as glucuronic and galacturonic acid. The hemicelluloses exist as linear polymers and also as branched chain molecules. The degree of polymerization of the hemicelluloses is much lower than that of cellulose.

Lignin is a complex three-dimensional polymer whose building blocks are phenyl-propane units. Lignin is a generic term as the name is applied to the material in plants as well as the material from pulping and hydrolytic processes and whose properties differ from each other. The lignin from softwoods contains coniferyl groups, while the lignin from hardwoods is made up of both coniferyl and syringyl groups *(49)*. Lignin is not hydrolyzed by acids as are cellulose and hemicellulose. Lignin appears to have little effect on the acid hydrolysis of wood, but it greatly inhibits hydrolysis of associated polysaccharides by the enzyme, cellulase *(8, 128)*.

The individual sugars obtainable from wood or other forms of biomass can be quantitatively determined by strong acid hydrolysis *(132)* followed by chromatographic separation *(42, 137)*. The carbohydrate composition of a given species of wood is not strictly constant. Fairly large variations can occur. However, the agreement among various workers *(40, 44, 164)* is fairly good.

Table 3 contains representative results from the literature on the distribution of the sugars in softwoods, hardwoods, and two agricultural residues. Softwoods, as a class, contain more mannose and less xylose than do the hardwoods. Galactose and arabinose are minor constituents in all the materials. The agricultural residues closely resemble the hardwoods in the distribution of the sugars. Softwoods contain more lignin than do the hardwoods, which in turn contain more lignin than do the agricultural residues.

Of the sugars produced by the saccharification of wood, only glucose, mannose, and galactose are fermentable to ethyl alcohol by yeast *(167)*. A rapid method for determining the potential fermentable sugars for ethyl alcohol production from biomass is quantitative saccharification by strong acid followed by sorption of the fermentable sugars by *Saccharomyces cerevisiae (90, 133, 134)*. A number of wood species have been analyzed in this way with the results shown in table 4. There are significant differences in yields of sugar from the various woods. Because of the lower lignin content, hardwoods tend to give higher yields of total reducing sugars than the softwoods. However, the fermentability of the sugars from the softwoods is higher than that of the hardwoods. The net result is that the potential fermentable sugar yield from softwoods is higher than from the hardwoods. Thus, for ethyl alcohol production, the softwoods would be the species of choice.

SACCHARIFICATION

Because only simple sugars can be used by yeasts in ethyl alcohol and most other fermentations, it is necessary to hydrolyze the wood polysaccharides. The hydrolysis of cellulosic materials to the monomer sugars appears to be a simple hydrolytic cleavage of glycosidic bonds. As such, one would expect the reaction to be simple, analagous to starch hydrolysis, and the manufacturing costs to be low. Such is not the case, for cellulose is extremely resistant to hydrolysis; this

resistance is the most important factor in determining the cost, methods of production, and character of wood sugar solutions. The glycosidic bonds of solubilized cellulose can be readily hydrolyzed, but the crystalline organization of untreated cellulose results in low accessibility to the dilute acid commonly used as a catalyst *(48)*. As a result, the conditions of temperature and acid concentration necessary to carry out the reaction in a reasonable time cause serious degradation of the sugars produced, resulting in low yields and solutions which are difficult to ferment.

The kinetics of batch saccharification of cellulose with dilute acid was first studied by Luers *(82, 83)*. This early work showed that the yield of sugar from cellulose followed a growth and decay curve that was well represented by consecutive first-order reactions. Changing reaction conditions were believed to affect the reaction rates, but not the maximum yield obtainable. Later work by Saeman *(129)* showed that the maximum yield does change with changes in the reaction conditions. The first-order rate constants for the hydrolysis of cellulose and for the decomposition of glucose were found to be of similar magnitude. The maximum yield of glucose is a function of the ratio of the rates of hydrolysis and decomposition. Saeman's work showed that increasing the temperature and acid concentration resulted in increased efficiency in the conversion of cellulose to reducing sugar *(61)*.

Wood hydrolysis processes which have been carried out on a commercial scale fall into the following categories *(48)*:

1. A single-stage dilute-acid hydrolysis carried out at elevated temperatures without separation of the product as it is formed.

2. A percolation process using a dilute acid at elevated temperatures in which yields are higher than from the single-stage hydrolysis, because the sugars produced are continuously removed as they are formed.

3. A concentrated acid process in which the crystalline nature of the cellulose is destroyed; the cellulose is solubilized, after which it is completely hydrolyzed by dilute acid.

At present, the only industrial wood hydrolysis plants are in Russia where the percolation process is used.

As noted earlier, the two major deterrents to the utilization of lignocellulosic residues for chemical, enzymatic, or microbiological conversion processes are cellulose crystallinity and lignin. Lignin acts by restricting enzyme access to the cellulose, while crystallinity restricts the rate of all three modes of attack on the cellulose. Chemical and physical pretreatments have been investigated to overcome these problems. Tsao et al. *(165)* have shown that cellulose solvents, such as cadoxen or an iron sodium tartrate solution, can decrystallize and dissolve cellulose from biomass and that the precipitated cellulose is easily hydrolyzed enzymatically. Millett et al. *(92)* reviewed the physical and chemical methods for pretreatment of lignocellulosic materials that have been used to increase the reactivity of cellulose toward acid or enzymatic hydrolysis. Chemical methods which induce swelling or dissolution of cellulose, and physical methods such as vibratory ballmilling or electron radiation, can markedly increase the rate of cellulose hydrolysis. Commercial feasibility of any of the pretreatments remains in doubt until the costs of the pretreatment can be shown to be economically viable.

Research on ethyl alcohol production from wood residues has been carried on intermittently at the U.S. Forest Products Laboratory since 1910 with the main emphasis on dilute acid hydrolysis. The earliest work was done in response to the vast amounts of wood residues accumulated at sawmills in excess of their power requirements. Kressmann, in 1922 *(74)* stated that this waste could be obtained for 30 to 50 cents per ton, making the raw material cost per gallon of alcohol from sawdust about 2 cents. This included the fuel charge, as the residue after sugar extraction was available for fuel. In the 1940's the motivating factor was the need for ethyl alcohol in the synthetic rubber program. In the 1970's the primary objective was the use of renewable resources for the production of liquid fuel and chemicals as at least a partial replacement for those produced from petroleum.

Batch Hydrolysis

Simonsen's paper in 1898 *(152)* described the single-stage batch hydrolysis of cellulose and wood residues. Later work by Classen *(17)*, Ewen and Tomlinsen *(27)*, Kressmann *(74)*, Sherrard *(148)*, and others were more or less variations on Simonsen's work. Simonsen concluded that the best conditions for hydrolyzing sawdust were:

Time of hydrolysis	- 15 minutes
Acidity	- 0.5 percent H_2SO_4
Ratio wood to liquid	- 1 to 4
Pressure	- 9 atmospheres

At the Forest Products Laboratory, Kressmann *(74)* conducted a long series of experiments on a pilot-plant scale in which he investigated: (1) the influence of temperature and pressure of digestion, (2) time of digestion, (3) ratio of water to sawdust, (4) concentration of sulfuric acid in water, (5) ratio of acid to dry sawdust, (6) size of wood particles, (7) yields of alcohol from different wood species, (8) fermentation variables, and (9) steam consumption. A porcelain-lined rotary digester with a capacity of 100 pounds of dry sawdust, heated by direct steam, was used for the experimental hydrolysis. The resulting sugars were extracted from the hydrolyzed wood in diffusion batteries, neutralized with lime, filtered, and prepared for fermentation. Maximum yields of fermentable sugars were obtained in 20 minutes at 7.5 atmospheres with a water-to-wood ratio of 1.25 to 1 and a sulfuric acid concentration of 2 percent. Twelve species of softwoods and 10 of hardwoods were hydrolyzed and gave about the same yield of reducing sugars. However, the sugars from softwoods were about 70 percent fermentable while those from hardwoods were 30 percent fermentable by yeast. This is understandable, since under the conditions of hydrolysis, most of the hemicellulose would be hydrolyzed and little of the true cellulose, so the hardwood hydrolyzate would consist chiefly of xylose.

The single-stage batch hydrolysis, which became known as the "American Process" was applied on

an industrial scale in a plant at Georgetown, S.C., in 1913 and then in one at Fullerton, La., in 1916. The Forest Products Laboratory assisted in the development and pilot-plant work of both these plants *(161)*, and in fact Kressmann, formerly at the Forest Products Laboratory, became plant manager of the Fullerton plant *(151)*. Both plants operated successfully during World War I and for several years after. They produced from 5,000 to 7,000 gallons of ethyl alcohol a day from southern yellow pine sawdust and chips obtained from nearby sawmills. During the war the sawmills overcut their holdings so that, as a result of the curtailment of mill operations and a decrease in the price of blackstrap molasses, both hydrolysis plants were forced to close *(151)*.

The Georgetown plant has been described by Foth *(35)* and by Demuth *(22)*. An engineering study of the process was made in which the cost of 95 percent alcohol was estimated at $0.25 per gallon *(148)*. The raw materials required for the production of 3,600 gallons of alcohol per day were estimated *(131)* at:

Wood (dry basis)	- 180.0 tons
Sulfuric acid (80 pct)	- 3.9 tons
Lime	- 2.2 tons
Molasses	- 150.0 gallons
Malt sprouts	- 317.0 pounds
Ammonium sulfate	- 175.0 pounds

At the Georgetown plant, the wood was processed on a 1-hour cycle in four spherical rotary digesters each holding 4,700 pounds of dry wood. The hydrolyzed mass was transferred to a diffusion battery consisting of nine cells, arranged for countercurrent extraction with hot water. Ninety-six percent of the sugar was extracted to give a solution containing about 12 percent total solids, 9 percent reducing sugar, and 6 percent fermentable sugars. The yield of 20 gallons of ethyl alcohol per ton of dry wood was somewhat less than that obtained in the pilot-plant work. The lower yield was attributed to the loss of sugar solution in transferring the hydrolyzed mass from the digester to the diffusion battery.

Further experimental work with the rotary digester continued at the Forest Products Laboratory. Successive treatments with dilute sulfuric acid resulted in sugar yields of about 40 percent of the weight of the wood *(150)*. This, in effect, was a forerunner of the Scholler percolation process. Plow et al. *(120)* showed that, by successive acid treatments with water wash between the acid cycles, yields of ethyl alcohol of over 50 gallons per ton of wood could be achieved. However, the involved operating schedule and time required for the completed run were considered to be serious disadvantages that could be overcome by use of the vertical stationary percolator.

Percolation Process

The rates of sugar production and destruction are of similar magnitudes in the single-stage hydrolysis of cellulose. Thus, the yields ordinarily are limited to about one-third of theoretical. To make the ratio of production to destruction more favorable, Scholler *(142, 143)* developed a dilute-acid percolation process using a vertical stationary digester. The Scholler process has been described by Luers *(82, 83, 84)*, Fritzweiler and Karsch *(36)*, and by Saeman et al. *(135)*. Basically, the process involves the hydrolysis of wood in a vertical cylindrical pressure vessel by a percolation process in which dilute acid is injected into the top of the vessel; acid addition is stopped and the hydrolyzate is withdrawn from the bottom of the vessel. This cycle of addition and withdrawal is repeated until the hydrolysis is complete, which requires from 14 to 20 cycles.

The Scholler plants constructed in Germany contained six to eight digesters of 50 cubic meters capacity. The digester is loaded with chips, sawdust, or shavings from the top. The digester holds about 10 tons of wood, which is packed to a density of 12.5 pounds per cubic foot by steam shocking. The bottom of the digester is equipped with a filter cone, lines for removing the hydrolyzate, and a quick-opening valve through which the lignin is discharged. After the percolator is filled, the wood is heated with direct steam to 135° C. A charge of 1.4 percent sulfuric acid is injected at a temperature 5 to 10° C lower than the percolator contents. After the acid is added, the charge is brought back to temperature. The solution is then "pressed" from the percolator by applying steam to the top of the charge. This operation is then repeated the required number of times with the temperature gradually increased to a maximum of 185° C. The complete percolation cycle requires about 15 hours. Yields of 95 percent alcohol in a well-run plant amounted to about 53 gallons per ton of wood.

Madison Wood-Sugar Process

The rights to the Scholler process in the United States were acquired by the Cliffs Dow Chemical Company of Marquette, Mich., in 1935. Pilot plant studies were made of a modified Scholler process but never commercialized.

Due to the need for ethyl alcohol in the synthetic rubber program, the War Production Board in 1943 requested the Forest Products Laboratory to study the Scholler process. Arrangements were made to use the pilot-plant at Marquette, Mich. but, after some preliminary work, the facilities were transferred to Madison, Wis. The Vulcan Copper and Supply Company was requested to follow the pilot-plant work and to prepare an engineering report which could serve as the basis for the design of a commercial plant suitable for the manufacture of alcohol from wood waste *(28)*.

The pilot-plant equipment for the hydrolysis and fermentation is shown, diagrammatically, in figure 1. The original pilot-plant hydrolyzer was a 27-cubic-foot, 350-pound capacity, vertical digester, made of silicon bronze with a length of 11 feet and a diameter of 2 feet. A second hydrolyzer also used was a 60-cubic-foot, 600-pound-capacity digester made of Monel metal, with a length of 13 feet and a diameter of 2-1/2 feet.

Preliminary work with the pilot-plant equipment was by the Scholler process *(52)*. Taking advantage of the kinetics of the process *(129)*, modifications were soon introduced to reduce the time required and to increase the yield of reducing sugars *(51)*. A rapid percolation process, the Madison Wood-Sugar Process, was developed which is similar to the

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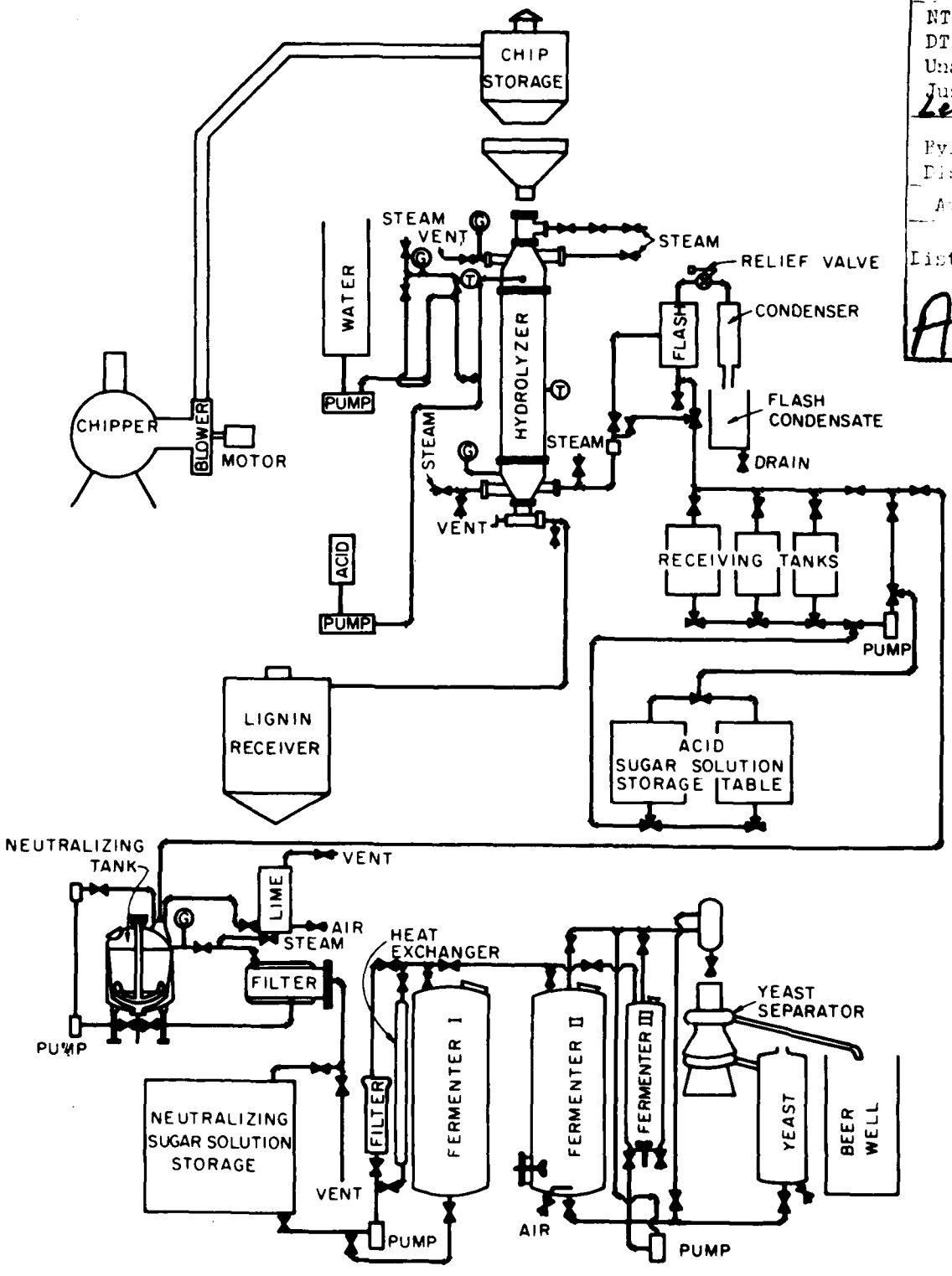

Figure 1.—Equipment for saccharification of wood and fermentation of wood sugar solutions.
(M 149 123)

Scholler method of hydrolysis in that dilute sulfuric acid is percolated through a bed of wood chips in a stationary digester. The Madison Process differs in that, after an initial period of low-temperature hydrolysis, the acid solution is pumped in continuously at the top of the hydrolyzer and hydrolyzate is removed at the bottom with no interruptions until the hydrolysis is complete. A description of the Madison Wood-Sugar Process follows *(80)*:

Madison Process

Wood waste in the form of shavings, sawdust, or chips is loaded into the percolator. When filled, the hydrolyzer is temporarily closed and steam is rapidly injected above the chip bed. This packs the bed and permits more wood to be added. The procedure is repeated once or twice. With Douglas-fir, a density of about 14 pounds per cubic foot was found to be satisfactory. Dense hardwoods could be charged in amounts up to 20 pounds per cubic foot.

With the cover closed securely, steam is admitted to the digester and allowed to pass through the chip bed and out the bottom to remove air and heat the chips. When steam begins to flow rapidly from the bottom vent, this line is closed and steaming continued until the charge reaches a temperature of 145° C. When this temperature is reached, steaming is discontinued and dilute sulfuric acid at 145° C is injected. A calculated quantity of hydrolysis solution containing 2.5 percent acid is first injected to compensate for the diluting effect of the condensate from the steaming operation and the moisture content of the wood. The composite acid concentration in the digester is thus brought to 0.7 percent. The entering acid solution is then readjusted to a concentration of 0.7 percent and a constant flow rate is maintained to give a 2.5 to 1 liquor-to-wood ratio in the hydrolyzer in 45 minutes. The temperature of this entering stream is held at 145° C for 30 minutes and then taken to 170° C in the next 15 minutes.

After 45 minutes of elapsed time, the hydrolyzate control valve is opened at the bottom of the digester and the takeoff rate is adjusted so the liquor level coincides with the chip-bed surface. The hot acid liquor flow rate is adjusted to equal 0.08 times the weight of the dry charge per minute. The temperature of the acid liquor is increased at the rate of 1° C per minute so that, after 1 hour's elapsed time, it is at a maximum of 185° C. At this time, most of the low-temperature hydrolyzate has left the reactor, and hydrolysis of the resistant cellulose is underway in the upper part of the chip bed. The high through-put rate is continued until the sugar concentration in the hydrolyzate stream reaches about 3 percent. The flow rates are then halved and maintained until the sugar concentration drops below 2 percent, when addition of the acid is stopped. Hydrolyzate removal continues until the liquor level in the vessel is 10 to 12 inches below the surface of the chip bed. The blow-down valve at the bottom of the hydrolyzer is opened and lignin residue is discharged by steam pressure into the lignin receiver.

The sugar solution withdrawn from the hydrolyzer is passed to a flash tank where the pressure is reduced to 30 pounds per square inch. The solution is neutralized with lime and filtered at this pressure. Because of the temperature-solubility relationship of calcium sulfate, this procedure avoids subsequent scaling in the stills.

The condensed steam from the flash tank contains the following volatiles *(52)*:

Acetic acid	- 1.50 grams per liter
Formic acid	- 0.19 grams per liter
Methanol	- 0.15 grams per liter
Acetone	- 1.59 grams per liter
Furfural	- 2.4 grams per liter

The lignin recovered in the cyclone has a moisture content of about 70 percent. On pressing to about 45 percent moisture content, sufficient sugar solution could be recovered to raise the total yield of reducing sugars by about 3 percent. The lignin residue from various runs amounted to 25 to 40 percent of the original wood. In representative runs on Douglas-fir, the lignin residue amounted to 29 percent of the original wood and contained 17.7 percent of cellulose. The heating value of the dried residue is approximately 10,800 Btu's per pound.

Yields of reducing sugars obtained from various residues and wood species by the Madison Wood-Sugar Process *(161)* are listed in table 5. Residues high in bark reduce the sugar yield. The potential sugar yields from Douglas-fir residues containing various amounts of bark are shown in table 6.

Experimental Operation

In 1944, design and construction of a Government-financed plant at Springfield, Oreg., was begun by the Vulcan Copper and Supply Company, based on data obtained at Madison. A description of the plant is given by Saeman *(131)*. The plant was designed with a through-put of 220 tons of dry bark-free Douglas-fir wood residues per day, with a production of 10,700 gallons of 190° proof alcohol. The plant with its five percolators 40 feet high and 8 feet in diameter was not completed when World War II ended. One digester was put into experimental operation to determine conditions necessary for successful operation. The production of wood sugars presented some problems, none of which were considered fundamental to the process. The fermentation of the wood hydrolyzate was carried out successfully and confirmed the pilot-plant results. Some 50,000 gallons of 190° proof alcohol were produced. Unfortunately, the plant operators were not financially able to put the plant into full operation so that data on costs were never obtained.

WOOD SUGAR FERMENTATIONS

Wood sugar solutions prepared by the acid hydrolysis of wood have been used in fermentations on a commercial scale to produce only ethyl alcohol or fodder yeast. Many other fermentations have been investigated on a laboratory scale. A schematic representation of the products formed from wood on acid hydrolysis is shown in figure 2, along with the products that have been obtained by fermentation of the hexoses and pentoses *(43)*. Wood sugar solutions have generally been used as prepared with no attempt to separate the hexoses from the pentoses. Many bacteria can utilize both the hexoses and pentoses. On the other hand, while

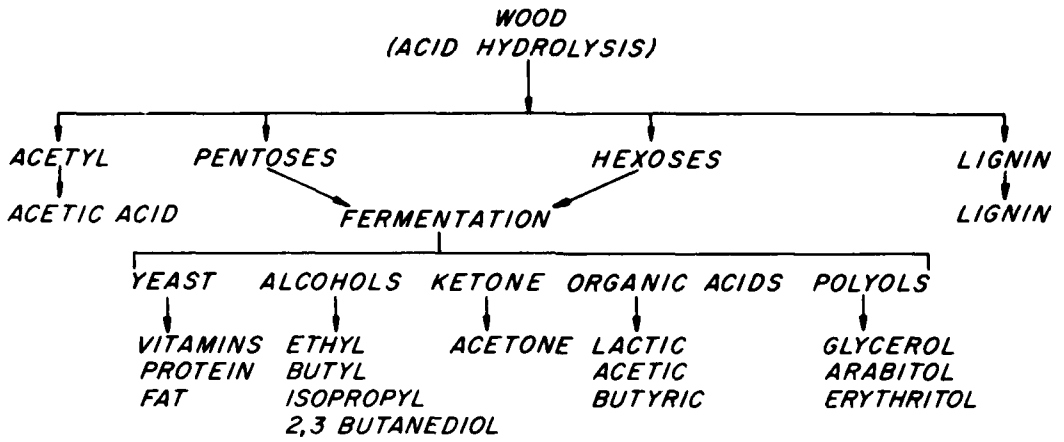

Figure 2.—Potential fermentation products from wood hydrolyzates.
(M 149 065)

yeasts can only use hexoses in anaerobic fermentations *(161)*, some yeasts can also utilize the pentoses in aerobic fermentations *(116)*.

Ethyl Alcohol Fermentation

Wood saccharification processes were developed with the view of obtaining maximum sugar yields and not with the view of obtaining easily fermentable solutions. Patents on treating wood sugar solutions admit the difficulties of fermentation *(29, 108, 144)*, and laboratory procedures have been developed for inducing more rapid fermentations *(33, 75, 85, 116, 153)*. Wood sugar hydrolyzates will differ in their ease of fermentation due to differences in the severity of the hydrolysis conditions and the method used to discharge the sugar solution from the digester. The solution may be cooled to ambient temperature, flashed to atmospheric pressure, or flashed to about 30 pounds pressure and neutralized at this pressure before being brought to atmospheric pressure.

At the two plants which used the "American Process" for hydrolysis, the sugar solution from the diffusion batteries was pumped to a neutralizing tank where milk of lime was added and the liquor at 80° to 90° C was agitated by introduction of air at the bottom of the tank. To avoid "burning" of the sugar, the liquor was circulated by air up through a central cylinder in the tank and returned to the bottom of the tank outside the cylinder. Neutralizing at the high temperature produced a liquor that was easier to ferment than if neutralized at ambient temperature. The neutralized liquor, after settling for about 8 hours and cooling to 30° C, was ready for fermentation. A fresh yeast inoculum was prepared for each fermentation. The inoculum was built up over 24 hours with a cane molasses and wood sugar solution together with malt sprouts, ammonium sulfate, and calcium phosphate. Wood sugar solution was then gradually added. The entire fermentation required about 96 hours for completion.

The slow-feed technique is a means of indirectly using a large initial cell count for inocula and overcoming an unfavorable oxidation-reduction potential *(75, 122)*. Still, 96 hours is a very long fermentation time to produce a beer containing about 2 percent ethyl alcohol. Whereas grain and molasses fermentations give a ten- to twentyfold increase in the number of cells, wood sugar worts give only a two- to fourfold increase during the course of fermentations under similar conditions. Thus, the rate of fermentation is correspondingly low. Although 500 to 1,000 cells per milliliter are sufficient to initiate a slow fermentation in grain wort, untreated wood sugar worts require as many as 100 million cells to initiate the fermentation.

Although the major constituents of wood hydrolyzates are known *(52, 70)*, minor constituents, as yet undetermined, may be the major inhibitors of yeast fermentations. The composition of hydrolyzate from the percolation process is approximately as follows:

	Percent
Reducing sugar as glucose	- 3.0 to 6.0
Sulfuric acid	- 0.4 to 0.8
Organic acids as acetic	- 0.1 to 0.3
Furfural, hydroxymethylfurfural	- 0.1
Wood extractives, lignin products, etc.	- 0.1 to 0.2

Furfural is a decomposition product of the pentoses while hydroxymethylfurfural is formed from the hexoses. Hydroxymethylfurfural can be further decomposed to formic and levulinic acid. Levulinic is relatively nontoxic, while formic acid is toxic *(68)*.

Luers *et al. (85)* determined the concentration of various substances that might be present in wood hydrolyzates required to cause a 25 percent inhibition in yeast propagation or in alcoholic fermentation (table 7). They found that hydrolyzates prepared in the laboratory often were more difficult to ferment than those produced by industrial percolation. This they attributed to the fact that a steam distillation occurs during the flashing of the hydrolyzate during discharge

from the percolator and thus the volatile and most toxic constituents are removed.

Leonard and Hajny *(75)* listed four potential sources of toxic substances which may be encountered in wood hydrolyzates: Carbohydrate decomposition products, lignin decomposition products, extractives present in the wood, and metal corrosion products from the equipment.

Treatments to reduce the toxicity of wood hydrolyzates are many and the work of different researchers are sometimes at odds because the hydrolysis conditions have been different.

Steam distillation *(75)* or steam stripping proved to be beneficial to the fermentation. The stripping was continued until the distillate gave no test for furfural with aniline hydrochloride. Volatiles other than furfural were also removed during the distillation and no doubt contributed to the better fermentation results.

A simple but not commercially practical method of preparing hydrolyzates for fermentation is to adjust the pH to 9 or 10 and then acidify to a pH suitable for fermentation *(108, 153)*. The solution had to be held for only a few minutes at the high pH to obtain a favorable reaction. The original reason for the use of the high pH was to remove heavy metals, which were known to be present, by filtration at the high pH. The filtration step had only a negligible effect. Because the filtration did not improve the fermentation, it was assumed that, instead of removing heavy metals, the action taking place was alkaline reduction of sugar degradation products. The hydroxides of calcium, sodium, barium, and ammonium were equally efficacious for the treatment of the hydrolyzates.

Adsorption on surface-active materials *(116, 144)* or on ion exchange resins have also improved the fermentations. These treatments are especially effective when hydrolyzates containing up to 20 percent reducing sugars are used. As shown in figure 3, a 19 percent wood sugar stock solution was not fermented at all when untreated but, after treatment with activated charcoal or with ion exchange resins, the rate of fermentation was similar to that of a glucose solution.

Treatments that reduce the oxidation-reduction potential of fermentation media and so improve the rate of fermentation are

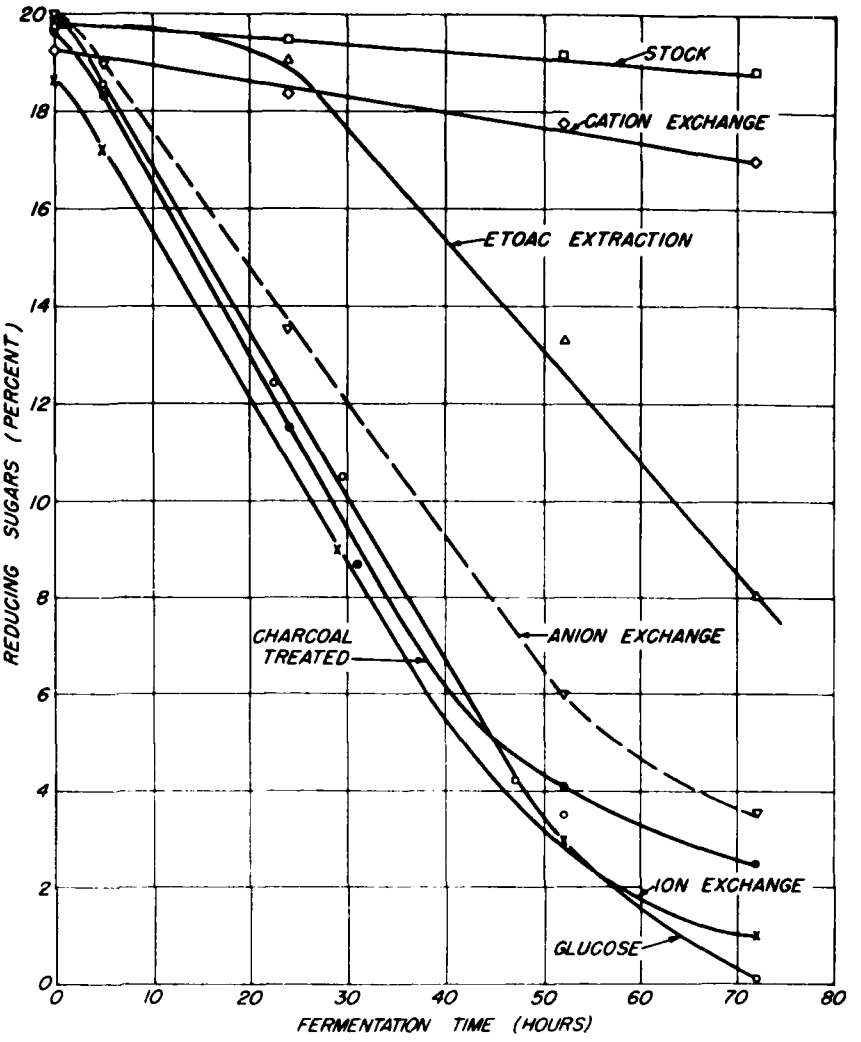

Figure 3.—Rate of fermentation of variously treated wood sugar solutions compared to a glucose solution.

(M 149 218)

phytochemical reduction *(78, 79, 122)*, reducing agents *(18)*, and reducing substances produced by the caramelization of sugars by heat *(31, 39, 122)* or alkali degradation of sugars *(26)*. The oxidation-reduction potentials of various wood sugar worts were found to be between +200 and +250 millivolts. Sodium sulfite, sodium bisulfite, sodium hyposulfite, reduced iron filings, and ascorbic acid were all beneficial in improving the fermentability of wood sugar worts. As little as 0.3 percent of sodium hyposulfite, based on the reducing sugar, resulted in complete utilization of the fermentable sugars in a wort that was not fermentable without treatment *(75)*.

Large inocula can be used to overcome the unfavorable oxidation-reduction potential *(75)*. Since aerobic yeast production on wood sugar solutions is a well-known process *(50, 116, 135, 161)* and because very little contamination is encountered even in unsterilized wood sugar solutions, it is industrially practical to produce a large inoculum and recycle the yeast in subsequent alcoholic fermentations. Recycling of yeast in alcoholic fermentations is a well-known industrial practice *(25, 138, 159)*. Within reasonable limits, the rate of sugar utilization depends on the quantity of yeast (table 8). Rapid fermentations can be made at high yeast concentra-

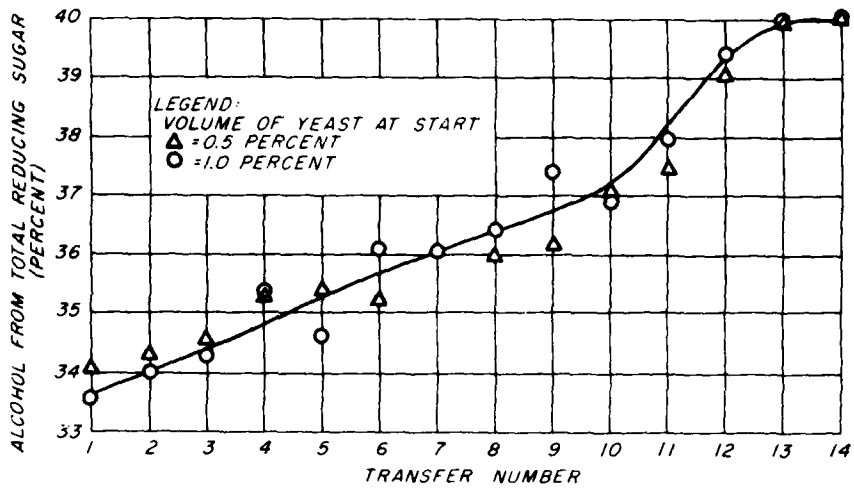

Figure 4.—Increase in alcohol yield by repeated transfer of Saccharomyces cerevisiae. Seven-liter fermentations of Douglas-fir hydrolyzate. Reducing sugar concentration—5 percent.

(M 149 066)

tions; however, agitation is necessary to keep the yeast in suspension. Large inocula are thus desirable both from the standpoint of overcoming the toxicity of the wood sugar solution and of increasing the rate of fermentation.

As many organisms can adapt to their environment, much work has been done to acclimate various yeast species and strains to wood sugar solutions *(53, 57, 66, 116)*. Johnson and Harris *(66)* studied the acclimatization of a number of yeasts for ethyl alcohol production. Inocula of the various yeasts were grown in a glucose-malt sprouts medium. Yeast sufficient to make an inoculum of 1.0 percent wet volume was suspended in 200 milliliters of the wood sugar solution in 1-liter Erlenmeyer flasks with Bunsen valve closures to exclude air. The medium contained approximately 5 percent reducing sugar, 0.1 percent urea, 0.05 percent potassium dihydrogen phosphate, and the pH was adjusted to 5.8. The inoculated medium was fermented at 30° C on a reciprocal shaker for 24 hours. The entire amount of yeast from each flask was recovered by centrifuging and used as the inoculum for the next transfer.

The results of the acclimatization procedure are shown in table 9. All of the cultures showed improvement in the utilization of the reducing sugars and ethyl alcohol yields. Thirty transfers were made, but the maximum alcohol yield was reached by the twelfth transfer and then remained constant. Seven-liter fermentations were made with *Saccharomyces cerevisiae* through 14 transfers and the gradual increase in the alcohol yield with each transfer is shown in figure 4 *(53)*. Fully acclimated cultures of *Torula utilis* No. 3 were used in different sized fermentations to determine if different methods of mixing might affect the efficiency of alcohol production. The results shown in table 10 indicate that, from bench-scale to pilot-plant volumes, consistent results are obtained *(66)*.

In fermentations with several species of yeast, there was no evidence of production of ethyl alcohol from the pentose sugars. The pentoses from softwoods, which represent 15 to 20 percent of the total sugars in solution, constitute a considerable disposal problem in any plant producing only ethyl alcohol from wood sugars. The pentosan content of hardwoods, which is still higher, eliminates the use of hardwoods for alcohol production by the commonly used yeast cultures, except in cases where the pentoses could be profitably recovered and utilized. White and Willaman *(172)* reported that *Fusarium* produced ethyl alcohol from pentose sugars. Later, Nord *et al. (81, 101)* confirmed these results. Using *Fusarium lini* Bolley on the spent beer from a wood hydrolyzate fermentation, about 60 percent of the previously unfermented sugars were fermented but required 16 days *(161)*. Leonard and Hajny *(75)* also investigated the fermentation of xylose and wood sugar solutions with *Fusarium lini*. Yields of alcohol from xylose were in the range of 30 to 35 percent on the sugar used, but the fermentations were extremely slow. Much development work will be required before *Fusarium* can be considered for an alcohol plant.

Wood sugar worts are reputed to contain only inositol and para-aminobenzoic acid *(75)* as growth factors. For recycling of yeast, the only nutrients found necessary to be added to the medium were nitrogen and phosphorus. Nitrogen in the form of urea at 0.1 percent concentration was found satisfactory along with about 0.05 percent of a phosphorus salt. With small inocula, malt sprouts proved to be the best source of growth factors. With large inocula, no added growth factors were found necessary although cane molasses, corn steep liquor, yeast extract, and distillers' solubles gave a slight stimulation to the fermentation.

The most practical method of treating wood sugar solutions industrially for fermentation is by flashing the hydrolyzate to about 30 pounds per square inch, neutralizing with lime at this pressure, and filtering off the calcium sulfate under pressure. Harris and Beglinger *(51)* describe the process as follows:

> The sugar solution being withdrawn from the hydrolyzer is passed into an expansion chamber or flash tank to drop the pressure to about 30 pounds per square inch. The drop in pressure is accomplished by a pressure-regulating relief valve which allows the steam containing vapors of furfural and methanol to pass through a condenser for heat and chemical recovery. From the flash tank, the sugar solution flows under 30 pounds per square inch pressure to the neutralizing tank. An automatic pH controller introduces lime from a tank under air pressure through a proportioning valve to a pH of about 5. The solution, still under pressure, is led to a steam-jacketed leaf filter where the precipitated calcium sulfate is removed. From the filter, the solution is flashed to atmospheric pressure and cooled to 30° C, when it is ready for fermentation.

Neutralizing at the high temperature not only resulted in a sugar solution that was readily fermented by yeast, but a second important feature was the decrease in the solubility of the calcium sulfate. This is particularly important in the distilling section of an alcohol plant where in the interest of heat economy, the primary beer still might be operated under pressure; low levels of calcium sulfate are necessary if scaling of the still is to be prevented. In table 11, results are reported for neutralizing wood sugar solutions at three temperatures. Only a few minutes' holding time is necessary to reduce the calcium sulfate levels to near those reported in the literature for its solubility at higher temperatures.

At the Forest Products Laboratory, it was necessary to use an alcoholic fermentation procedure to evaluate the wood-sugar liquors from the pilot plant before much of the information was available on the behavior of the yeast in the wood sugar solution *(52)*. The yeast used was *Saccharomyces cerevisiae* No. 26 from the University of Wisconsin collection. It was carried in pure culture on glucose and wood-sugar slants. Inocula were prepared by diluting cane molasses to 5 percent sugar, adding 0.05 percent urea and 0.025 percent potassium acid phosphate, adjusting the pH to 4.8 to 5.0, sterilizing and, after cooling, inoculating with the yeast.

The wood sugar solution was prepared by treating with calcium hydroxide at room temperature to a pH of 10 to 10.5. The sludge was then removed by filtration, decantation, or centrifugation. The clear solution was acidified with concentrated sulfuric acid to a pH of 4.8 to 5.6 and nutrients in the same concentration as used in preparing the inoculum were added. In addition, malt sprouts equal to 0.5 gram per 100 milliliters were steeped in the wood sugar solution at 15 pounds per square inch steam pressure and removed.

The fermentation was started by adding the molasses inoculum, equal in volume to 10 percent of the total solution to be fermented, with an equal volume of wood sugar solution. Carbon dioxide evolution was usually apparent after 1 hour and, at 2 hours, another increment of wood sugar was added. At 2-hour intervals, wood sugar solution was added until a total volume of 8 liters was obtained. Specific gravity determinations were made to follow the course of the fermentation. From 30 to 60 hours were required to complete the fermentation. Reducing sugars and alcohol were corrected for the added inoculum.

In this early work, the Scholler method of hydrolysis was used to obtain the wood sugar solutions. A number of wood species, both softwoods and hardwoods, were investigated for reducing sugars and alcohol yields. The results are reported in table 12. From all but one species, the total reducing sugar yields obtained were between about 45 and 50 percent based on the weight of the ovendry wood. The exception was redwood, with a low yield because of the high extractive content, which in the heartwood can amount to 15 to 20 percent *(176)*.

There is a clear distinction between the softwoods and the hardwoods in the fermentability of the reducing sugars produced on acid hydrolysis. Whereas the sugars produced from softwoods are 75 to 80 percent fermentable, those from the hardwoods are in the range of 52 to 62 percent fermentable. This, of course, reflects the difference in the hemicellulose composition between the hardwoods and softwoods as shown in tables 2 and 3. Alcohol yields from the softwoods were in the range of 50 to 55 gallons per ton of ovendry wood, except for redwood. The yields from the hardwoods were lower, of the order of 35 to 40 gallons, again due to the high pentosan content of the hardwoods. Thus, if no profitable outlet can be found for these nonfermentable sugars, only the softwoods could be candidates for an alcohol plant that uses wood as the raw material.

With the development of the previously described Madison Wood-Sugar Process with neutralization of the sulfuric acid under pressure, a sugar solution was obtained that required no further pretreatment, other than addition of nutrients, pH adjustment, and a preformed inoculum. Sterilization of the substrate was not necessary and not used. No serious contamination of the wood sugar solutions was ever encountered. After the Madison Wood-Sugar Process was well in hand, fermentations were made in 55-gallon volumes to more closely simulate industrial conditions. Emphasis was placed on hydrolysis of Douglas-fir residues because amounts of residues of this species, sufficient to supply large hydrolysis plants within reasonable hauling distances, were then available at several locations. A large number of hydrolyses and fermentations were made with the Douglas-fir residues. In table 13, results are shown both for the hydrolysis and yields of alcohol that can be expected when using the Madison Wood-Sugar Process *(53)*.

Although the average yield of 63 gallons of ethyl alcohol per ton of ovendry wood was satisfactory, the use of a new preformed inoculum for each fermentation would be too costly on an industrial scale. The recycling of yeast from fermentation to fermentation was thus investigated. This is a well-known industrial operation *(25, 138, 159)*, but there was concern that, due to the impurities in the wood sugar wort, there might be a loss in yeast vitality. When the wood sugar wort is exposed to air, a fine black precipitate forms which separates with the yeast. What effect this would have on the yeast was unknown.

The pH of the wood hydrolyzate was adjusted to 5.2 and sufficient urea (0.02 pct concentration) and monopotassium phosphate (0.003 pct concentration) were added to allow for a 20 percent increase in yeast when 1 percent yeast by volume was used as the inoculum. This amount of growth had to be allowed for because 15 to 20 percent of the yeast was lost in handling due principally to losses in centrifuging. The temperature of the fermentation was controlled at 30° C in 70-gallon stirred tanks containing 55 gallons of wort. The results are reported in table 14.

In these fermentations, the yeast from the first fermentation was recycled through each of the subsequent fermentations. A 1 percent wet yeast volume was desired as the inoculum. Such a 1 percent wet yeast volume equates to about 100 million cells per milliliter. During the series of fermentations, this yeast volume was maintained. Ethyl alcohol production ceased after 18 to 20 hours but, for convenience of scheduling, the fermentations were run for 24 hours. Approximately 83 percent of the sugars were fermentable and the alcohol yield based on fermented sugars for the 49 fermentations was 47.2 percent. Assuming a theoretical yield of 51.1 percent, a fermentation efficiency of 92.6 percent was obtained. The ethyl alcohol yield of 47.2 per-

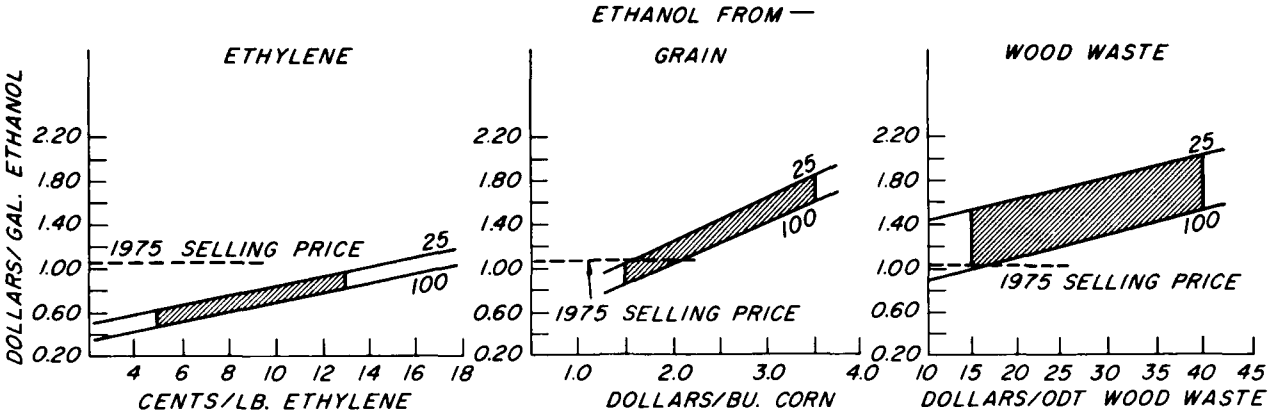

Figure 5.—Selling price of ethanol as made from ethylene, grain, and wood waste (69) at plant capacities of 25 and 100 million gallons per year of 190 proof ethanol.
6(M 149 067)

cent equates to a yield of about 60 gallons per ton of ovendry wood. Although the yeast turned dark in the presence of wood sugar solutions, in the series of 49 transfers, no loss in vitality of the yeast inoculum was evident. It is assumed that the recycling of the yeast can be repeated indefinitely.

A second series of 31 transfers was made in which 300-gallon batches of wood sugar solutions were fermented in a 500-gallon fermentor. The same procedures were used in this series as those for the 55-gallon fermentations. The average values for the 31 transfers were fermentable sugar, 81.1 percent; yield of alcohol on total reducing sugar, 39.4 percent; and on fermentable sugar, 48.2 percent.

The beer from the 300-gallon fermentations was used to obtain information concerning the behavior of these solutions when subjected to distillations in a pressure still. Some 1,200 gallons of this fermented liquor, containing about 1.99 percent alcohol, was shipped to the Vulcan Copper and Supply Company, Cincinnati, Ohio, and distilled in their pilot plant's 61 plate, bubble-cap column. The following information is adapted from their report on this distillation *(53)*:

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. The 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 a 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 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 need for making adjustments in the distillation equipment, but a satisfactory balance between input and yield was obtained. No appreciable amounts of fusel oil were recovered.

The same still was used for the purification of the 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 the rate of 8.3 pounds per hour. Reflux was at the rate of 14.4 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.

The heads were distilled in a glass still and found to contain about 50 percent ethanol, 25 percent methanol, 10 percent acetaldehyde, and 15 percent of a yellow-colored material having some of the properties of diacetyl, but which had a boiling point just below ethanol. Treatment with a small amount of permanganate or strong caustic removed the material.

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 potassium permanganate according to Army Specification F-4f. The material easily met specifications which required that the color remain for 20 minutes.

In 1975 the U.S. Forest Products Laboratory commissioned Raphael Katzen and Associates to study the economics of producing ethyl alcohol and other chemicals from wood waste. The Madison Wood-Sugar Process and fermentation results were the basis for estimating investment and operating costs for an ethyl alcohol plant. These economics were compared with those for producing ethanol from more conventional raw materials *(69)*. The basic design parameter was a production rate of 25 million gallons per year of 190° proof ethanol. The softwood residue mixture was assumed to consist of 25 percent bark and 75 percent wood. The ethanol yield was assumed to be 49 gallons per ovendry ton of the wood and bark mixture. The quantity of wood required to meet the design production rate would be 1,480 ovendry tons per day.

In addition to the ethanol, other products which can be recovered are furfural, methanol, unfermented sugars, and the lignin residue, table 15. The flash condensate contains the furfural and methanol in substantial quantities. The condensate is passed to a distillation column for recovery of the methanol. From the base of this tower, the bot-

toms pass to a second distillation tower from which a furfural-water azeotrope is recovered. Upon condensation, the azeotrope is decanted to obtain a crude furfural. The bottoms from the beer stripping tower contain the pentose sugars. Rather than dispose of these through a waste treatment system, they can be converted to furfural, yeast, or concentrated to a 50 to 60 percent solution for sale as a feed supplement. The lignin residue has a heating value of about 10,000 Btu's per pound. Burning of this residue in a recovery boiler to generate steam at 900 pounds per square inch gage is expected to make the overall facility self-sufficient with regard to steam and power requirements.

Estimates of investment requirements for a 25-million gallon per year ethanol wood waste plant totaled $70 million in 1975. A breakdown of this estimate is given in the report *(69)*. The estimate covers a complete facility, including off-site utilities, bulk storage for raw materials and finished product, and office and laboratory buildings. It includes a contingency of 25 percent and working capital of 5 percent. Scaleup of the plant to produce 100 million gallons of ethanol per year resulted in an investment requirement of $185 million.

In figure 5 the selling price of ethanol from a wood waste facility is plotted as a function of the cost of wood waste and the size of the facility and compared to the selling price of ethanol from ethylene and corn. The selling or delivered price of ethanol includes costs for fixed expenses, raw material, labor, overhead, chemicals, and profit. Fixed costs are based on an allowance of 8 percent for depreciation, 4 percent for maintenance (including labor and material), and 2 percent for local taxes and insurance. A credit is derived from the sale of furfural and pentose. It is assumed that the crude furfural can be sold for 20 cents per pound. The pentose sugar in the form of a concentrated solution was assumed to have a selling price of 10 cents per pound. Profit is based on a nominal return of 30 percent on investment, equal to 15 percent after Federal Income Tax.

Wood waste at $34 per ovendry ton results in an estimated selling price of $1.90 per gallon for a 25-million gallon per year facility. Scaleup of the facility to produce 100 million gallons per year reduces the selling price of ethanol at a cost of wood waste of $34 per ton to $1.42 per gallon (cost estimates as of 1975). The minimum cost of wood waste will be determined by its fuel value with higher or lower costs being site specific. At present, the production of ethanol by dilute acid percolation is technologically feasible but the economic viability is marginal.

Glycerol Fermentation

Glycerol as a normal minor product in alcoholic fermentations was first reported by Pasteur in 1858 *(109)*. The yield of glycerol in the alcoholic fermentation is approximately 2.5 to 3.0 percent of the sugar fermented.

The possibility of increasing these yields significantly was not realized until about 1911 when Neuberg and coworkers *(97, 98)* investigated the mechanism of the alcoholic fermentation. These workers were interested in the occurrence of acetaldehyde as an intermediate in the fermentation. They added sodium sulfite and other chemicals to the fermentations to bind the acetaldehyde formed and to prevent it from undergoing any further reaction. This resulted in a marked increase in glycerol and acetaldehyde and a decrease in ethyl alcohol. For each mole of acetaldehyde bound, one mole of glycerol was produced.

Neuberg and coworkers showed that there was an effect of other alkaline salts that was different from that of the alkaline sulfite. In the alkaline fermentation, alkali causes acetaldehyde to undergo the Cannizzaro reaction to form acetic acid and ethanol along with glycerol.

As a result of these studies, Neuberg proposed the following three forms of fermentation:

1. $C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2$

2. $C_6H_{12}O_6 + Na_2SO_3 + H_2O \longrightarrow C_3H_8O_3 + NaHCO_3 + NaHSO_3 \cdot CH_3CHO$

3. $2\ C_6H_{12}O_6 + H_2O \longrightarrow 2\ C_3H_8O_3 + 2\ CO_2 + CH_3COOH + CH_3CH_2OH$

The first form is the normal alcoholic fermentation. The second is the sulfite fermentation and the third the alkaline fermentation.

Following Neuberg's work, the mechanism of the alcoholic fermentation was intensively studied by many workers. This research culminated in the Embden-Meyerhof-Parnas scheme outlined in figure 6. No attempt will be made to go into the history of its development or a detailed discussion of the various reactions.

Initially, the fermentation of glucose involves the stepwise phosphorylation of glucose to fructose-1, 6-diphosphate. Fructose-1, 6-diphosphate is formed whether glucose, mannose, or fructose is the starting sugar. The diphosphate is broken down into two triose units, dihydroxyacetone phosphate and 3-glyceraldehyde phosphate, which are in equilibrium with each other.

The formation of 1,3-diphosphoglyceric acid from d-glyceraldehyde phosphate (step 6) is the only oxidative step in the formation of ethyl alcohol. This results in the reduction of diphosphopyridine nucleotide (reduced coenzyme I), which must be reoxidized in order to participate in further oxidation. Reduction of dihydroxyacetone phosphate to glycerol phosphate (step 13) and accumulation of acetaldehyde takes place in the "initial phase" of the fermentation. This reduction accounts for the small amount of glycerol found in the normal alcoholic fermentation. When enough acetaldehyde has been formed in this "initial phase" of the fermentation to serve as hydrogen acceptor, the reduced phosphopyridine nucleotide is oxidized in step 12, where acetaldehyde is reduced to ethyl alcohol. At this time, the "stationary condition" of the fermentation is reached and the normal alcohol fermentation continues.

With this mechanism of the alcoholic fermentation, anything which prevents the acetaldehyde from acting as a hydrogen acceptor, without interfering with the alternate pathway for glycerol formation, will tend to result in increased glycerol yields. In the sulfite fermentation, the acetaldehyde sodium bisulfite complex is formed. The enzyme system *cannot* reduce this complex, so the alternate pathway is used and glycerol becomes the major fermentation product.

Theoretical yields of glycerol have never been achieved, for some of the

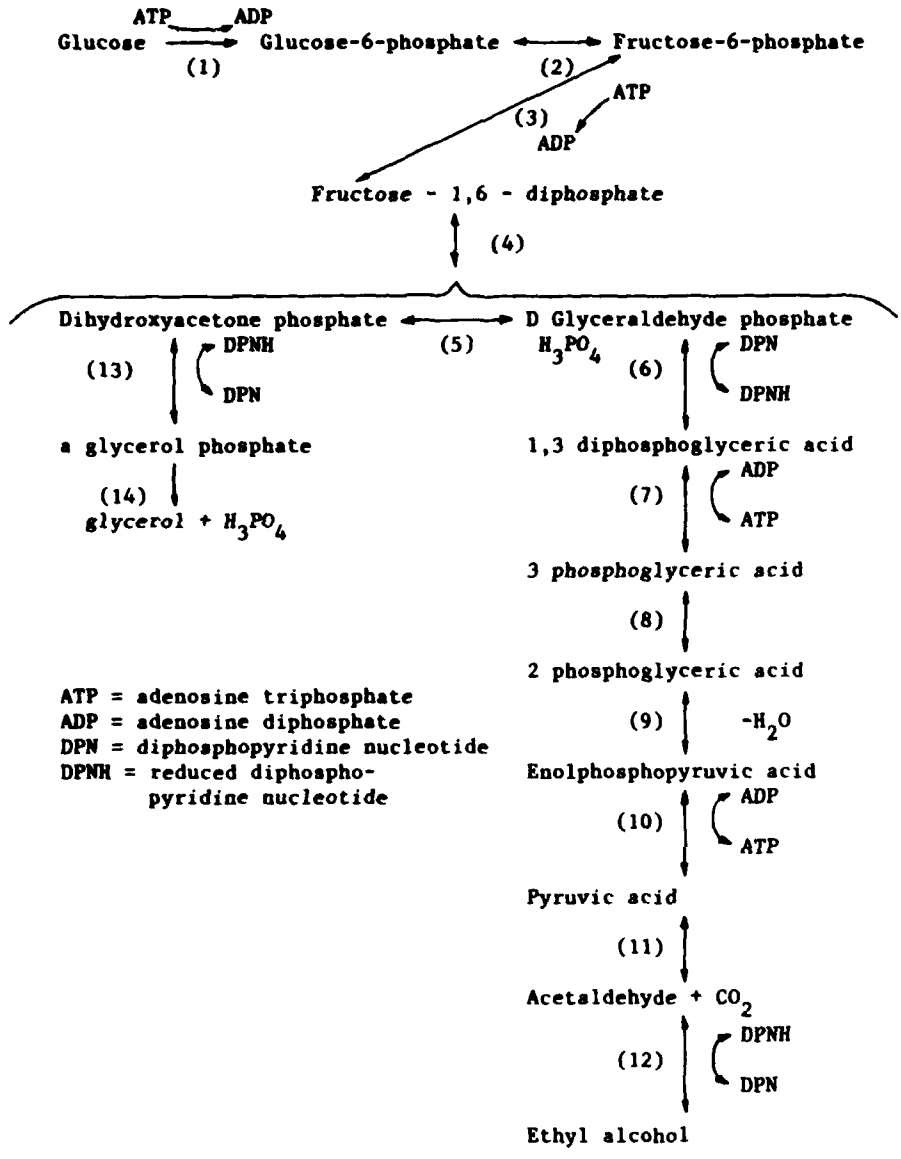

Figure 6.—Embden-Meyerhof-Parnas fermentation scheme.
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acetaldehyde is still reduced to ethyl alcohol. The bisulfite ion is toxic to yeast, so that high concentration of the free bisulfite must be avoided. On the other hand, too low a concentration of bisulfite results in lowered glycerol yields. High concentrations of bisulfite result in long fermentation times and high yields, while low concentrations of bisulfite result in short fermentation times and low glycerol yields. Thus, satisfactory fermentations are the result of compromises on length of fermentation and yield of glycerol.

A review of the development of the fermentation procedures for glycerol has been given by Underkofler *(167)*. The fermentation method employed in the Forest Products Laboratory (FPL) investigation was fundamentally similar to the various bisulfite addition fermentations Underkofler described, but the FPL version had several important modifications. The process was put on a continuous basis with automatic control of pH, bisulfite ion concentration, and temperature *(45, 60)*.

In any fermentation, the most important consideration is the organism used. From the mechanism of the alcoholic fermentation (fig. 6), it would seem that any yeast which is a good alcohol producer would be a good glycerol producer if it were tolerant to the chemical used to block the reduction of acetaldehyde to alcohol. The yeast used in this study was *Saccharomyces cerevisiae* No. 49 from the Laboratory's culture collection. This culture was chosen for its proven ability to produce ethyl alcohol in high yields. Cultures of *Saccharomyces cerevisiae, Debaryomyces* sp., *Monilia murmanicum, Schizosaccharomyces pombe, Torulopsis utilis, Torulopsis pulcherrima, Hansenula anomala, Zygosaccharomyces acidifaciens, Eudomyces vernalis, Geotrichum lactis,* and *Rhodotorula gracilis* were screened, with and without bisulfite addition, for their glycerol-producing ability. *Saccharomyces cerevisiae* No. 49 proved to be one of the best cultures for glycerol production.

The early work on the fermentation was done on a batch process. Later, when fermentation conditions had been determined, the process was made continuous.

Whereas the bisulfite ion is toxic to yeast, the sulfite ion is much less so. However, the bisulfite ion is necessary in the fermentation, since it, and not the sulfite ion, combines with the acetaldehyde. The equilibrium between the bisulfite and sulfite concentration is determined by the pH of the solution. In the presence of bisulfite, the yeast grows very little; thus the usual method for an alcoholic fermentation, in which only a small amount of yeast is added to the medium and in which the yeast will grow up to a suitable concentration, is not feasible. The full amount of yeast necessary for the fermentation is grown in bisulfite-free solution and then added to the medium at the start of the fermentation.

It would obviously be uneconomical to grow a new inoculum for each fermentation, so efforts were made to reuse the yeast from one fermentation to another. In the batch process, the fermentation time for a 20 percent sugar solution was approximately 5 days. This long contact time with the bisulfite made the yeast quite inac-

tive. It was found, however, that if the inactive yeast were placed in a sugar solution which contained no bisulfite, it was reactivated in 2 to 3 hours. Bisulfite could then be added and the glycerol fermentation repeated. Handling the yeast in this manner, it was found that about a 10 percent increase in yeast occurred in each cycle. Yeast has been reused in this way for 6 months at a time with no apparent decrease in the vigor of the yeast or yields of glycerol.

Careful analysis of the beer revealed that, in addition to the glycerol, acetaldehyde, ethyl alcohol, and residual sugar, small amounts of acetic and lactic acids were present. The acetate in the beer can result either from the action of bacteria or from small amounts of the acetaldehyde undergoing the *Cannizzaro* reaction. The *Cannizzaro* reaction becomes more prominent if the pH is raised and will be insignificant if the pH is lowered. However, a decrease in pH results in a pronounced decrease in the rate of fermentation.

The presence of sodium lactate in the fermentation product indicates the presence of bacterial contamination. These bacteria are not active in the fermentor, since the presence of the bisulfite has an inhibiting effect. They are active in the recycling equipment where the bisulfite ion is not present in sufficient concentration to retard their activity. It has been found that, if the yeast cream is given an acid wash at a pH of 3 to 3.5 *(12)* prior to rejuvenation, the lactic acid bacteria can be kept at a very low level. Proper handling of the recycle yeast will eliminate the lactic acid bacteria almost entirely. In the experimental work, yeast was recycled daily for a year with no change in strain or accumulation of contaminating organisms.

Aside from the sodium bisulfite and sodium carbonate necessary to control the pH, the medium used for the bisulfite glycerol fermentation is as follows:

Constituent	Percent
Sugar	20.0
Urea	0.075
Disodium phosphate	.048
Potassium chloride	.016
Magnesium sulfate	.004
Blackstrap molasses	.25

The medium is made up with tap water and is not sterilized. Blackstrap molasses, which is added as a source of growth factors for the yeast, is diluted with four volumes of water and sterilized before being added to the medium.

Sodium bisulfite and sodium carbonate are added to the fermentation to control the pH and bisulfite ion concentration of the medium. There is interaction between these variables and it is important to maintain close control of the bisulfite ion concentration for several reasons. As already noted, low concentrations are ineffective in binding the acetaldehyde so that glycerol yields decrease and alcohol yields increase. High bisulfite levels decrease the activity of the yeasts, resulting in slower fermentation rates and higher levels of residual sugar. This results in lower yields on sugar charged and increased difficulty in recovering the glycerol. It is important to control the pH because at low pH levels (6.0 and below), fermentations are slow and erratic and, in fact, the fermentation may stop. If the pH is increased, more sodium ion than is necessary is added to the medium, which results in increased costs of chemicals and product recovery. The most suitable pH range has been found to be between pH 6.3 and 6.6.

It is impractical to add all the bisulfite necessary at the start of the fermentation, since the activity of the yeast would be very low. Likewise, manual addition of bisulfite throughout the course of the fermentation results in cyclic variations in the concentration of the bisulfite ion. An automatic control for maintaining the bisulfite ion concentration was, therefore, developed. This is accomplished by the continuous withdrawal and titration with iodine solution of a small sample stream from the fermentor. The mixed iodine and fermentation medium is led into a cell which contains a saturated calomel electrode and a platinum electrode. The electromotive force developed by this cell is used to activate a controller. There is a well-defined change in the electromotive force from a solution containing an excess of iodine to one containing an excess of bisulfite. At the slightly acid pH of these solutions, the bisulfite bound to the acetaldehyde has no effect.

Optimum fermentation conditions were studied with controlled bisulfite and pH. In table 16, the effect of bisulfite concentration at constant pH, sugar concentration, and yeast volume is shown on the yield of glycerol and time of fermentation. Increase in sugar concentration does not have a large effect on glycerol yield. Some variations in bisulfite concentration can be tolerated with little effect on the fermentation if pH is held at a proper level. Increase in amount of yeast present in the fermentation results in a faster fermentation. From the results of these studies, conditions chosen for the fermentation were a yeast volume of 2 to 3 percent, a free bisulfite concentration of 0.5 to 1.0 percent, and a 20 percent sugar solution.

A fermentation balance was made to determine whether or not all the products of the fermentation were accounted for. A 25-gallon fermentation was run in the usual manner, except that a closed fermentor was used and the carbon dioxide evolved was measured in a wet test meter. The results of the fermentation balance are given in table 17. The products account for 98.9 percent of the carbon in the sugar charged. Thus, these compounds were assumed to be the only components present except for small amounts of protein coming into solution from the yeast. This assumption was further substantiated by calculating the oxidation-reduction balances that gave an O/R ratio of 0.993, while theory calls for a ratio of 1.0.

From the background information gained on batch fermentations, a continuous process was developed. Two fermentors were connected in series. Sugar solution of 20 percent W/V concentration and yeast cream was metered to the first fermentor. Bisulfite ion level was maintained in the fermentor through use of the titration controller previously described; pH was controlled with an industrial model pH controller, which fed a solution of sodium carbonate to the fermentor as required. Both fermentors were jacketed, and the temperature was maintained at 32° C. Nutrients were added to the fermentation by combining the necessary ingredients, as discussed previously, with the yeast cream.

The holding time in the first fermentor was 24 hours and, at this throughput rate, the sugar feed of 20 percent was reduced to an effluent of approximately 1 percent sugar. The effluent from the first fermentor was

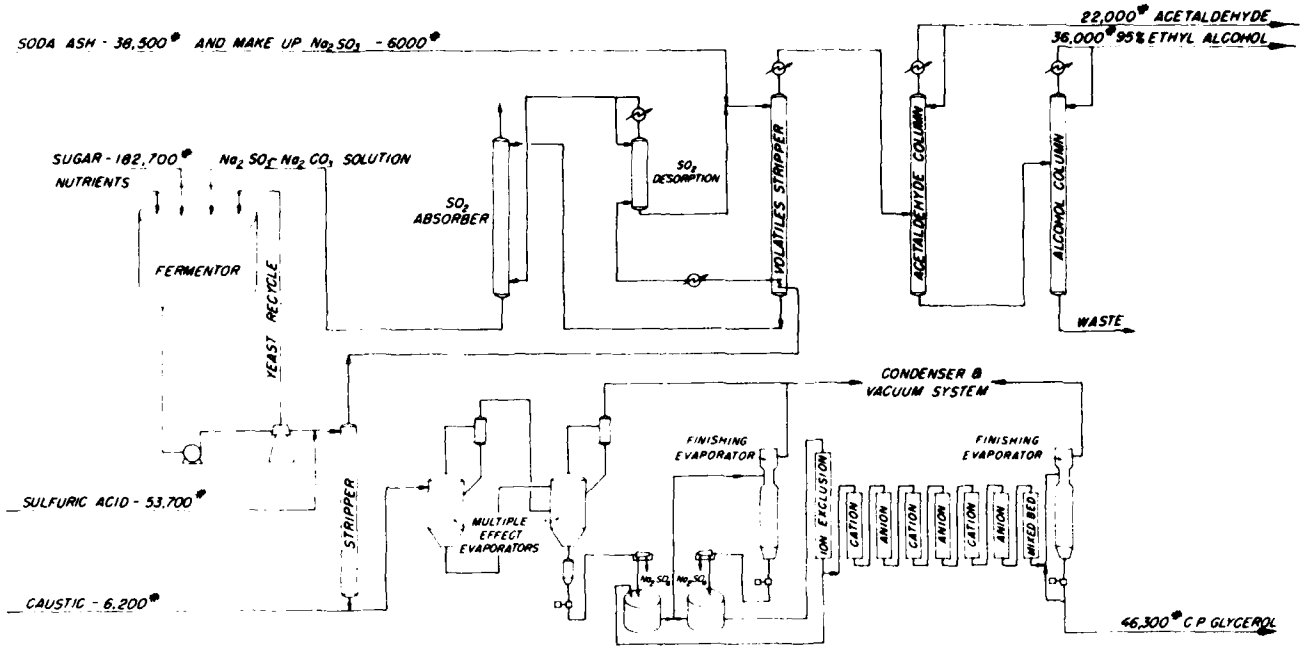

Figure 7.—Flow sheet for 15-million-pound-per-year fermentation glycerol plant. (ZM 107 749)

held in the second fermentor for an additional 12 hours. Introduction of a small amount of air during this period reduced the residual sugar concentration to less than 0.1 percent. The beer from the first fermentor contained about 0.7 percent free sodium bisulfite. No additional bisulfite was added to the second fermentor so that, at the end of the second holding period, the amount of free bisulfite had declined to approximately 0.3 percent.

The beer from the second fermentor was sent to a centrifuge which removed the yeast as a thick cream. The clarified beer was sent to recovery while the yeast was recycled. The yeast cream was first acidified with sulfuric acid to a pH of about 3.0 and held at this pH for 1 hour. The nutrients, including the blackstrap molasses, were then added to the yeast cream. This treatment brought the pH of the yeast to about 5.0. The cream was then ready for recycling through the processes. This method of handling the yeast resulted in a net increase of about 10 percent per day. The excess yeast was discarded.

The handling of the yeast for recycle is a very important step in the process, for here is the phase where the minor constituents of the beer, which become very important in the recovery process, are controlled. In table 18, an analysis of the centrifuged beer for carbon content is given, together with a determination by combustion of the total carbon in the beer. The carbon analysis, made by determination of the individual compounds in the beer, agrees well with the total carbon determined by combustion analysis. The values in the table indicated that all the compounds in the beer have been determined, and that the minor constituents have been held at a satisfactory low level. This analysis was made after the continuous process had been in operation for several months and indicates that no buildup of undesirable compounds had occurred.

The materials and the products formed in the continuous fermentation are shown in table 19. Yields of products are average values taken during extended runs of the fermentation. The yield of glycerol on sugar charged is 55.5 percent of that theoretically attainable and that of alcohol, 40.8 percent of the theoretical. Acetaldehyde is produced in almost a mole per mole ratio with glycerol, its yield being 55.2 percent of theoretical. These yields of products changed only slightly over rather large changes in fermentation conditions. Thus, control of the fermentation is comparatively simple. After some familiarity with the process, it was found that adequate control of the fermentation could be achieved by merely metering the feed, yeast cream, sodium bisulfite, and sodium carbonate solutions. Quick daily analysis for free and bound sulfite, pH, and yeast volume, with slight adjustments to the metering pumps if necessary, was sufficient to keep the fermentation running smoothly.

Underkofler *(167)* points out that the main problem in commercial development of the fermentation production of glycerol is the difficulty in separating the product from large amounts of salts and other materials present in the fermented beer. The recovery procedures suggested by the patent literature are nearly all based on solvent extractions or distillation. Investigation proved that these methods had serious disadvantages when used with this feedstock. In the case of solvent extraction, suitable solvents with high selectivity could not be found. With distillation, losses of glycerol in the still bottoms and decrease in product quality were excessive.

In the recovery method developed at this laboratory, the solution is first acidified, enabling all volatiles to be removed. Sodium sulfate is then

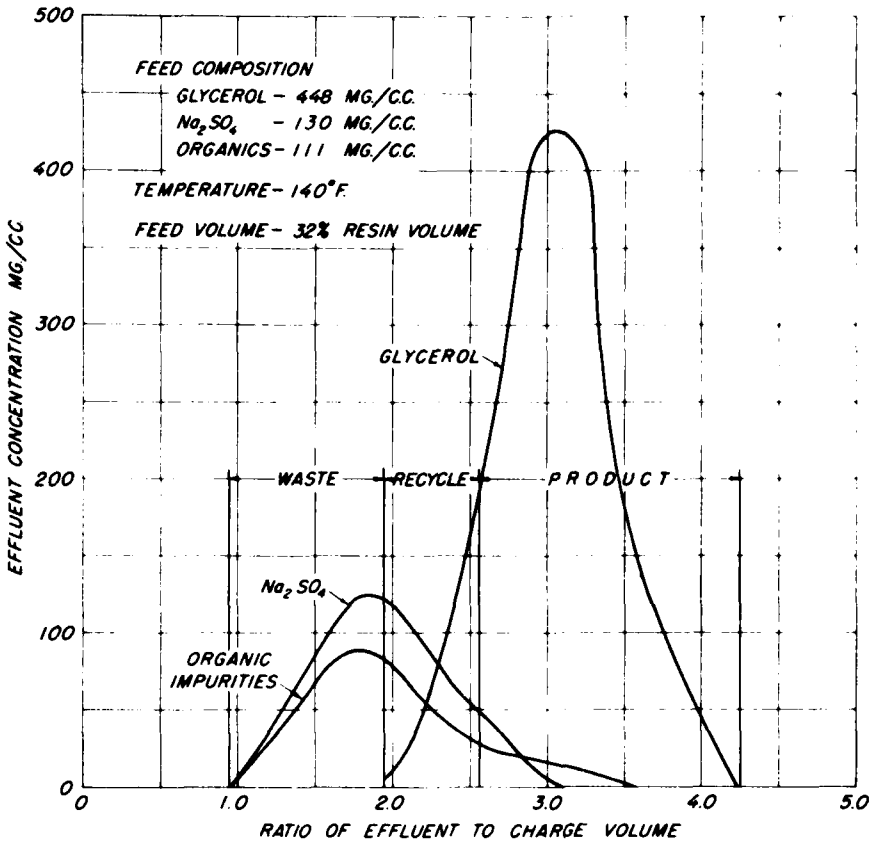

Figure 8.—Effluent concentrations from the ion-exclusion column (60). (M 118 337)

removed by crystallization and the product finished using an ion-exclusion, ion-exchange combination *(121)*. The process flow sheet is shown in figure 7.

The centrifuged beer from the fermentation is acidified with strong sulfuric acid and charged to a stripping column. The quantity of acid added must be sufficient to reduce the pH to less than 1.0. At this pH, the acetaldehyde-bisulfite complex is highly dissociated and the sulfur dioxide and acetaldehyde exert their normal vapor pressures. During the stripping operation, ethyl alcohol, acetaldehyde, sulfur dioxide, and carbon dioxide are removed in the overhead. The dissociation rate of the complex is high and is not a limiting factor in the stripper operation. Using the conditions described, over 95 percent of all volatile components can be easily removed.

Construction of the stripping tower and packing must be of corrosion-resistant material because, after acidification, the solution is quite corrosive. Immediately after the volatiles are removed, the solution is neutralized. The neutralizing stream can be introduced into the still portion of the column so that the corrosive conditions are limited to the tower and packing.

The composition of the streams associated with the stripper is shown in table 20. The overhead stream is processed to recover the sulfur dioxide, acetaldehyde, and ethyl alcohol. The first step in the recovery is the condensation of approximately 25 percent of the sulfur dioxide as an aqueous solution. The acetaldehyde and alcohol is separated from the remaining sulfur dioxide by adding sodium carbonate, which effectively binds the sulfur dioxide and allows the acetaldehyde and alcohol to be removed and rectified. The alkaline stream is then used to absorb the sulfur dioxide removed previously. This method of recovery is necessary to avoid adding excessive amounts of sodium in the process.

The neutral solution from the stripper is evaporated to a 60 percent glycerol concentrate as a feed for the ion-exclusion column. The first step is the concentration to about 13 percent glycerol, which is the saturation point. This glycerol solution is then sent to the crystallizing evaporators where the glycerol concentration is maintained at 30 percent. At this concentration, the sodium sulfate crystallizes as the decahydrate. At higher concentrations of glycerol, it precipitates as small crystals of the anhydrous salt which are difficult to free from the viscous glycerol solution. For this reason, the solids precipitated in the finishing evaporator, where the concentration of glycerol is brought to 60 percent, are recycled back to the crystallizer and all the sodium sulfate removed during evaporation is in the form of the decahydrate. The composition of the various streams involved in the evaporation step are presented in table 21.

The glycerol solution from the finisher evaporator is then fed to the ion-exclusion column. The ion-exclusion technique is basically a chromatographic separation. Non-ionic components are strongly absorbed by ion-exchange materials, whereas ionic components are almost totally excluded from the resin phase. The resin used in this work was Dowex 50, X- 8, 50- 100 mesh in the sodium form. The method differs from the usual ion-exchange as no chemical reaction takes place. The process consists of alternately feeding the solution, which contains the solutes to be separated, and the pure solvent (water in this case), to a column of ion-exchange material. As the solution proceeds down the column, a separation occurs because the non-ionic components will be absorbed on the resin and be retarded.

Typical effluent concentration curves for this feedstock are shown in figure 8. The separation of the glycerol from the sodium sulfate and extraneous organic impurities is apparent. Separation of the organic impurities indicates that they are either ionic impurities, such as acetates or lactates, or have too-large a molecular structure to enter the pore structure of the resin.

The product stream from the ion-exclusion column is de-ionized to obtain an aqueous glycerol solution of high purity. The ion-exchange unit consisted of two cation-anion pairs and a mixed bed. The product obtained on evaporating the ion-exchanged solution was water white.

It met all U.S. Pharmacopeia specifications except for the silver reducing test.

Polyol Production by Osmophilic Yeasts

At the Forest Products Laboratory, the program for the investigation of fermentation glycerol followed three main paths: (a) the sodium bisulfite-steered fermentations, (b) production of yeast mutants, and (c) glycerol production by osmophilic yeasts.

The work on the production and testing of yeast mutants has been published *(178)*. As enzymes are under genetic control, it seems possible that a genetic block at one of several points might result in higher yields of glycerol. A mutant producing large amounts of acetic acid should give high yields of glycerol, because formation of acetic acid is coupled with glycerol production. No such mutant was found but two of them did give somewhat more glycerol than the parent; one of these two grew faster and tolerated bisulfite better than a high-yielding strain of *Saccharomyces cerevisiae*.

Osmophilic yeasts have generally been studied with respect to their ability to grow in high concentrations of sugar, but very little attention has been paid to the kind and amount of products formed. Nickerson *(99)* isolated a yeast from sour wine that he classified as *Zygosaccharomyces acidifaciens* and later with Carroll *(100)* studied the products formed by this culture. Besides ethyl alcohol and carbon dioxide, this yeast produced lactic and acetic acids and glycerol. Spencer and associates *(127, 154, 155, 156, 157, 158)* investigated this and other species of *Zygosaccharomyces* as well as osmophilic isolates from honey and other sources specifically for the production of glycerol. In addition to glycerol, D-arabitol, erythritol, and mannitol were produced. A urea-yeast extract medium of low inorganic phosphate content at 39° C, with adequate aeration, promoted formation of the polyhydric alcohols. The use of oxygen in the fermentation is a distinctive feature of their work. Later, Onishe *(104, 105, 106, 107)* studied the characteristics of the osmophilic soy yeasts and found that isolates identified as *Saccharomyces rouxii* and *Pichia miso* produced polyhydric alcohols in aerobic fermentation. D-arabitol was the principal polyhydric alcohol found, although glycerol was also an important product.

At the Forest Products Laboratory, investigations were undertaken with the objective to find, if possible, a yeast strain that would produce glycerol as the only high-boiling constituent in the beer so that recovery problems would not be complicated by other high-boiling or nonvolatile products *(15, 46, 114)*. Several species of *Zygosaccharomyces* were examined in this study. The classifications of these are shown in table 22. The numbering and classification are those which were on the cultures when they were received. Cultures *Zygosaccharomyces priorianus* IP-22 and *Z. wyocena* IW-1 were obtained from the Department of Bacteriology of the University of Wisconsin; cultures *Z. barkeri* Y-12, *Z. mellis*, *Z. mussbaumeri* Y-6, and *Z. rugosus* came from the Prairie Regional Laboratories, Saskatoon, Saskatchewan, while all others were obtained from the Northern Regional Research Center, Agricultural Research Service, Peoria, Ill. Stock cultures were carried on a medium consisting of 20 percent glucose, 1 percent yeast extract, 0.1 percent urea, and 2 percent agar. Fermentation medium was the same as the stock slant medium with the agar omitted. The fermentations in 500-milliliter Erlenmeyer flasks were incubated at 35° C on a rotary shaker. Aeration rate was approximately 42 mM oxygen per liter per hour. As can be seen, ethyl alcohol, glycerol, and arabitol were the principal products of the fermentation, table 22.

The effects of aeration rate, temperature, glucose concentration, yeast extract concentration, phosphate concentration, vitamins, and trace minerals on the fermentation were examined. Production of glycerol and arabitol is probably the result of the interrelation of many factors. Foremost among these is the inherent factor, that is, the kind of yeast. External factors that affect polyol production appear to be:

A. Limited but adequate growth. There is more growth than under anaerobic conditions, but much less than under truly aerobic conditions. A yeast extract level of 1 percent was too high, and 0.2 percent was too low. The lower concentration resulted in increased polyol yields, but prolonged the fermentation unduly.

B. Adequate but not excessive aeration. Aeration is necessary to produce the hydrogen-carrying enzymes that are required for the formation of the reduced products. Aeration is also interrelated with glucose concentration. While 42 mM oxygen per liter per hour is adequate for 10 percent glucose, it is not for 20 percent glucose. Similarly, if the aeration of a 10 percent glucose medium is aerated at 360 mM oxygen per liter per hour, polyol yield will decrease.

C. Limited inorganic phosphate. Under the conditions of the fermentation, 0.001 M phosphate was sufficient. If larger quantities are used, polyol formation will be depressed.

Because no *Zygosaccharomyces* culture was found that produced only glycerol as a high boiling product, osmophilic isolates were surveyed to find a culture meeting this requirement *(46)*. Yeasts were isolated from fresh pollen, brood comb honey, brood comb pollen, and from clover heads growing near a bee colony. The carrier material was placed in Erlenmeyer flasks containing a 40 percent glucose, 1 percent yeast extract medium, and incubated with shaking at 30° C until good growth had taken place. Then the cultures were streaked on 20 percent glucose, 1 percent yeast extract, 2 percent agar plates, and individual colonies were picked from these and transferred to stock slants. The medium for the stock slants was the same as for the isolating plates with the addition of 0.1 percent urea. Stock cultures were transferred every 2 weeks because the ability of some of the cultures to produce polyols was lost if the cultures were not transferred every 3 or 4 weeks.

Fermentation media were made up with approximately 10, 20, and 30 percent glucose concentration and 0.5, 1.0, and 1.5 percent yeast extract, respectively. The urea concentration was held constant at 0.1 percent. Departures from these standard media are noted where necessary. The media were sterilized by autoclaving at 121° C for 15 minutes. The pH of the media was about 6.2 to 6.4 both before and after autoclaving.

Inocula were prepared by loop transfers from stock slants to 50 milliliters of the 20 percent medium in 500-millliliter Erlenmeyer flasks. These

were incubated at 35° C for 72 hours on a rotary shaker that describes a 1-inch circle at about 250 revolutions per minute. Two and one-half milliliters of inoculum were used with 50 milliliters of fermentation medium in 500-milliliter Erlenmeyer flasks and incubated on the rotary shaker. Analyses were made at 24- hour intervals during the fermentation and incubation was continued until most of the glucose had been used.

No quick test for determining glycerol production, as by color reactions by the colonies on agar plates, could be developed. Therefore complete fermentations and analyses for products had to be made with each of the isolates. The results of the screening fermentations are given for 21 of the cultures in table 23. All of the isolates, including those not listed in table 23, have the common characteristic of producing one or more of the polyhydric alcohols, although in several cases only trace quantities were produced.

As was to be expected, yeast growth and rate of sugar utilization varied widely among the cultures. There was a three-fold variation in yeast growth as measured by cell volume. Some cultures utilized a 20 percent glucose solution in 72 hours, whereas other cultures did not utilize this amount of glucose in 144 hours. All cultures produced ethyl alcohol as one of the products. Glycerol was produced by most cultures but, in most cases, only in small amounts. The majority of cultures produced D-arabitol. Erythritol was produced by a few.

The cultures of outstanding interest in table 23 are I_2B, I_{11}, I_2A, I_3C, and $I_{22}A$. I_2B is of interest because glycerol is the only polyhydric alcohol produced by it in any quantity and I_{11} is of interest because of its outstanding ability to convert glucose to arabitol. I_2A, I_3C, and $I_{22}A$ are of interest since they produce the rare alcohol, erythritol.

Culture I_2B was selected for further study because of its glycerol-producing ability and of the absence of other polyhydric alcohols in the beer. A transfer of this yeast was identified by Dr. Lynferd J. Wickerham of ARS as *Torulopsis magnoliae*, Lodder et Kreger-van Rij.

The results of a study of the effect of temperature on fermentation with culture I_2B are shown in table 24. A temperature of 42° C is obviously too high, for yeast growth was small and sugar utilization was very slow. Glycerol yield was little affected by a change in temperature from 30° to 35° C. However, because sugar utilization is considerably faster at 35° C than at 30° C, 35° C was chosen as the standard temperature for this fermentation. It will be noted that glycerol yields at 35° C were considerably higher at 48 hours than at 72 hours. This culture is able to utilize glycerol quite well so, to obtain maximum yields, the fermentation must be stopped as soon as the sugar content of the medium is exhausted.

The chief ingredient in the medium aside from glucose is yeast extract. Yeast extract concentrations were varied from 0.125 to 1.0 percent as shown in table 25. At the lower levels of yeast extract, yeast growth was poor and sugar utilization was very slow. With 0.125 percent yeast extract, 80 percent of the sugar remained after 72 hours; with 0.5 and 1.0 percent yeast extract, substantially all of the glucose was used in 48 hours.

Yeast extract concentration has a pronounced effect upon the formation of products. High concentrations favor alcohol formation with lower glycerol production, whereas at low concentrations of yeast extract, only trace amounts of ethyl alcohol are formed. A yeast extract concentration of 0.5 percent was chosen as a standard for obtaining optimum yields of glycerol in reasonable lengths of time.

Urea is the third component in addition to glucose and yeast extract. The results of a study of the effect of urea concentration upon the fermentation are shown in table 26. Varying the concentration of urea in the medium from 0.0 to 0.2 percent had little effect upon the growth of yeast as measured by yeast volume. The yields of ethyl alcohol and glycerol produced in the fermentation were essentially constant at all levels of urea investigated. The chief effect of adding urea to the medium is to increase the rate of fermentation. With no urea in the medium, considerable glucose still remained in the beer after 72 hours; at the 0.1 percent level, the fermentation was essentially complete in 48 hours. With 0.5 percent yeast extract in the medium, approximately one-half of the nitrogen is supplied by urea at the 0.1 percent level. Approximately one-third of the urea is broken down during sterilization, presumably to the ammonium ion, but no nitrogen is lost from the medium. Therefore, it is not readily apparent whether the urea caused the increase in the rate of fermentation. It can be said, however, that if the medium is prepared as described, urea appears to hasten the fermentation.

During some work in which fermentations were being made with two lots of yeast extracts produced by different manufacturers, one lot consistently produced low yields of glycerol and higher yields of ethyl alcohol and increased cell growth. Analysis for inorganic phosphate revealed that the one producing high yields of glycerol contained 6.3 milligrams of phosphorous per gram and the second lot, 17.7 milligrams of phosphorous per gram. With the yeast extract of the low inorganic phosphorous as a base, media were made containing several levels of added phosphate. Fermentation results with these media are recorded in table 27. An increase in the phosphate level in the medium causes a decrease in glycerol production and an increase in ethyl alcohol yield. A small increase in cell volume also occurs. In this fermentation, phosphate levels must be severely limited if good glycerol yields are desired.

One of the striking features of this fermentation is the fact that aeration is necessary to produce good yields of the reduced product, glycerol. To determine whether adequate aeration was being achieved, fermentations were made at three aeration levels and three sugar levels. Different degrees of aeration were obtained by using smooth and indented flasks and varying the speed of the rotary shaker. Oxygen availability was determined by the method of Cooper *et al. (21).* The data for these fermentations are given in table 28.

In the anaerobic fermentation, yeast growth was small and the yield of glycerol was only 12 percent. Ethyl alcohol was also produced with a yield of 16 percent of the sugar consumed. Oxygen availability of 42 mM O_2/L/hr was adequate for the 10 percent glucose level, but not for the higher concentrations as shown by the drop in glycerol yields. A level of 200 mM O_2/L/hr appeared to be sufficient for the 9 and 18 percent levels of glucose, but may be low for the 27 percent level. At the highest level of aeration, 360 mM O_2/L/hr, yields of

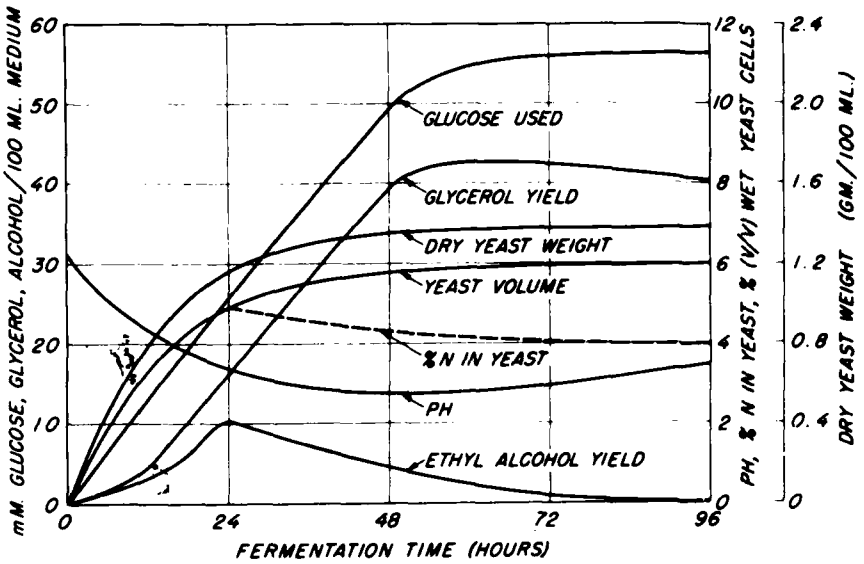

Figure 9.—Glycerol fermentation with Torulopsis magnoliae I_2B. (M 114 483)

glycerol at all three levels of glucose were comparable, although they were somewhat less than at 200 mM O_2/L/hr. In most cases increased aeration resulted in increased yeast growth with perhaps a slight decrease in the rate of sugar utilization. For 10 percent sugar concentration, oxygen availability at 42 mM O_2/L/hr was adequate, but increased aeration is necessary for higher sugar levels.

In table 29 data are given on the aerobic fermentation of various substrates by *Torulopsis magnoliae* I_2B. Glucose, levulose, mannose, and sucrose are all readily fermented and glycerol is produced in good yields from these sugars. Arabinose, galactose, and xylose are slowly utilized by the culture with no production of glycerol. The polyhydric alcohols—glycerol, mannitol, and sorbitol—are also utilized for growth. Under the conditions of the fermentations, glycerol produced the greatest growth of yeast. Maltose and lactose were not used at all.

Carbon balances were made on fermentations using *T. magnoliae* to determine whether any major products of the fermentation were not being detected. The fermentations were run in a closed system with the carbon dioxide being trapped in sodium hydroxide solution. Aeration was accomplished by a stream of air flowing into the fermentation flask at the rate of 1 volume of air per 1 volume of medium per minute. Before entering the flask, the air was freed of carbon dioxide by passing it through a soda-lime tube, humidified by bubbling through water, and sterilized by passing through a glass fiber filter. The cells for the inoculum were washed twice in sterile distilled water and resuspended in water before use as the inoculum. The fermentations were incubated at 35° C on a rotary shaker for 48 hours. The data for this fermentation are given in table 30. The wet cell volume of yeast was 6 percent and the final pH was 6.5. Identified products accounted for a recovery of 94.4 percent of the carbon. A small undetermined amount of gum is formed in this fermentation and this, together with alcohol that escaped during the fermentation, could account for the 5.6 percent deficit.

The sequence of events in a glycerol fermentation using *T. magnoliae* is depicted in figure 9. Glycerol production follows glucose utilization closely throughout the fermentation. Ethyl alcohol is produced early in the fermentation. Because of aeration, ethyl alcohol is being lost throughout the fermentation, so the values for this product should not be considered as true yields. Eighty percent of the yeast growth occurs in the first 24 hours of fermentation and thereafter is slow until the glucose is exhausted. The nitrogen content of the cells is highest early in the fermentation and thereafter it gradually decreases. The pH of the medium constantly decreases as long as the sugar remains, with a slight increase thereafter. The change in pH can be used to give a rough indication of the completion of the fermentation.

Before going to pilot-scale fermentations, it was desired to replace the yeast extract in the medium with a less costly source of nitrogen. Replacement of yeast extract with distiller's dried solubles, corn steep liquor, malt, malt extract, or blackstrap molasses resulted in fermentations in which glycerol yields were low. To determine the nutritional needs of the culture for glycerol production, an investigation was undertaken to develop a completely synthetic medium. The synthetic medium was patterned after that of Olson and Johnson *(103)*, although it was immediately apparent that the phosphate level was much too high for our purposes. The synthetic medium finally developed is shown in table 31. If tap water were used in making up the medium, it was found that the trace minerals, zinc, iron, copper, and manganese could be omitted. Nitrogen may be supplied as ammonium salts or urea. Potassium and magnesium are necessary but, in contrast to phosphorous, high levels have no deleterious effect on the fermentation. Of the growth factors, only biotin and thiamin are necessary. Of these thiamin is necessary for yeast growth and sugar utilization, while biotin stimulates glycerol production. With the synthetic medium, glycerol yields of 35 to 40 percent based on the sugar were consistently obtained.

With the knowledge that only an excess of phosphorous in the medium lowered glycerol yields, attention was again turned to corn steep liquor as a substitute for yeast extract. Untreated corn steep when used in the fermentation gave good yeast growth and fast sugar utilization, but low glycerol yields. Analysis showed that the particular lot of corn steep contained 54.0 percent solids and 3.02 percent of total phosphorous. The phosphorous level was reduced by precipitating phosphorous with calcium chloride from a 1 to 10 dilution at pH 5.5. One percent of the treated corn steep liquor, based on the original concentration, 0.2 percent urea, and 10 percent glucose provided a medium with which yields of glycerol of 40 percent based on the sugar were obtained in 48-hour fermentations. If untreated corn steep liquor were used in a similar fermen-

tation, glycerol yields of only 17 percent were obtained. Pilot plant fermentations of 15-gallon size were made using the treated corn steep medium and glycerol yields confirmed those obtained in shake flasks.

A slow-feed batch fermentation was devised in which the cell growth process was separated from the glucose-to-glycerol phase for an efficient process *(15)*. The synthetic medium described earlier was used in this study for the start of the fermentation; thereafter only a glucose solution was added. By this means, the desired cell population was rapidly achieved and the terminal cell population was in the proper phosphate-deficient condition. The sugar added periodically was converted from glucose to glycerol by the static cell population.

A conventionally stirred, aerated, and baffled 60-liter stainless steel fermentor was used for the slow-feed process. Oxygen transfer efficiency was a relatively high 160 mM O_2/L/hr. Sparged air flow was adjusted so that about 5 percent of the oxygen was removed from the input air. The pH was maintained at 4 by addition of potassium hydroxide on demand. Respiration was quantitatively monitored with a paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer. Sugar concentration was maintained by addition of a warm 70 percent glucose solution at about 36-hour intervals. When the glucose fell to near 0.1 percent, sufficient glucose solution was pumped in to raise its concentration to about 8 percent. The fermentation was continued for 10 days. Metabolic activity fell off at about 8 days but was quickly restored by replenishing the depleted nitrogen source. After 10 days of operation, the glycerol concentration in the fermentor had reached 17 percent. The supernatant liquor was clear and nearly colorless. Glycerol yield on total glucose was 45.9 percent.

Arabitol Fermentation

One of the isolates, I_{11}, was shown in table 23 to be an excellent producer of the rare chemical, D-arabitol. It was of interest in conjunction with our work on the glycerol fermentation to determine the fermentation conditions necessary for high yields of D-arabitol *(44)*. A transfer of this culture was identified as *Endomycopsis chodati* (Nechitch) Wickerham et Burton by Lynferd J. Wickerham.

To determine the effect of various nutrients on the course of the fermentation, the synthetic medium described in table 31 was used. Urea and ammonium lactate proved to be the best synthetic sources of nitrogen. The influence of the concentration of nitrogen clearly showed that excessive nitrogen in the medium causes a drastic decrease in the yields of arabitol. At low levels of nitrogen, yeast growth is restricted and sugar utilization is slow. As the nitrogen level is increased, yeast growth is increased, with a leveling off of growth at about 0.12 percent nitrogen in the medium. The rate of sugar utilization parallels yeast growth so that fast fermentations were obtained at the high nitrogen levels. Good yeast growth and fast fermentations are not synonymous with good fermentations, because the criterion for a good fermentation in this case is a good yield of arabitol. Compromise must be involved in choosing fermentation conditions so that good product yields may be obtained in reasonable fermentation times. On this basis, nitrogen content of the medium should be about 0.08 percent, which will result in a fermentation in which the arabitol yield will be about 30 to 35 percent in about 72 hours.

Phosphorous is, of course, an essential nutrient in the medium. Arabitol yields increase as the phosphorous content is increased from very low levels in the medium to a maximum and then decrease. The maximum arabitol yield was obtained at 0.3 percent potassium dihydrogen phosphate in the medium. Both potassium and magnesium are essential to the fermentation, but above a minimum concentration, 0.1 gram of potassium chloride and 0.05 gram magnesium sulfate per liter, neither has any effect upon the fermentation. Trace minerals are essential but could be supplied by tap water.

Yeasts as a class of organisms have widely varying requirements for growth factors, so the synthetic medium was made up with biotin, calcium pantathenate, nicotinic acid, thiamine hydrochloride, pyridoxamine hydrochloride, and inositol which are commonly required by a wide variety of yeasts. Fermentations were made in which all the growth factors were omitted, and in which none were omitted. The results showed that none of the growth factors were essential. To guard against carryover of growth factors in the inocula, seven serial transfer fermentations were made with the synthetic medium in which all the growth factors were omitted. Yields of arabitol were essentially constant in these fermentations, ranging from 32 to 37 percent of the glucose.

For pilot-scale fermentations, a search was made for inexpensive natural products which could substitute for the synthetic medium or yeast extract. Corn steep liquor could be used at the 0.25 percent level to produce satisfactory arabitol yields but with long fermentation times. If the level of corn steep were increased, arabitol yields dropped. A medium consisting only of 10 percent glucose, 10 percent blackstrap molasses, and 0.1 percent urea gave a satisfactory fermentation. The data for such a fermentation are given in table 32. The results of this larger fermentation are comparable to those in shake flasks, with the exception of time for complete sugar utilization. The arabitol concentration in the beer was 5.7 percent for a yield on total sugars of 41.9 percent.

Erythritol Fermentation

Four of the osmophilic yeast isolates (table 23) had been shown to produce the polyhydric alcohol, erythritol. These four isolates were again examined for their ability to produce erythritol using the standard medium with the results shown in table 33. Culture I_2A was chosen for further study because on paper chromatography no evidence was found for either glycerol or arabitol in the beer, whereas they were found in the beer from the other cultures *(47)*.

A transfer of culture I_2A was also sent to Dr. L. J. Wickerham who gave the following general description of the culture:

> Culture I_2A is a filamentous, nonascosporogenous, yeastlike fungus that produces brown or black pigment and reproduces mainly by budding, but also by septation, and probably belongs in, or is closely related to, the dematioid genus *Torula*.

The synthetic medium was again used to determine the effects of the various nutrients on the yield of products. Again, it was found that both nitrogen and phosphorous must be limited in the medium for good polyhydric alcohol yields. Nitrogen concentration of 0.05 percent in a 10

percent sugar medium was adequate. Ammonium lactate was satisfactory as the sole nitrogen source. Phosphorous gave satisfactory fermentations at a level of 70 milligrams as phosphorous per liter. Higher concentrations led to lower yields of erythritol. In contrast to the other osmophilic yeast fermentations, potassium had a strong effect on the erythritol fermentation. As the concentration of potassium chloride was increased from that in the synthetic medium to molar levels, increasing amounts of glycerol were produced with decreasing amounts of erythritol. Thus, with no added potassium chloride to the standard medium, 38.7 percent erythritol was produced with no glycerol; with potassium chloride at the 3 molar level only 17.7 percent erythritol was produced along with 31.0 percent glycerol. The only growth factor found necessary for the erythritol fermentation was thiamin. When thiamin was omitted from the synthetic medium, yeast growth was poor, sugar utilization was low, and erythritol yields were nil.

A survey was made of natural products that might take the place of yeast extract or the synthetic medium for this fermentation. Corn steep liquor, blackstrap molasses, malt sprout extract, and distillers' dried solubles all proved to be effective nutrient sources with erythritol yields in the range of 35 to 40 percent on glucose used. Table 34 contains the results of fermentations in which corn steep liquor and urea supply all the nutrients. For the highest sugar concentrations, the aeration rate of 42 mM O_2/L/hr is probably suboptimal. Nevertheless, the concentration of erythritol of 13 percent in the beer with a yield of 43 percent on sugar used makes this an interesting fermentation from the standpoint of recovery of products.

Butanol-Acetone Fermentation

The butanol-acetone fermentation was commercialized during World War I because of the acute need for acetone. Butanol was a byproduct for which there was little demand at the time so that storage and disposal presented a problem. When, however, butyl acetate was found to be an excellent solvent for nitrocellulose lacquers, butanol became an important article of commerce with acetone becoming the byproduct. The raw materials for the industrial fermentations were either corn mash or cane molasses. As with many fermentation processes which produced simple organic chemicals, petrochemical routes were found that produced these chemicals more cheaply than by fermentation so that, at present, there is no production of these substances by fermentation.

The organisms that produce butanol and acetone by fermentations are various species of the *Clostridia*. These organisms would seem to be well suited for the fermentation of wood sugars since they can convert pentose as well as hexose sugars. These organisms, however, are inhibited by furfural and other substances in wood sugar solutions so that pretreatments are necessary before good fermentations can be obtained *(77, 153, 174)*. Sjolander *et al. (153)* neutralized the hydrolyzate with calcium carbonate, filtered off the precipitate, made the filtrate alkaline to about pH 10, filtered off the resulting precipitate and clarified the solution with active carbon, and finally adjusted the pH to 6.5. Leonard *et al. (77)* believed that furfural was the principal inhibitory substance. This was not the only toxic substance involved because, once furfural was removed by steam distillation, if furfural was added back to the medium in the same concentration as originally present, the fermentations were not inhibited to the same extent as initially.

Sjolander *et al. (153)* screened a number of micro-organisms on wood sugar solutions before settling on *Clostridium felsineum* and *Clostridium butylicum* for more intensive study. Wood sugar solutions in concentrations up to 5 percent were almost completely fermented. Thirty to 35 percent by weight of the sugars were converted to neutral volatile products as shown in table 35. The yields and distribution of products formed by *Clostridium felsineum* were similar from wood sugars and glucose. Butyl alcohol was the principal product with smaller amounts of acetone and ethyl alcohol. *Clostridium butylicum*, in addition to these products, produced considerable quantities of isopropyl alcohol.

Leonard *et al. (77)* used *Clostridium butylicum* on wood hydrolyzates from different species of wood. Ease of fermentation varied among the species; and, since the solutions had been steam stripped until furfural was no longer detected, it was assumed that extractives were the cause of any inhibition. The results of the fermentation are shown in table 36. Although the distribution of products is quite similar from the different woods, the yield of total products from oak was quite low.

Wiley and coworkers *(174)* studied the same fermentation, using spent sulfite liquor as a substrate. It was necessary to pretreat the liquor with lime to pH 12 to precipitate sulfite and lignin complex before good fermentations could be obtained. Some 70 to 80 percent of the reducing substances were utilized in the fermentation, and yields of neutral solvents of about 26 percent of the sugars were obtained.

The yields of neutral solvents from these experimental fermentations are comparable to those obtained in commercial fermentations. On the industrial scale using cane molasses, total yields ranged from 29 to 33 percent based on the sugar, with about 70 percent butanol, 26 to 32 percent acetone, and 1 to 3 percent ethanol in the mixture of products *(112)*.

Lactic Acid Fermentation

Marten *et al. (86)* investigated the lactic acid fermentation of the sugars remaining in the still bottoms from the ethyl alcohol fermentation of wood sugars produced by the one-stage American process for hydrolyzing wood. The sugars remaining in the still bottoms are chiefly pentoses. The number of strains of lactic acid organisms is large and all of them produce lactic and acetic acid. They vary in their abilities to ferment pentoses and the ratios of the acids produced depend on the particular strain of the organism employed. Several cultures were studied to find a strain which fermented pentoses vigorously with lactic acid as its chief product. One culture, later identified as *Lactabacillus pentosus* 124-2, was selected for further work.

Theoretically, the lactic acid bacteria should produce 2 moles of lactic acid from 1 mole of hexose and 1 mole of acetic acid and 1 of lactic acid from 1 mole of pentose. Using *Lactabacillus pentosus* 124-2 in fermentations of pure sugars, the results very closely approach those predicted by the theory.

In the fermentation of still bottoms, it was found necessary to add nitrogen as a nutrient and to buffer the solution with calcium carbonate. With Douglas-fir still bottoms, about 75 percent of the reducing substances were utilized with a yield of acetic and lactic acids of 94 percent of the sugars used. Lactic acid comprised about 90 percent of the mixed acids. When still bottoms from a hardwood hydrolyzate were used, the ratio of lactic to acetic acid was 75 to 25.

The same organism was used by Allgeier and associates *(2)* on the total hydrolyzate of Douglas-fir, spruce, and yellow pine. A nitrogen source and a buffering agent must be used in the fermentation. From 7 to 10 percent concentrations of sugar can be fermented in 5 to 7 days with a utilization of 82 to 88 percent of the total reducing material. A yield of acid equivalent to over 95 percent of the sugar used can be obtained. The acid mixture consists of 90 to 95 percent lactic and 5 to 10 percent acetic acids.

The production of lactic acid from spent sulfite liquor was reported on by Leonard *et al. (76).* The spent liquor was prepared for fermentation by steam stripping followed by precipitation of the remaining sulfite with lime at pH 8.5. After filtration, neutralization, and addition of malt sprouts to provide nitrogen, the liquor was inoculated with *Lactabacillus pentosus* 124-2. The fermentation required about 48 hours for completion at 30° C and, during this time, the pH was controlled at 5.8. On the basis of 1 ton of pulp, it was estimated that 300 pounds of lactic acid and 80 pounds of acetic acid could be produced by this fermentation.

2,3-Butylene Glycol Fermentation

The production of 2,3-butylene glycol from acid hydrolyzates of both softwoods and hardwoods has been investigated by Perlman *(113).* A culture of *Aerobacter aerogenes*, obtained from the ARS Northern Regional Research Laboratory, was used in this work. This organism is able to ferment both pentose and hexose sugars and was shown to utilize about 90 percent of the reducing substances in wood hydrolyzates.

Pretreatment of the hydrolyzates was found necessary to obtain satisfactory fermentations. Two rather simple pretreatments were found. In one the pH was raised to 6 with lime and the precipitate filtered off. In the second or alkaline treatment, the pH was raised to 10 with lime and the hydrolyzate was filtered and the pH adjusted to 6. With low concentrations of sugar in the hydrolyzate, both methods of pretreatment gave good results on fermentation. With higher sugar concentrations, the alkaline method proved more satisfactory. Recovery of 2,3-butylene glycol is difficult from dilute solutions so hydrolyzates were concentrated by vacuum distillation and these more concentrated solutions were fermented. The results of these fermentations are shown in table 37. The nutrient requirements of this organism are quite simple. Nitrogen was supplied by 3 grams of urea per liter and potassium and phosphorous by 3 grams of potassium dihydrogen phosphate per liter. Wood sugar solutions of up to 18 percent concentration were fermented satisfactorily with yields of 2,3- butylene glycol of about 35 percent based on the total reducing substances present before fermentation.

FODDER YEAST

Fodder yeast, single cell protein, can be produced with only wood carbohydrates and inorganic salts as the raw materials. This is an excellent method of converting carbohydrates to protein with the added advantage of producing vitamins of the B-complex.

During the 1930's, the German government set up an extensive research program for the production of food yeast from spent sulfite liquor and wood hydrolyzates *(32, 33, 34, 141, 146).* The organism chosen for industrial development on wood sugar substrates was *Torula utilis (Torulopsis utilis, Candida utilis)* or simply called torula yeast in many publications. Ease of maintenance, fast growth, good pentose-utilizing abilities, and good nutritional properties are its chief characteristics. It has been officially accepted as a wholesome food and feed in the United States and other countries. Commercial development of food and feed yeast in Germany was rapid with the development of three aeration systems. Wood hydrolyzates in contact with yeast foam excessively on aeration. The aeration systems developed attempted to control the foam mechanically. The systems developed were the Waldhof fermentor based on work by Claus *(16),* the second by Scholler and Seidel *(145),* and the third by Vogelbusch *(170).*

The amount of air and its method of distribution are critical factors in the efficiency of yeast production. Generally, the use of diffusers which deliver very fine bubbles of air have been used successfully with most substrates. Wood sugar solutions have undesirable foaming properties which complicate aeration with diffusers even with the use of antifoam. Mechanical methods for aeration and foam control are a necessity with wood sugar solutions, although some mechanical systems are better than others. The Waldhof system used no antifoam while the Vogelbusch system required antifoam to the extent of 10 percent by weight of the dry yeast produced *(50, 135).* The mechanical aeration systems produce emulsions having about two volumes of air per volume of liquid and give very high efficiencies of gas transfer at the air-liquid-yeast interface.

Air supplied for yeast growth is critical for the efficiency of the process. Too much air can be accompanied by increased respiration, increased heat production, and lowered yeast yields. Insufficient air can lead to anaerobic conditions with alcohol production and decreased yeast yields. With mechanical aeration methods, satisfactory yeast yields can be obtained with the use of 150 to 200 cubic feet of air per pound of dry yeast produced.

Initial work at the Forest Products Laboratory on growing yeast on wood sugar solutions was done in shake flasks or in fermentors equipped with stirrers and diffusers *(58, 116).* The results were unsatisfactory from several points of view. The wood sugar solutions had to be diluted to 1 percent reducing sugar content before satisfactory growth could be achieved. Although sugar utilization in the shake flasks was usually over 90 percent, yeast yields were low. When fermentors equipped with stirrers and diffusers were used, the stiff foam was difficult to control in spite of the large amounts of antifoam used; the amount of air required was

also excessive, being of the order of 1,200 cubic feet per pound of dry yeast.

Yeasts belonging to several genera were studied for their ability to grow in wood hydrolyzates and to determine whether acclimatization would increase yields and sugar utilization. The results are reported in table 38. Acclimatization resulted in increases in both yeast yield and sugar utilized in all cases. Peterson *et al. (116)* had indicated that *Torula utilis* No. 3 (University of Wisconsin collection) appeared to be suited for single cell protein from wood sugar hydrolyzates. This culture was selected for all subsequent work at the Forest Products Laboratory.

When the design of the Waldhof fermentor became available *(55, 130, 135)*, a small laboratory fermentor model, somewhat modified from the industrial scale equipment, was built at the Laboratory. Figure 10 is a drawing of this fermentor. This fermentor design is characterized by an arrangement for mechanically dispersing air and simultaneously forcing emulsion circulation through a central draft tube. This prevents the accumulation of nonbreaking foam on the top of the liquid but breaks the foam, releasing air high in carbon dioxide when the emulsion contacts the aeration wheel at the bottom of the draft tube. The fermentor is designed for continuous addition of feed and withdrawal of product so that the yeast is maintained in a vigorous condition.

The laboratory fermentor had a working capacity of 9 gallons (34 liters) and was constructed of stainless steel. The central draft tube is supported from the sides so that these supports also act as baffles. The diameter of the fermentor is 16 inches and the draft tube 6 inches. The aeration wheel disk must be slightly larger than the draft tube. The air ducts are welded to the disk and extend beyond it to insure distribution of the air outside the draft tube. Air is fed to the ducts through a 3/4-inch hollow central shaft. The hub of the disk fits a vulcanite sleeve bearing in the bottom of the propagator, while a brass cap for introduction of air fits the top of the shaft and is bored to a tight running fit. Air pressures of 3 to 5 pounds per square inch are sufficient for the air supply. A variable-speed motor supplies the power to the shaft. The speed of the aeration disk as well as volume of air can both be used to control the characteristics of the foam. An overflow tube of adjustable height is the means of withdrawing the product and maintains a constant level of foamed liquid in the fermentor during continuous operation. Temperature control is obtained with a cooling coil of 1/2-inch tubing, two 150-watt contact heaters, and a bimetallic switch. Heating is necessary only at startup for sufficient heat is produced during growth of yeast to maintain the temperature.

This propagator worked very well and a larger one was constructed with a working capacity of 150 liters. The only change was to use a water jacket in place of the cooling coil. With this larger equipment, the medium, containing 4.0 to 5.0 percent reducing substances, could be fed at 16 to 20 liters per hour, with an air rate of 2 to 3 cubic feet per minute so that 15 to 20 pounds of dried yeast could be produced in 24 hours. The holding time in the fermentor is approximately 3 hours, based on the rate of feed and the amount of actual liquid in the propagator.

In the operation of the propagator, the successful handling of the foam problem depends upon maintaining a proper relationship between the height of the liquid and that of the draft tube. Operation of the fermentor varies with the medium used. A tendency to foam is desirable, for it assures that a foam with a high density will move down the draft tube. The liquid moving down the draft tube loads the aeration wheel and helps produce good dispersion of the air. The maximum amount of air that can be used is limited by the amount of foamed liquid that can move down the draft tube. When the tube is overloaded, a dense foam collects on the surface and rises over the top of the fermentor.

The foaming characteristics of wood sugar change with depletion of sugar, increase in yeast content and with continued aeration; for this reason, the equipment has not proved satisfactory for batch operation. In continuous growth of yeast, only a small concentration of sugar is in the medium at all times so anaerobic fermentation does not take place as it would with high sugar concentrations.

The foaming characteristics of wood sugar solutions depend on the pH during the fermentation. The lower limit at which satisfactory yeast growth occurs is about pH 4. As the pH is increased, yeast growth becomes more rapid and utilization of sugar improves, but the yeast becomes progressively darker in color. The propagator was routinely operated at a pH of 5 to 5.5. At this pH, no contamination was noted even when working with unsterilized wood sugar solutions and open fermentors. This, however, is characteristic of wood sugar solutions. With other substrates, a closed fermentor and sterile technique may be required.

Startup with this equipment has proved to be very simple. An inoculum of such size as to supply about one-tenth as much yeast as would be present under equilibrium conditions was put into the fermentor and continuous addition of a 2 percent feed was begun. The overflow product was centrifuged and the recovered yeast returned to the fermentor. After about 10 hours, the feed was increased to full strength, about 5 percent reducing sugar, and it was no longer necessary to add recycle yeast to the fermentor. Runs have been made for as long as 6 months with no need for a fresh inoculum and no evidence of contamination.

The Waldhof fermentor is a much underrated and unappreciated piece of equipment. Its main virtues are: (a) it allows for continuous fermentation, and (b) it successfully controls foam mechanically and indeed makes a virtue of the foaming tendencies of many media by the superior aeration obtained in the emulsion. The Waldhof process for fodder-yeast production permits fermentations to be carried out continuously at a high rate of speed and maintain the yeast in a vigorous condition. The principle involved of successful foam control by the rapid turning over of the medium through a central draft tube has proved to be sound.

Prospective users of the Waldhof fermentor should bear the following points in mind:

1. The fermentor works best as a continuous unit, because in a batch fermentation foam characteristics change with time.

2. Under optimum operating conditions, the medium in the fermentation will be present as an emulsion with a specific gravity of about 0.35 for wood sugar solutions.

3. *Never add antifoam.* Proper operation of the fermentor demands that the medium be present

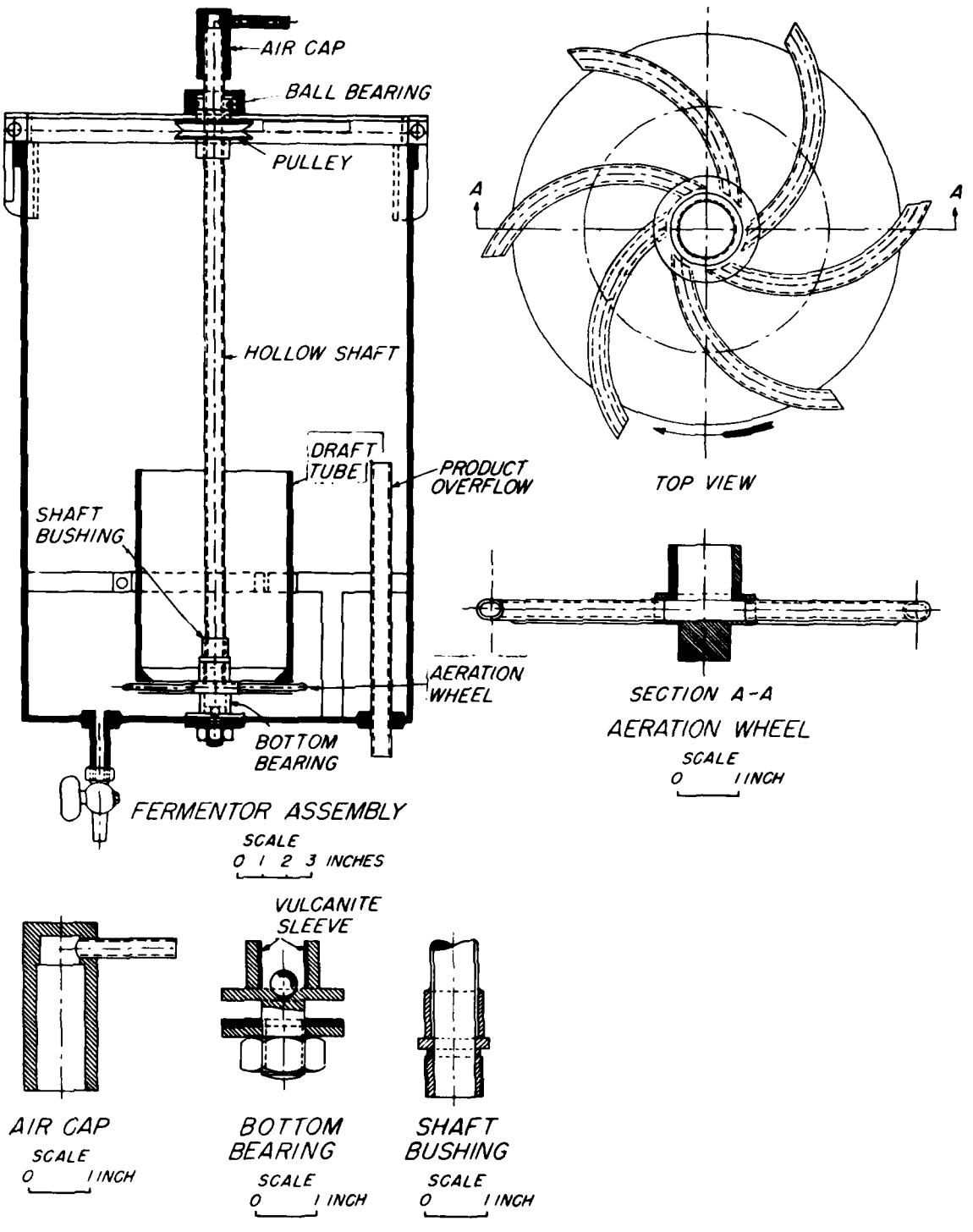

Figure 10.—Laboratory-scale experimental fermentor with mechanical aerator built at the Forest Products Laboratory with design altered somewhat from industrial-scale equipment used in Germany.
(ZM 70406 F)

as an emulsion to obtain high aeration efficiency. Use of antifoam in the belief that the increased capacity of the fermentor will result in increased yeast production is false. When changes are made in the media, foam characteristics may change, but proper operation of the fermentor may be maintained by adjusting the speed of the aeration wheel.

The nutrient requirements for yeast growth were determined by analysis of dried yeast for nitrogen, phosphorous, potassium, and magnesium. Media were then made up to supply one nutrient at levels above and below those calculated to give good yeast growth, assuming a 50 percent yield of yeast on sugar available. The other nutrients were held at the calculated values until the actual value for that nutrient had been experimentally determined *(50, 55, 56)*.

For example, the calculated value for nitrogen was about 3 pounds of nitrogen for each 100 pounds of sugar in the feed. In table 39, the results of varying the nitrogen from 2.5 to 12 pounds per 100 pounds of reducing sugar are shown. Sugar utilization was constant at all levels of nitrogen at 3.2 pounds and above per 100 pounds of sugar. At levels of nitrogen above about 3.5 pounds per 100 pounds of sugar, the nitrogen content of the yeast increased but the nitrogen recovered in the yeast from the medium decreased drastically.

Similarly, the amounts of phosphorous and potassium required for maximum yeast yields and maximum sugar utilization are shown in tables 40 and 41. One and one-half pounds of phosphorous pentoxide and 1.1 pounds of potassium chloride appeared to be sufficient. There appeared to be no difference whether or not magnesium was added to the medium. It was concluded that there was sufficient magnesium in the wood hydrolyzate for the yeast's requirements. The air rate was varied from 0.2 cubic foot to 1 cubic foot per minute per cubic foot of medium in the propagator. About 0.5 cubic foot of air per minute per cubic foot of medium gave the maximum yields of yeast and sugar utilization.

Much of the previous work on yeast growth suggested that dilute sugar solutions, 1 percent or less, should be used. In table 42 the results of a series of tests are reported in which the sugar concentration in the feed was varied from 1 to 8 percent. At the 8 percent sugar concentration, aeration was inadequate and alcohol was produced along with the yeast. There is a trend to higher yeast yields at lower sugar concentrations. Compromises must be made between yeast yields on sugar used and the increased costs incurred in handling the larger volumes of solution and increased size of equipment.

The medium adopted as standard contained these nutrients per 100 pounds of sugar in the feed: 3.4 pounds of nitrogen as nitrogen in ammonia, ammonium sulfate, or urea; 0.71 pounds of phosphorous as phosphorus in sodium phosphate salts, and 0.58 pounds of potassium as potassium in potassium chloride. The air requirement was set at 80 cubic feet per each pound of sugar in the feed. The pH of the propagator was held at 5 to 5.5 and the temperature at 30° C. Throughput time of a 5 percent sugar solution was 3 hours.

These fermentation conditions were used on hydrolyzates from several wood species to determine whether any large differences existed between species. The results are given in table 43. Essentially no differences were found as yields from hardwoods and softwoods are similar.

Torula yeasts are grown in commercial-sized Waldhof propagators on spent sulfite liquor at the Lake States Yeast Company in Rhinelander, Wis. *(63, 65, 173)* and by Boise Cascade Corporation at Salem, Oreg. *(3)*. Under proper operating conditions, the yeast growth utilizes a quantity of the sugars sufficient to reduce the biological oxygen demand of the effluent by 50 to 70 percent. Yeast propagation is thus a promising means of reducing stream pollution.

As the name "fodder yeast" implies, torula yeast was produced to be used as a food. It is now also being used for its flavor properties, yeast extractives, nucleic acids, as well as for its protein and vitamin content. The composition of torula yeast grown on wood hydrolyzate, spent sulfite liquor, and blackstrap molasses is shown in table 44 *(160)*. The protein content of yeast can be varied somewhat by changes in the amount of nutrient and growth conditions. Generally, the crude protein content of torula yeast is about 50 percent. The vitamin content of yeasts grown on wood hydrolyzate is comparable to that of brewers' yeast with the exception of thiamin. This has been shown to be due to the lack of thiamin in the medium. Yeasts have a great capacity to take up thiamin from the medium far in excess of their needs.

In nutrition the quality of the protein is an important consideration. "Complete" proteins are those that contain all the essential amino acids required by the test animal in sufficient quantities for growth. In general, meat and milk proteins are complete while plant proteins are not. Yeast proteins appear to fall between the meat and plant proteins. Table 45 compares the amino acid content of torula yeast and casein. Of the amino acids essential for man, in the protein of torula yeast, methionine would appear to be the limiting amino acid.

Protein quality of six strains of yeast grown on wood sugar hydrolyzate was evaluated by feeding rats with rations in which the yeasts were the sole source of protein *(54)*. The yeasts were grown in the continuous propagator using the standard medium described earlier. The yeast strains, yields, and protein content are shown in table 46. Ninety to 96 percent of the reducing substances in the media were utilized, with yeast yields of 42 to 51 percent, and crude protein content of the yeasts ranging from 45 to 51 percent.

The first tests of protein availability from yeast grown on wood sugars were made with weanling rats, by comparing *Torula utilis* with casein as the total source of protein. The gain in weight of the rats fed the yeast averaged only 60 percent of that for casein. Fur became thin on the backs of the male rats on the yeast diet. Female rats showed this condition to a lesser extent. The loss of hair was taken to indicate a lack of some essential amino acid. The yeast ration was, therefore, supplemented with various amino acids in further feeding tests. The only amino acid that produced improvement in weight gain and hair restoration was methionine.

In evaluation of the six strains of yeasts as sources of protein, the protein depletion method of Frost and Sandy *(37, 38)* was used. In these tests, adult rats weighing 160 to 190 grams were fed a nonprotein diet for protein depletion for 12 days. During that time, the rats lost 40 to 50 grams in weight. Then 1.5 grams of casein

as a source of protein was added to the diet of each rat for 3 days, followed by the nonprotein diet for 4 days. The rats were then fed the nonprotein diet supplemented with the yeast protein for 5 days. Assuming that all the nitrogen in the samples was protein, yeast or casein equivalent to 0.2 gram of nitrogen was added to the nonprotein diet. At the end of 5 days, weight gain was measured for each rat. The average gain in weight per day is shown in table 47, run 1. In this series, gains in weight on the yeast diets were 60 to 80 percent of those on the casein diet. After this, the rats were prepared for a second trial by feeding a standard rat feed for 2 days, and then the protein depletion diet for 7 days. They were then fed the same as in run 1 for 5 days and gains in weight recorded. The average gain in weight per rat per day agrees with run 1 as shown in the table.

It was apparent that one or more amino acids was lacking or present in suboptimal amounts. On the basis of the feeding tests with weanling rats, the diet was supplemented with methionine. Rose *(126)* has shown that methionine should be present at the 0.6 percent level. This amount was added to the yeast ration with no correction for methionine in the yeasts. An excess of methionine was later shown to have no effect on increase in weight gain. The rats were depleted of protein as before and the methionine-supplemented diets fed. The results are recorded in table 47 as run 3. Yeast supplemented with methionine is the equivalent of the complete protein, casein. These tests also indicated little difference among the strains of yeast from the growth and feeding standpoints.

Feeding tests have been made with poultry, swine, and cattle using torula yeast as the source of protein and vitamins. Dr. J. S. Carver at Washington State University fed torula yeast to white leghorn pullets with torula yeast supplied by the Forest Products Laboratory. Gain in weight, egg production, and hatchability were determined for rations containing fish meal, soybean meal, and torula yeast. All groups gained weight and egg production was about the same for all rations. Hatchability was low on the soybean meal ration, but approached that of fish meal when supplemented with torula yeast. This result was verified by Ringrose *(123)*.

Experimental feeding of yeast to swine has been reported *(6)*. Swine are fed primarily on cereals so there is a need for a protein supplement of good quality to make up for deficiencies of essential amino acids in the cereal proteins. It was concluded that torula yeast cannot completely replace fish meal in swine rations, but that a mixture of yeast and fish meal is a good protein supplement. As a result of the feeding trials, it was recommended that two parts of torula yeast and one part of animal protein be used in making up the protein supplement.

The protein requirements of ruminants differ from those of monogastric animals. All essential amino acids can be made by the bacteria in the rumen from simple forms of nitrogen *(95)*. Although dairy cattle require large quantities of protein for growth and milk production, their digestive systems are such that high-quality proteins are not as necessary as for poultry and swine. Ritzman *(125)* in an extensive investigation on the value of yeast grown on wood sugars for lactating dairy cows concluded that: (a) torula yeast is equal in chemical composition to the usual concentrated feed protein used for livestock feeding, and (b) the dried yeast is equal to soybean meal as a protein supplement for production of milk.

FEEDING WOOD SUGAR MOLASSES

The dilute sugar solutions from the wood hydrolyzer may be concentrated in suitable equipment to produce a molasses. In the Forest Products Laboratory pilot plant, the sugar solutions were neutralized to a pH of 3.5 and filtered under pressure to suppress the solubility of calcium sulfate. The solutions from the filter were passed directly to a long-tube multiple-effect vacuum evaporator or to a vapor-recompression evaporator. In the Tennessee Valley Authority research, wood sugar solutions were concentrated to 25 percent reducing substances by submerged combustion after which a long-tube vacuum evaporator was used to produce a 50 percent molasses *(41)*.

From a ton of wood about three-fourths of a ton of molasses can be obtained. The average composition of the molasses, as determined by the analysis of samples from various species of wood, is shown in table 48 *(168)*. The molasses from a softwood, such as Douglas-fir, contains about 85 percent hexose and 15 percent pentose sugars. The sugars in a hardwood molasses, such as maple, are composed of about 65 percent hexose and 35 percent pentose sugars.

There are storage problems with wood sugar molasses. During the hydrolysis process, unstable soluble products are formed which undergo chemical and physical changes and slowly precipitate. These deposits vary in character from spongy sludges to tar-like products. In a few weeks of storage, the amount of this material formed is seldom enough to cause problems in pumping. After several months of storage, troubles of this sort may be encountered. This problem can be avoided if fresh molasses is processed into mixed feeds.

Some 200 tons of wood sugar molasses were produced at the Forest Products Laboratory and sent to various agricultural experiment stations throughout the country for feeding trials. Feeding tests were made with the molasses in mixed feeds, sprayed on hay, and as a preservative for grass silage. Milk cows, beef cattle, sheep, swine, and poultry were fed in these experiments.

Palatability is an important characteristic for feeds fed to farm animals. Wood molasses was compared to blackstrap molasses under the supervision of N. N. Allen at the University of Wisconsin Agricultural Experiment Station for palatability and to determine any effects on the cattle with long-term feeding. When wood or blackstrap molasses was mixed with grain or roughage and offered on a free-choice basis, both were equally acceptable. When wood molasses was fed on a long-term basis, there were no detrimental effects on the cattle or their offspring *(168)*.

Grain mixed with either Douglas-fir wood molasses or blackstrap molasses at the 5 percent level was accepted equally well by cattle as reported by Willard *(175)*. At the 10 percent level, cane molasses appeared to be preferred to the wood molasses. At the 10 percent level, neither molasses was as well liked as at the 5 percent level.

Colovos *et al. (19)* compared the nutritive value of wood molasses to

that of cane molasses at the New Hampshire Agricultural Experiment Station by means of protein and energy digestion balance experiments with dairy heifers. Southern pine wood molasses and cane molasses were added to a field-cured grass-legume mixture and fed on an equivalent energy basis to the cattle. The metabolizable energy per gram of dry matter was the same for both molasses. Wood molasses was concluded to be comparable to cane molasses when fed to dairy cattle.

Douglas-fir molasses was used by Jones *(67)* at Oregon State University in feeding experiments with dairy cattle. The control ration consisted of 2 pounds of ground barley per heifer per day plus oats and vetch hay. Three pounds of wood molasses or cane molasses was substituted for the 2 pounds of ground barley in the experimental rations. Growth on the two types of molasses was similar, but less than that on barley. Hay consumption was highest for the animals fed the barley. No differences were found in milk production.

Keyes *(71)* concluded from his work with dairy cows that wood molasses could be used in place of cane molasses with no loss in milk production.

Jersey and Holstein heifer calves, 3 to 10 months old, have been shown by Blosser *et al. (11)* to gain faster when 2 to 4 pounds of wood molasses per day per calf were used in a ration of grain and hay which was 5 percent below Morrison's *(95)* minimum standard for growing dairy cattle.

Wood sugar molasses has also been used in feeding trials for beef cattle. Heinemann *et al. (62)* fed long-yearling steers on rations containing 16.6 and 24.1 percent wood molasses as a substitute for a barley-beet pulp concentrate. Data on daily gain and cost per unit of gain were obtained. The steers receiving wood molasses made smaller daily gains than did those on the control ration and, although the cost of gains on the molasses was higher than that of the control, this cost was regarded as reasonable.

Burkitt *et al. (14)* fed a wood molasses, prepared from Douglas-fir, lodgepole pine, and western larch, to range steers and compared the weight gains to steers receiving cane molasses. Native meadow hay with only a trace of legume was fed free-choice with no record of the amount of hay consumed. A concentrate containing oats, corn, barley, soybean oil meal, alfalfa meal, and iodized salt was fed in the form of pellets at the rate of 10 pounds per head per day. When molasses was incorporated in the pellets, it was substituted for some of the oats, corn, and barley. The average gain per animal per day for the 84-day feeding trial was as follows *(168)*:

Ration	Weight gain
	Lb/day
Control	1.62
10 Percent cane molasses	1.43
10.7 Percent wood molasses	1.67
7.0 Percent wood molasses	1.36

These cannot be considered as rapid gains for steers of the size used. Gains were probably reduced by cold weather conditions and by limiting the consumption of concentrates to 10 pounds per day.

A molasses made from blackjack oak was found by Barrentine and Leveck *(10)* to be a satisfactory replacement for part of the corn in steer rations. Feeding trials were conducted with three lots of steers. Lot I received cottonseed meal, hay, and 14 pounds of ground corn per day. Lot II received cottonseed meal, hay, a three-fourths ration of corn, and oak molasses equivalent on a dry matter basis to one-fourth of the weight of the corn. Lot III received cottonseed meal, hay, and a three-fourths ration of corn per day. Table 49 presents the data for the feeding trial which was conducted for 120 days *(168)*. The steers on the molasses feed made somewhat better daily gains than did those on the control ration and used less feed per pound of gain. A 30-day reversal feeding trial was made in which the steers in Lot III were put on the molasses ration and those in Lot II on the ration with three-fourths corn. The results are presented in table 50. The results of the reversal test indicate a higher nutritional value for molasses than did those in table 49. The shorter period of the reversal test should be regarded as only qualitative, although it supports the fact that the feed value of wood molasses is comparable to that of corn on a dry matter basis.

A number of trials have been made to test the value of wood molasses as a preservative for grass silage. Allen *(1)* reported that wood molasses was as satisfactory as cane molasses for making grass silage.

Alfalfa silage preserved with 3 percent wood molasses was compared to that with 3 percent cane molasses by Jones *(67)*. The two silages were of equal quality as determined by odor, appearance, and chemical analysis. The silage with wood molasses appeared to be preferred slightly by dairy cows in production.

Blosser *et al. (11)* reported that a silage of alfalfa, brome and orchard grass with 60 pounds of wood molasses per green ton as the preservative was better preserved than silage with no preservative. The wood molasses silage appeared to be more palatable than that to which no preservative had been added.

Peet and Ragsdale *(110)* compared alfalfa-brome silage prepared with 60 pounds per ton of wood molasses with one prepared with 60 pounds of cane molasses for preservative value, palatability, and milk and fat production. It was concluded that wood molasses has a preservative value equal to that of cane molasses. The silage preserved with wood molasses was as palatable and as readily consumed as silage preserved with cane molasses. In reversal feeding of the two silages to the milking herd, milk and fat production on the basis of average daily production was almost identical for the two types of silage.

In feeding trials at Oregon State University *(179)*, swine were fed a basal ration of barley, tankage, and alfalfa and an experimental ration with 15 percent of Douglas-fir molasses replacing an equivalent amount of barley. Normal gains were obtained with the experimental rations. When the wood molasses was increased to 30 percent of the ration, the feed was unpalatable to the pigs.

Burkitt *et al. (14)* reported on 4 years of feeding trials in which part of the barley and oats was replaced with wood molasses in feedlot rations of lambs. As high as 20 percent of the grains in lamb fattening rations could be replaced by wood molasses. On an equal dry matter basis, the wood molasses was comparable to cane molasses in feeding value. Digestibility trials showed no differences in the coefficients of apparent digestibility of individual nutrients or in the total digestible nutrients of the total ration when the concentrate contained no molasses, cane molasses, or wood molasses.

Average daily gain and feed per pound of gain were similar on all rations. As judged by a tasting panel, the palatability scores of roasted lamb from the various lots were identical. Pfander *et al. (119)* fed Masonex *(166)*, a wood molasses obtained as a byproduct of hardboard production, to sheep and lambs and showed that palatability, digestibility, daily gains, health of the animals, and quality of carcass was such that the molasses should be considered as a valuable new feedstuff.

McGinnis *et al. (87)* fed New Hampshire chicks from 1 week to 4 weeks of age on diets in which 5, 10, 15, and 20 percent Douglas-fir molasses replaced corn and wheat in the ration. Wood molasses proved a satisfactory substitute for cereal grains when additional protein in the form of soybean oil meal was added to the diet. Levels of wood molasses up to 20 percent did not have a laxative effect. In other tests turkey poults were fed rations containing 5 to 20 percent wood molasses. Weight gains and mortality for the test ration and the basal ration were comparable.

Cooney and Parker *(20)* have reported results on feeding S. C. White Leghorn pullets rations containing 7.5 and 15 percent Douglas-fir molasses. Egg production, feed conversion, and mortality data are presented in table 51. Seven and one-half percent wood sugar molasses fed to the pullets as a replacement for an equal amount of cereal grains in the ration brought about an increase in egg production. Fifteen percent wood molasses in the ration failed to sustain egg production. Both rations, however, were readily consumed by the birds and did not adversely affect body weight, livability, or color of the egg yolk.

It is apparent from the reported feeding trials that the carbohydrates in wood molasses are efficiently utilized by various animal species and that the molasses could become an important feedstuff under the right economic conditions.

WOOD AS AN ANIMAL FEEDSTUFF

Although wood is composed of 65 to 75 percent polysaccharides, the cellulose and hemicelluloses in wood are generally unavailable as nutrients to ruminants because of the lignin-carbohydrate complex and the highly crystalline nature of the cellulose. If simple, economic ways could be found to make these polysaccharides susceptible to enzymatic or microbiological attack, wood residues could not only supply an energy feed for ruminants that is now provided by hay and grains, but would also permit utilization of cull and weed trees and cellulosic residues that occur during harvesting and manufacture of wood products.

A summary of research on the use of wood in animal feeds conducted by the Forest Products Laboratory in cooperation with Federal and state agencies has been published *(9)*. These research efforts began in 1920 when a severe drought in the Pacific Northwest resulted in shortages and high prices for grain and fodder. Great quantities of unutilized wood residues then existed at sawmills throughout the region, so it seemed desirable to determine if a nutritive feedstuff could be prepared from these residues. Sawdust was hydrolyzed as in the American process, with 1.8 percent sulfuric acid for 20 minutes at 120 pounds per square inch steam pressure at a water:wood ratio of 1.25 *(149)*. The treated mass was centrifuged to remove a portion of the acid-sugar solution and then leached with hot water. The leachate and centrifuged liquor were combined and neutralized with calcium carbonate. The filtered liquor was evaporated to a thick syrup. The air-dried hydrolyzed sawdust was mixed with the syrup and the whole dried to about 12 percent moisture, after which it was ready for feeding.

Several feeding experiments with sheep and dairy cows were made with this material *(5, 96, 177)*. Dairy cows could be fed rations containing up to 15 percent of the hydrolysis mixture without noticeable effect on milk production. Satisfactory results were obtained with maintenance rations containing up to one-third of the hydrolyzed sawdust mixture.

More recent work in the feeding of wood and bark residues to farm animals has examined these materials for their use as first, pure roughage and secondly, as a source of metabolizable energy. All ruminants need roughage in their diets; roughage helps minimize rumen lesions and liver abscesses in steers on high-concentrate rations and helps prevent abnormally low milk fat from dairy cows *(139)*.

Roughage in the ration provides tactile stimulation of the rumen walls and promotes cud chewing which maintains salivation and the buffer supply for maintenance of rumen pH. Hay is the roughage in general use, although corn cobs, cottonseed, oat, and rice hulls and polyethylene pellets have been used. A roughage substitute should be effective in maintaining the health of the animals, be readily obtainable at low cost, be capable of being handled mechanically for transport, storage, and mixing purposes and, in the case of dairy rations, of maintaining normal milk fat. Wood and bark residues could supply more than enough roughage for all the concentrates fed in the United States *(147)*.

Sawdust has been compared with hay as a roughage in feeding experiments with beef cattle and sheep *(4, 23, 24, 73)*. Weight gain, efficiency of feed conversion, carcass grade, fat marbling, and health of animals as revealed by inspection of the livers and rumen indicated that wood sawdust was equal to hay as a roughage. From the several feeding trials, it may be concluded that sawdust at levels of 5 to 15 percent of the total ration is an effective roughage.

Aspen sawdust was fed to dairy cows on a high grain ration to determine whether it could be a partial replacement for hay and still maintain the normal fat content of milk *(139, 140)*. A ration composed of 5.1 pounds of hay and 37.5 pounds of pelleted grain, one-third of which was aspen sawdust, maintained a normal milk fat level. When a similar ration with the sawdust omitted was fed, the milk fat level decreased by 50 percent. Ruminating time doubled when aspen sawdust was included in the high-concentrate ration. Sawdust was thus shown to be a usable roughage for dairy cows, especially when hay is in short supply.

As mentioned previously, the carbohydrates in wood are not generally available as nutrients for farm animals. To increase the digestibility of these components, physical and chemical treatments were tested for their ability to break down the materials. Treatments investigated were electron radiation, vibratory ball milling, gaseous and liquid ammonia, gaseous sulfur dioxide, pulping, dilute sodium hydroxide, and white-rot

fungi. Ideally, to determine the effectiveness of the treatments, the treated material should be used in feeding trials to determine acceptability, palatability, and digestibility. Feeding trials, however, require rather large quantities of material and are time consuming. An *in vitro* rumen method was adapted by Mellenberger *et al. (88)* to determine the digestibility of the products. Later an enzyme method using the cellulase from *Trichoderma viride* was developed *(94)*. These two procedures were used to determine the digestibility response to the various treatments. Feeding trials to determine the *in vivo* digestibility were made with those products which showed promise in the *in vitro* trials.

The digestibilities of the wood and bark of several tree species by the *in vitro* rumen method are shown in table 52 *(91)*. Aspen is unusual in having a significant digestibility. As a class, hardwoods are somewhat digestible while softwoods are essentially indigestible. Feeding trials with sheep and goats were done on red oak and aspen at several levels in the ration *(88, 91)*. By extrapolation of the data to 100 percent wood, oak was shown to be zero percent digestible and aspen about 40 percent. Thus, the *in vivo* and *in vitro* results are in close agreement.

High-energy electron irradiation has been suggested as a means of increasing the digestibility of wood for ruminants or as a pretreatment before enzymatic hydrolysis of the polysaccharides in wood. Millett *et al. (91)* investigated the effect of increasing levels of electron irradiation on the digestibility of aspen and spruce. For aspen irradiated at 10^8 rep, (roentgen equivalent physical) digestion, determined by rumen assay, was essentially complete; it is assumed that only carbohydrate is solubilized. Saeman *et al. (136)* had found in earlier work on the use of electron irradiation to enhance wood saccharification that carbohydrate destruction was about 15 percent at a dosage of 10^8 rep and increased to 45 percent at 5×10^8 rep. The response of spruce to electron radiation was very low; the maximum digestibility was only 14 percent at 10^8 rep. Although electron irradiation appears to provide a means for enhancing the digestibility of some woods, the authors considered the costs to be too high to be of commercial interest.

In studying the lignin-cellulose bond in wood, Pew *(117)* and Pew and Weyna *(118)*, ground aspen and spruce wood in a vibratory ball mill for up to 8 hours. On treatment of the milled material with cellulase enzymes, up to 96 percent of the carbohydrates were removed, in sharp contrast to the untreated wood. In addition to hydrogen and covalent bonding between the carbohydrates and lignin, this work suggested that "incrustation" or "snake cage resin" effect is an important factor in the lack of activity of cellulase enzymes on the carbohydrates in untreated wood. The three-dimensional lignin network acts as a barrier that prevents the large enzyme molecule from entering the structure. If the particles produced by vibratory milling are small enough, the lignin barrier is destroyed and the enzyme can remove most of the cellulose.

Millett *et al. (91)* determined the effect of vibratory ball milling on the *in vitro* rumen digestibility of a number of hardwoods and softwoods. The digestibility of the hardwoods increased rapidly during the first 20 minutes of milling, after which there was a gradual increase until a plateau was reached at about 2 hours' milling time. In this work, species specificity was apparent. Aspen and sweetgum showed maximum dry matter digestibilities of about 75 percent; red oak, 57 percent; hickory, 40 percent; and red alder, 20 percent. Softwoods were less responsive to vibratory ball milling than were the hardwoods. The maximum *in vitro* digestibility of five softwoods (Douglas-fir, western hemlock, ponderosa pine, slash pine, and redwood) after 2 hours of milling was about 18 percent. The reason for this selective response of the various wood species is unclear. Particle size would seem to be ruled out since all wood samples received the same degree of milling and settling tests indicated similar particle size distribution. Quantity and distribution of lignin in the cell wall may be the controlling factor.

The selective response of the various woods to vibratory ball milling makes this technique of limited value for upgrading wood residues for animal feedstuffs. In addition, this finely ground material would probably pass too quickly through the rumen so that the *in vivo* digestibility would be considerably less than the *in vitro* results. This has been shown with forages where fine grinding actually resulted in a decrease in digestibility of cellulose in the rumen. Fine grinding might be of interest as a pretreatment for responsive forms of biomass which are to be enzymatically hydrolyzed to the simple sugars for further conversion by fermentation to selected chemicals such as ethyl alcohol.

A method of increasing the digestibility of low-grade roughages, such as straw, is a steeping treatment with a dilute sodium hydroxide solution *(147)*. The digestibility of the resulting product is about 30 percent greater than the original straw. This method has been used, principally in Europe, during times of severe shortages of conventional feeds. It was of interest to determine whether wood would respond to this treatment with an increase in digestibility. Five grams of sawdust of various wood species were treated at room temperature for 1 hour with 100 milliliters of a 1 percent sodium hydroxide solution *(91)*. The sawdusts were then washed with water to neutrality, dried, and assayed. Under these conditions, the yield was about 95 percent.

In vitro digestion of the products showed a range of response and species specificity. Aspen and basswood were outstanding in their response with digestibilities of 55 percent. Black ash, white birch, and soft maple showed an intermediate response with digestibilities of 35 to 40 percent. Red and white oaks, elm, yellow birch, and cottonwood ranged between 10 and 20 percent. The two softwoods, Douglas-fir and Sitka spruce, showed no response to the treatment.

Further work showed that the minimum amount of sodium hydroxide, 6 grams per 100 grams of wood, necessary to produce the maximum response to *in vitro* digestibility, was roughly equivalent stoichiometrically to the combined acetyl and carboxyl content of the wood. This supports the contention *(30, 163)* that the principal effect of alkali treatment is the saponification of intermolecular ester bonds. Breaking of these crosslinks allows the swelling of wood in water beyond normal waterswollen dimensions. Thus, penetration of enzymes into the fine structure of wood would be enhanced.

Alkali-treated aspen sawdust was fed to goats in a roughage ration and also incorporated in a concentrate ration *(89)*. In the roughage rations, the

alkali-treated aspen was 52 percent digestible and in the concentrate ration, it was 48 percent digestible. In the concentrate ration, untreated aspen was found to be 31 percent digestible. Alkali treatment can thus upgrade aspen sawdust to a low-quality hay. The cost of treatment is low, but its chief shortcoming is the fact that it only works well on a few species of hardwoods.

Treatment of aspen sawdust with either gaseous or anhydrous liquid ammonia substantially increased its *in vitro* digestibility. The treatment is rapid since maximum digestibility was achieved in 1/2 hour. The treatment is simple; after removal of the gaseous or liquid ammonia, the treated sawdust is air d ied and ready for use. An added benefit is the increased nitrogen content of the product. Kjeldahl analysis of the ammonia-treated aspen showed 9 percent crude "protein" compared to a control value of 0.5 percent. Again the process is species specific because ammonia-treated red oak was 7 percent digestible and spruce 2 percent. A digestion trial of the treated aspen material with goats indicated an *in vivo* dry-matter digestibility of 50 percent which was in close agreement with the *in vitro* data *(9)*.

Vapor phase treatment of wood with sulfur dioxide disrupts the lignin-cellulose complex and yields a product with a high digestibility *(9)*. Wood in the form of sawdust is treated with an initial pressure of sulfur dioxide of 30 pounds per square inch at room temperature; the temperature is raised to 120° C and held for 2 to 3 hours. There is no free liquid present as the water to wood ratio is 3 to 1. After blow-down and evacuation to remove sulfur dioxide, the treated material is neutralized to pH 7 and air dried. Since no washing is involved, the yield of sulfur dioxide-treated product is 106 to 112 percent, a result of sulfonation and neutralization. Although all of the lignin was retained in the products, the Klason lignin analysis of the hardwoods showed greatly decreased lignin values. This suggests that the lignin was extensively depolymerized by the treatment and was converted in part to soluble products. Table 53 presents analytical data for lignin and carbohydrates as well as values for digestibility data by the use of the cellulase digestion method. Enzymatic conversion of the carbohydrates to the simple sugars was almost quantitative for the hardwoods and somewhat less so for the softwoods. The sulfur dioxide treatment is one of the few methods which is not species specific.

Three hundred pounds of the treated material was prepared from red oak sawdust and fed to goats in a pelleted ration containing 0, 20, 35, and 50 percent of the sulfur dioxide-treated sawdust over an 8-week period. Extrapolation of the data to 100 percent sulfur dioxide-treated wood yielded values of 52 percent for *in vivo* dry matter digestion and 60 percent for carbohydrate digestion. Thus, the vapor phase sulfur dioxide treatment effectively converts red oak sawdust into a ruminant feedstuff having a digestible energy equivalent to a medium-quality hay.

In times of emergency, delignification of wood by chemical pulping has been resorted to as a means of producing a digestible fodder. During World War II, it is estimated that over 1.5 million tons of chemical pulps from spruce, pine, and fir were fed to livestock in the Scandinavian countries *(64, 102)*. In the post-war economy, these high-quality pulps could not compete economically with the usual feedstuffs so their use declined rapidly.

To obtain information on the degree of delignification necessary to produce an acceptable fodder from wood, Baker *(7)* prepared a series of kraft pulps having a range of yields and lignin contents. Pulps were prepared from two hardwoods, paper birch and red oak, and from two softwoods, Douglas-fir and red pine. Pulps were prepared in yields of 40 to 80 percent with lignin contents of 1 to 32 percent. Some of the high-yield pulps had lignin contents higher than the original wood, because hemicellulose is removed more rapidly than lignin in the early stages of pulping. The relationship between rumen *in vitro* digestibility and extent of delignification is presented in figure 11. Extent of delignification is the percent of lignin removed from the original wood.

The difference in the delignification-digestion response between hardwoods and softwoods is clearly shown in the figure. With the hardwoods, digestibility increases rapidly with delignification and approaches a plateau of about 90 percent digestibility. With the softwoods, considerable delignification must take place, 30 to 40 percent, before any significant increase in digestibility occurs. After this phase, digestibility rises rapidly to the maximum.

To obtain a product having 60 percent *in vitro* digestibility—that of a good quality hay—the lignin content of the hardwoods must be reduced by only 25 to 35 percent while that of the softwoods must be reduced from 65 to 75 percent. Saarinen *et al. (128)* prepared a series of birch and spruce pulps by 10 different pulping methods and determined the *in vivo* digestibility of the products with sheep. The results are comparable to those shown in figure 11. Degree of delignification and not the method of pulping governs the digestibility of pulps.

In the pulp and paper industry, it is estimated that about 80 pounds of fiber residues are generated for each ton of pulp produced. Thus, about 2 million tons of these residues are produced annually. Most of these residues have undergone at least partial delignification. Millett *et al. (93)* have investigated both the *in vitro* and *in vivo* digestibility of a number of representative samples of these commercial residues. As was to be expected, groundwood fines, with the exception of aspen, had very low digestibility as no delignification had taken place. Screen rejects and pulp fines from chemical pulps had digestibilities ranging from 40 to 70 percent. Thus, any of these residues from the chemical pulping processes could serve as energy nutrients for livestock.

Wood decayed by some white rots has a lower lignin content than that of the original wood. All the white-rot fungi decompose lignin as well as the cellulose and hemicellulose. Kirk and Moore *(72)* examined nine white-rot fungi for their ability to remove lignin faster than they removed polysaccharides from aspen and birch wood. In addition, one of the organisms, *Fomes ulmarius*, was examined with southern pine, Douglas-fir, and Sitka spruce.

During decay most of the fungi decreased the lignin content of the wood; that is, a larger percentage of the lignin was removed than the polysaccharides. Lignin removal was always accompanied by removal of polysaccharides. It is probable that catabolism of carbohydrates is necessary to provide energy for the breakdown of lignin. Both rumen and cellulase methods were used to determine the digestibility of the decayed

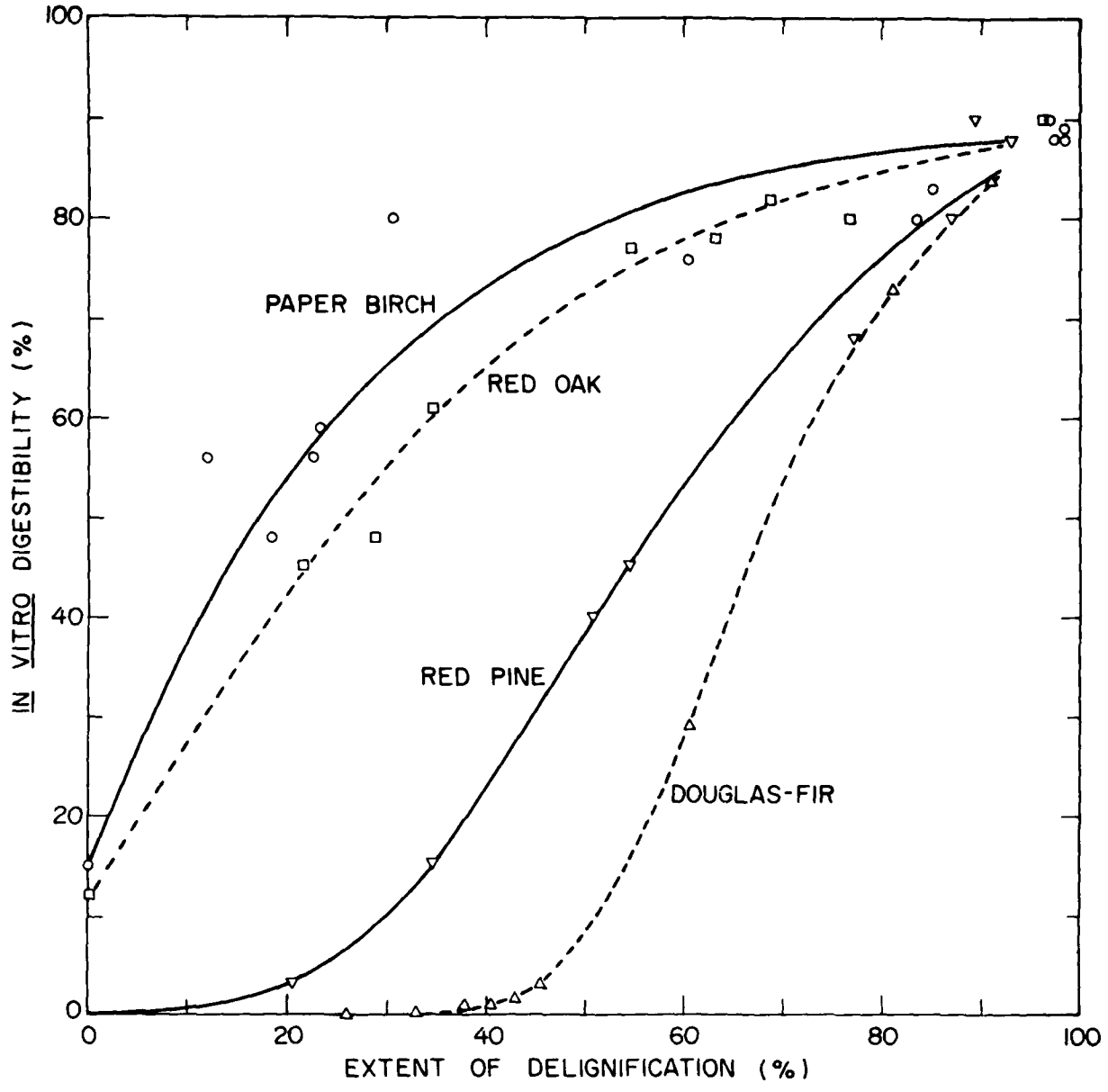

Figure 11.—Relationship between in vitro digestibility and extent of delignification for kraft pulps made from four wood species (9).

(M 140 060)

wood samples. As found in the pulping studies, digestibility is inversely related to the lignin content of the samples.

▲ ▲ ▲ ▲

SUMMARY

The key to successful utilization of wood as a raw material in the fermentation industries is the economic processing of wood to produce monomeric sugars. Cellulose, because of its highly crystalline organization, is extremely resistant to hydrolysis by dilute acids. The conditions of temperature and acid concentration necessary to carry out the reaction in a reasonable time seriously degrade the sugars produced, resulting in low yields. Concentrated acid hydrolysis can produce monomers in high yields but the necessary recovery of the acid is costly. Enzymatic hydrolysis of cellulose is possible, but the highly lignified nature of wood requires extensive pretreatment to open up its structure and make the cellulose accessible to the enzyme. It has been said that, if cellulose were as easily hydrolyzed as starch, a chemicals-from-wood industry would now be a reality. It is doubtful that with present technology a plant producing only chemicals would be viable in the United States without subsidy. At the present time, however, some $500 million worth of chemical byproducts or coproducts appear as part of the output of wood industries. Such products are expected to become more prominent. The rising cost of petrochemical feedstocks is intensifying the search for fully competitive wood hydrolysis processes. Much past work of the Forest Products Laboratory and other research organizations shows that wood carbohydrates are a potential source of many valuable and useful products.

Sugar solutions produced by the Madison Wood Sugar process from a number of wood species were fermented by the yeast, *Saccharomyces cerevisiae*, to ethyl alcohol. Because pentose sugars are not fermented by yeasts, sugars produced from softwoods were 75 to 80 percent fermentable, whereas those produced from hardwoods were only 52 to 62 percent fermentable. This reflects the difference in the hemicellulose composition between the hardwoods and softwoods. It precludes the use of hardwoods from ethyl alcohol production unless a satisfactory use can be found for the pentoses.

Due to inhibitory substances produced during hydrolysis, a preformed yeast inoculum must be used to obtain satisfactory fermentations. It was found, however, that the yeast could be recycled indefinitely with no loss in activity. In the pilot plant, yields of 65 gallons of alcohol could be obtained from 1 ton of ovendry Douglas-fir wood. The data from the pilot plant studies were used in an engineering cost study. For a 25-million-gallon-per-year facility with a wood cost of $34/ton, it was estimated that, at 1975 prices, the cost of ethyl alcohol would be $1.90 per gallon. Scaleup to 100 million gallons per year would reduce the cost to $1.42.

A glycerol fermentation using sodium bisulfite as a steering agent was investigated. Glycerol yields of 28 percent were obtained based on glucose in the fermentation together with 21 percent ethyl alcohol and 12 percent acetaldehyde. An ion-exclusion method was developed for recovery of the glycerol. Osmophilic yeast isolates were examined for their polyol-producing ability. *Torulopsis magnoliae* produced glycerol yields of 35 to 40 percent based on sugar in aerated fermentations with limited phosphate availability. Similar yields of arabitol were obtained with *Endomycopsis chodati*, while an unidentified isolate produced comparable yields of erythritol.

Wood sugar solutions were also used to produce butanol, acetone, lactic acid, and 2,3-butanediol in fair yields.

Fodder yeast, single cell protein, was produced from dilute wood sugar solutions. Both pentose and hexose sugars are utilized in an aerated medium containing only inorganic salts in addition to the sugars. A rapid continuous fermentation was developed with yeast *(Torulopsis utilis)* yields of 50 percent based on the sugar. Crude protein content of the yeast was approximately 50 percent. Of the amino acids essential to human nutrition, only the methionine content of the yeast protein was low compared to casein, a complete protein.

Some 200 tons of wood sugar molasses containing 50 percent reducing substances were produced in the pilot plant and used at a number of agricultural experiment stations in feeding studies with farm animals. Feeding tests were made with the molasses in mixed feeds, sprayed on roughage, and as a preservative for grass silage. Milk cows, beef cattle, sheep, swine, and poultry were used in these trials. As a result of these investigations, wood molasses was concluded to be comparable to cane molasses as a feedstuff.

The potential of wood and bark for use either as a non-nutritive roughage or as a source of digestible carbohydrates has been investigated. Wood has been shown to be a practical roughage replacement in beef cattle rations at concentrations of 5 to 15 percent in the feed. Aspen sawdust, as a roughage extender in dairy cattle rations, maintained the fat content of the milk.

Both *in vitro* and *in vivo* methods have been used to assay the digestibility of untreated and chemically and physically treated wood for ruminant nutrition. Except for a few species of hardwoods, untreated wood does not serve as an energy source in ruminant rations. Treating both coniferous and deciduous species by vibratory ball milling, electron irradiation, dilute alkali, or anhydrous ammonia to increase the digestibility of wood revealed a species specificity effect. Coniferous species were largely unaffected, but the digestibility of a few deciduous species, most notably aspen, could be raised to that of a medium-quality hay. Vapor phase treatment of sawdust with sulfur dioxide did markedly increase the digestibility of all wood species tested.

A series of kraft pulps with different degrees of delignification were prepared and their rumen digestibility determined. The extent of lignin removal and not the method of removal determines the digestibility of the product. Many pulp and papermaking residues are partially or completely delignified. These could serve as nutritive feedstuffs for ruminants.

Although the work on treating wood to increase its digestibility was done to produce a feedstuff for farm animals, it has relevance to much of the work now going on to produce a substrate for the enzymatic hydrolysis of biomass for the production of ethyl alcohol and gasohol.

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Table 1.—Land area and net annual photosynthetic production of dry matter[1]

Geographic division	Area (total = 510 million Km²)		Net productivity (total = 155.2 billion tons dry wt/yr)	
	Percent of Total		Percent of Total	
Forests				
Tropical rain	3.3		21.9	
Raingreen	1.5		7.3	
Summer green	1.4		4.5	
Chaparral	.3		.7	
Warm temperate mixed	1.0		3.2	
Boreal (Northern)	2.4		3.9	
Subtotal		9.8		41.6
Woodland		1.4		2.7
Dwarf and scrub				
Tundra	1.6		.7	
Desert scrub	3.5		.8	
Subtotal		5.1		1.5
Grassland				
Tropical	2.9		6.8	
Temperate	1.8		2.9	
Subtotal		4.7		9.7
Desert (extreme)				
Dry	1.7		0.0	
Ice	3.0		0.0	
Subtotal		4.7		0.0
Cultivated land		2.7		5.9
Freshwater				
Swamp and marsh	.4		2.6	
Lake and stream	.4		.6	
Subtotal		.8		3.2
Continents total		29.2		64.6
Reefs and estuaries		.4		2.6
Continental shelf		5.1		6.0
Open ocean		65.1		26.7
Upwelling zones		.08		.1
Oceans total		70.8		35.4
Grand total earth		100.0		100.0

[1] Percentages based on data presented by H. Lieth at the Second National Biological Congress, 1971. Saeman, J. F., Energy and materials from biomass. Symposium on Clean Fuels from Biomass and Wastes. Orlando, Fla. Jan. 1977.

Table 2.—The composition of certain woods[1]

	Lignin	Holo-cellulose	Alpha-cellulose	Hemi-cellulose	Pentosans	Uronic acid anhydride	Acetyl	Methoxyl in carbohydrate
					Pct			
White spruce	26.6	73.3	49.5	23.8	10.9	2.68	2.35	0.70
Red spruce	26.6	72.9	48.3	24.6	11.6	3.20	2.50	0.92
Eastern hemlock	31.5	68.5	48.2	20.3	10.0	3.40	1.87	0.84
Balsam fir	30.1	69.9	44.0	25.9	10.3	3.08	2.24	0.41
Jack pine	27.2	72.5	49.5	23.0	12.8	2.92	1.92	0.75
Aspen	17.3	82.5	50.7	31.8	23.5	4.28	4.65	0.93
Willow	22.0	78.3						
Maple	23.5	76.3	50.0	26.3				
White oak	24.1	75.4	49.5	25.9				

[1] All values based on ovendried, extractive-free wood *(169)*.

Table 3.—Analysis of biomass samples (bone-dry basis)

Component	White spruce[1]	Douglas-fir[1]	Southern red oak[1]	Aspen	Corn stalks[2]	Wheat straw[2]
			Percent			
Glucan	46.5	43.46	40.63	45.97	34.0	37.0
Mannan	11.6	10.76	1.97	2.10	0.5	0.3
Xylan	6.8	2.77	19.19	17.74	14.0	18.9
Galactan	1.2	4.66	1.22	.79	1.0	0.5
Araban	1.6	2.67	0.36	1.23	1.7	5.6
Total carbohydrate	67.7	64.32	63.37	67.83	51.2	62.3
Lignin	26.7	31.30	23.91	20.30	13.1	13.6

[1] Data from FPL files.
[2] Sloneker, J. H. Biotech. and Bioeng. Symp. No. 6, p. 246 (1976).

Table 4.—Yield of potential reducing sugars and fermentable sugars from samples of representative hardwoods and softwoods *(131)*

Species	Potential reducing sugars	Fermentability	Potential fermentable sugars
		Percent	
Hardwoods			
American beech	70.1	75.1	52.6
Aspen	75.1	76.3	57.3
Birch	69.9	67.8	47.4
Maple	68.2	71.0	48.4
Red oak	63.6	63.0	40.2
Sweetgum	66.4	73.8	49.0
Yellow-poplar	70.9	76.1	54.0
Softwoods			
Douglas-fir	66.6	86.2	57.4
Eastern white pine	66.5	86.3	57.4
Hemlock	66.1	88.2	58.3
Ponderosa pine	68.0	82.2	55.9
Redwood	52.4	77.1	40.4
Sitka spruce	70.1	85.3	59.8
Southern yellow pine	64.8	82.0	53.2
Sugar pine	64.3	82.4	53.0

Table 5.—Yield of sugar by hydrolysis of wood by the Madison wood-sugar process *(161)*

Type of wood product	Hydrolysis time	Yield of sugar	Average sugar concentration
	Hours	Percent	Percent
White spruce chips	3.1	54.2	5.1
Douglas-fir chips	3.0	52.5	5.3
Douglas-fir sawdust	3.1	44.7	4.9
Douglas-fir hog fuel	3.0	38.7	5.1
Douglas-fir bark	2.9	15.3	2.3
Southern yellow pine woods waste	3.3	50.0	4.8
Southern yellow pine sawdust	3.1	47.5	4.6
Ponderosa pine chips	3.0	51.5	5.35
Eastern white pine sawmill slabs	3.1	44.6	4.55
White fir chips	3.0	53.8	5.40
Western white pine chips	3.0	46.6	5.0
Sugar pine chips	3.0	46.9	4.0
Western hemlock chips	3.0	51.5	5.0
Western larch chips	3.0	54.0	4.9
Western larch sawmill slabs	3.0	42.0	4.9
Lodgepole pine chips	3.0	51.0	4.9
Spent turpentine chips, long-leaf pine stumps	3.1	40.0	4.8
Western redcedar chips	3.1	46.9	4.3
Redwood chips	3.1	42.6	4.0
Mixed southern oak shavings	3.0	51.0	5.0
Mixed southern oak sawdust	3.0	46.9	5.05
Mixed southern oak sawmill waste	3.1	42.9	4.70
Sugar maple sawmill waste	3.0	48.0	4.75
Aspen sawmill waste	3.2	50.2	5.0
Yellow birch sawmill waste	3.1	49.5	5.30
Hickory sawmill waste	3.2	42.8	4.9
Beech sawmill waste	3.1	46.5	5.04
Willow sawmill waste	3.2	50.0	5.0

Table 6.—Composition of Douglas-fir sawmill waste *(51)*

Material	Bark	Potential total sugar	Potential fermentable sugar
	Percent	Percent	Percent
Shavings	0.0	67.5	57.0
Hogged waste	52.0	50.9	42.1
Sawdust	13.5	63.2	53.4
Slabs	34.6	56.3	47.4
Bark	100.0	37.5	26.4

Table 7.—Toxicity of various compounds to yeast *(85)*

Substance	Concentration causing 25 percent inhibiting effect	
	In yeast propagation	In yeast fermentation
	g/100 ml	g/100 ml
Furfural	0.110	0.0740
5-Hydroxymethylfurfural	.140	.2600
Menthol	.011	.0096
Borneol	.033	.0130
d-Pinene	.002	.0085
Pyrogallol	Not tested	.3700
Phloroglucin	Not tested	1.3600
Gallic acid	Not tested	.2300
Tannin	Not tested	.0250
Terpineol	.037	Not tested
Vanillin	.063	Not tested
Eucalyptol	.065	Not tested

Table 8.—Effect of inoculum size on fermentation time *(75)*

Wet yeast volume	Sugar fermented	Alcohol yield	Fermentation time
———— Percent ————			Hour
1.6	77	36	14.0
2.5	80	42	8.5
3.1	81	44	6.0
5.0	80	45	4.0

Table 9.—Acclimatization of yeast for alcohol production in Douglas-fir wood hydrolyzate (66)

Yeast	Initial sugar utilized[1]		Alcohol production[1]	
	First transfer	Twelfth transfer	First transfer	Twelfth transfer
	Pct			
Torula utilis major	70	84	18	37.3
Torula utilis thermophilis	60	82	18	35.4
Torula utilis No. 2	64	75	18	31.4
Torula utilis No. 3	47	85	10	39.6
Torula utilis No. 660	74	82	33	35.4
Torula utilis No. 793	71	80	33	33.5
Torula utilis No. 900	50	80	13	36.3
Torula utilis No. 957	74	79	34	36.3
Candida albicans	72	80	32	36.9
Candida arborae	74	79	34	35.1
Candida arborae No. 197	64	81	24	34.0
Mycotorula lipolytica	5	77	1	34.9
Hansenula anomala	17	81	1	37.3
Hansenula suaveolen	5	80	1	37.5
Saccharomyces ananensis	53	78	20	36.3
Saccharomyces cerevisiae	27	81	10	32.0
Saccharomyces ellipsoidens	33	81	23	37.5
Baker's yeast	29	84	1	37.9

[1] Based on total reducing sugars.

Table 10.—Effect of volume of fermentation on alcohol yield by *Torula utilis* No. 3 (66)

Fermentation volume	Sugar utilization[1]	Alcohol yield[1]
	Percent	
150 milliliters	80	34.5
7 liters	82	38.6
50 gallons	82	40.0
350 gallons	78	40.1

[1] Based on total reducing sugars.

Table 11.—Crystallization of calcium sulfate in wood hydrolyzate at various temperatures (75)

	130°C[1]		140°C[2]		150°C[3]	
Time	pH	$CaSO_4$	pH	$CaSO_4$	pH	$CaSO_4$
Min		Parts Per million		Parts per million		Parts per million
0.0	1.2	7,980	1.2	7,700	1.2	7,720
5.5	5.4	1,006	4.7	6,800	5.5	690
11	5.5	950	5.3	710	5.1	670
19	5.4	930	5.4	690	5.0	670
30	5.3	960	5.2	690	4.9	650
40	5.2	980	5.3	680	4.8	630

[1] Reported solubility, 830.
[2] Reported solubility, 655.
[3] Reported solubility, 530.

Table 12.—Alcohol yields from wood sugars produced by the Scholler process from various species of wood *(52)*

Species	Reducing sugar yield	Fermentable sugars	Alcohol yield from fermentable sugars	Alcohol yield
	Pct			Gallons/ton[1]
Douglas-fir	49.4	80.8	47.2	56.5
White fir	51.5	74.6	45.2	52.6
Ponderosa pine	50.5	78.7	44.2	53.2
Yellow pine	48.7	76.7	44.6	51.8
Sugar pine	46.9	80.1	46.1	52.5
Western white pine	45.2	80.1	46.4	50.9
White spruce	49.5	77.2	44.1	51.1
Redwood	42.7	75.8	40.9	40.1
Western Hemlock	46.8	81.6	46.9	53.8
Beech	45.9	59.2	41.7	34.3
Yellow birch	49.3	52.4	44.6	38.3
Sugar maple	47.0	56.5	47.1	37.9
Red oak	46.9	62.4	44.6	39.6

[1] Based on ovendry wood.

Table 13.—Hydrolysis of Douglas-fir sawmill wood waste using the Madison Wood-Sugar Process *(51)*

Run No.	Hog fuel B (35 pct bark)	Shavings	Ovendry wood	Bark-free wood	Hydrolysis time	Acid on ovendry wood	Reducing sugar solution weight	Reducing sugar concentration	Yield, bark-free wood	Alcohol concentration	Yield
	Pct	Pct	Lb	Lb	Hour	Pct	Lb	Pct	Pct	g/100 ml	Gal/ton
243	100	—	451	294	3.0	5.8	3,020	5.05	52.0	1.96	65.0
245	100	—	465	302	3.0	6.2	3,000	5.08	50.3	1.97	63.0
246	75	25	448	328	3.1	5.3	3,100	5.18	48.8	2.03	61.0
249	75	25	470	340	3.0	4.5	2,900	5.25	45.0	2.04	56.0
250	72	28	478	344	2.7	4.9	2,700	5.65	45.6	2.20	57.0
252	50	50	460	370	3.0	5.0	2,980	5.15	41.5	2.01	52.0
253	50	50	436	357	2.8	5.4	3,100	4.90	42.5	1.90	53.0
254	52	48	434	350	2.9	5.6	3,100	5.40	48.0	2.12	60.0
255	54	46	447	364	3.1	5.6	3,200	5.48	48.2	2.14	60.2
256	54	46	447	364	3.1	5.6	3,300	5.18	47.0	2.01	58.9
257	100	—	444	290	2.9	7.0	3,200	4.85	53.3	1.89	65.6
262	70	30	385	293	2.8	6.7	3,000	5.01	51.5	1.95	64.3
268	62	38	457	358	3.2	6.3	3,640	4.96	50.5	1.94	63.2
270	56	44	380	314	2.6	5.9	3,100	4.95	49.0	1.93	61.3
271	59	41	383	313	2.7	6.5	3,000	5.06	48.5	1.97	60.7
277	65	35	380	296	2.7	6.7	3,100	4.92	51.5	1.92	64.3
279	70	30	417	319	2.8	7.0	3,500	5.00	54.8	1.95	68.5
280	65	35	412	322	2.9	7.0	3,500	5.00	54.5	1.95	68.0
281	64	36	405	318	2.8	6.5	3,500	4.80	52.7	1.87	66.0
282	67	33	392	302	2.8	6.8	3,200	4.85	51.4	1.89	64.2
283	67	33	386	299	2.5	6.2	3,200	5.10	54.5	1.99	68.2
290	67	33	415	319	2.8	6.8	3,200	5.00	50.2	1.95	62.7
291	72	28	450	339	2.5	5.5	3,200	5.30	50.2	2.06	62.7
297	68	32	405	308	2.7	7.0	3,120	5.04	50.8	1.96	63.5
298	49	51	387	303	2.9	7.2	3,300	5.10	55.5	1.99	69.0
299	67	33	449	343	2.7	6.4	3,650	4.80	50.8	1.87	63.5
302	70	30	391	285	2.6	7.3	3,200	4.85	54.5	1.89	68.2
303	71	29	407	305	2.8	6.8	3,315	4.90	53.1	1.91	66.5
304	70	30	401	301	2.6	6.8	3,100	5.10	52.5	1.98	65.5
Average at 29 runs.....					2.8	6.2		5.07	50.3	1.97	63.0

Table 14.—Fermentation of Douglas-fir wood sugar solutions with recycle of yeast (*Saccharomyces cerevisiae*) (53)[1]

Transfer No.	Reducing sugar	Final sugar	Fermentability	Yeast count cells/ml		Alcohol concentration	Alcohol on total reducing sugar	Alcohol on fermented reducing sugar
				Initial	Final			
	Pct			Million	Million	Pct		
1	3.67	0.75	79.6	35	68	1.36	37.1	46.6
2	4.40	.87	80.2	43	77	1.65	37.5	46.7
3	4.37	.72	83.5	59	74	1.70	38.9	46.6
4	4.22	.71	83.2	74	93	1.68	39.8	47.9
5	4.35	.72	83.4	88	—	1.72	39.5	47.4
6	3.93	.70	82.2	—	112	1.59	40.6	49.2
7	3.81	.55	85.6	93	76	1.59	41.7	48.8
8	3.92	.67	82.9	83	121	1.48	37.8	45.5
9	3.86	.54	86.0	80	98	1.58	40.9	47.6
10	3.76	.50	86.7	—	92	1.52	40.4	46.6
11	3.74	.59	84.2	96	—	1.51	40.4	47.9
12	3.74	.59	84.2	—	96	1.50	40.1	47.6
13	3.80	.62	83.7	76	109	1.51	39.7	47.5
14	3.75	.57	84.8	89	117	1.50	40.0	47.2
15	3.76	.60	84.0	112	109	1.48	39.4	46.8
16	3.76	.63	83.2	97	115	1.44	38.3	46.0
17	3.79	.67	82.3	100	129	1.45	38.3	46.5
18	3.71	.56	84.9	100	124	1.45	39.1	46.0
19	3.73	.64	82.8	84	121	1.42	38.1	46.0
20	4.19	.76	81.9	99	115	1.70	40.6	49.6
21	4.44	.79	81.8	87	99	1.72	38.7	47.1
22	4.57	.79	82.7	76	99	1.67	36.5	44.2
23	4.16	.93	77.6	100	92	1.58	38.0	48.9
[2]24	4.53	.66	85.4	67	77	1.88	41.5	48.6
Average of 24 transfers			83.2				39.3	47.2
Average of 49 transfers			83.5				39.3	47.2

[1] 55-Gallon fermentations.
[2] 25 Additional transfers not tabulated.

Table 15.—Products from wood-based ethanol facility (*69*)

Product	Yield[1]
	Percent
Ethanol	16.4
Furfural	1.0
Methanol	.8
Pentose sugars	3.3
Residue	61.3

[1] Based on ovendry Douglas-fir wood waste.

Table 16.—Effect of reducing sugar concentration, yeast volume, and free sodium bisulfite concentration on glycerol yield and fermentation time (pH of fermentation held at 6.5, temperature 30° C) (*45*)

Yeast volume	Reducing sugar concentration	Free $NaHSO_3$ concentration					
		0.5 percent		1.0 percent		1.5 percent	
		Glycerol	Time	Glycerol	Time	Glycerol	Time
Pct	Pct	Pct	Hour	Pct	Hour	Pct	Hour
1	12.5	29.3	72	27.7	72	[1]	[1]
2	12.5	30.3	48	29.3	48	32.3	72
3	12.5	25.8	30	30.5	30	33.2	48
1	20.0	30.7	96	29.1	96	27.9	96
2	20.0	33.3	80	31.0	72	30.9	96
3	20.0	28.6	72	30.4	40	31.4	72

[1] Did not ferment.

Table 17.—Fermentation balance for glycerol fermentation *(45)*

Compound	Pounds	Percent of reducing sugar	Moles	Moles of carbon[1]	Oxidation value	Oxidation product[2]	Reduction product[2]
Glucose	24.68	100.0	62.2	373.2	0	—	—
Glycerol	7.01	28.4	34.6	103.8	-1	—	34.6
Alcohol	5.33	21.6	52.6	105.2	-2	—	105.2
Acetaldehyde	3.02	12.2	32.6	65.2	-1	—	32.6
Acetic acid	.095	.38	.72	1.44	0	—	—
Lactic acid	.785	3.17	3.96	7.92	0	—	—
Carbon dioxide	8.30	33.60	85.5	85.5	+2	171	—
Total products	24.54	99.35	—	369.06	—	171	172.4

[1] Carbon recovery $= \frac{369.06}{373.2} \times 100 \approx 98.9$ pct.

[2] O/R ration $= \frac{171}{172.4} = 0.993$.

Table 18.—Carbon analysis for centrifuged beer *(45)*

Compound	Product	Carbon
	Pct	Pct
Glycerol	4.68	1.832
Alcohol	2.67	1.392
Acetaldehyde	2.32	1.275
Lactic acid	.09	.036
Acetic acid	.23	.092
Nitrogen:		
Total nitrogen	.045	
NH_3 nitrogen	.029	
Protein (calculated)	.100	.053
Sugar	.13	.052
Total carbon by analysis		4.732
Total carbon by combustion		4.715

Table 19.—Glycerol fermentation *(60)*[1]

Input		Output	
Component	Amount	Component	Amount
	Lb		Lb
Sugar (20 pct solution)	3,521	Glycerol	1,000
Yeast (80 pct moisture)	390	Acetaldehyde	473
Nutrients:		Ethyl alcohol	735
Urea	14.6		
Disodium phosphate	9.4	Sodium bisulfite:	
Potassium chloride	3.1	Bound	1,117
Magnesium sulfate	.8	Free	13
Blackstrap molasses	48.0		
		Residual sugar	32
Sodium bisulfite	1,130		
		Organic acids	36
Sodium carbonate	281		
		Inorganic salts	21

[1] Organism, *Saccharomyces cerevisiae* (FPL No. 49); temperature, 32° C; pH, 6.5.

Table 20.—Primary stripping column *(60)*

Component	Composition by weight		
	Feed	Overhead	Bottoms
	Pct		
Ethyl alcohol	3.33	17.63	0.08
Acetaldehyde	2.15	11.36	.05
Sulfur dioxide	3.15	16.69	.08
Carbon dioxide	.51	2.86	—
Acetic acid	.09	.05	.08
Water	79.68	51.41	86.09
Sodium sulfate	5.21	—	6.39
Residual sugar	.14	—	.18
Sulfuric acid	.76	—	.95
Protein	.10	—	.12
Lactic acid	.10	—	.12
Other organics	.26	—	.32
Glycerol	4.52	—	5.56

Table 21.—Evaporator-Crystallizer *(60)*

Component	Composition by weight, percent			
	Evaporator feed	Crystallizer feed	Finisher feed	Finisher product
Ethyl alcohol	0.08	—	—	—
Acetaldehyde	.05	0.12	0.29	0.57
Sodium sulfite	.15	.36	.84	1.65
Sodium sulfate	7.72	10.15	17.62	14.54
Sodium lactate	.12	.27	.64	1.26
Sodium acetate	.08	.19	.46	.90
Residual sugar	.18	.41	.97	1.89
Protein	.12	.27	.64	1.26
Other organics	.31	.74	1.74	3.41
Water	85.66	66.49	46.20	14.52
Glycerol	5.53	13.00	30.60	60.00

Table 22.—Products and yields obtained in screening classified cultures of *Zygosaccharomyces (114)*

Culture		Fermentation time	Final pH	Yeast volume	Final glucose[1]	Ethyl alcohol	Glycerol	Arabitol	Total products
		Hour		Pct					
Z. acidifaciens:	Y-1011	96	4.1	5.0	4.8	18.1	3.3	9.9	31.3
Z. barkeri:	Y-12	144	4.2	1.5	15.0	16.1	4.0	12.3	32.4
	Y-222	72	5.0	7.2	.4	14.1	3.3	46.9	64.3
Z. fermentati:	Y-1559	144	4.6	2.4	.5	26.0	.0	2.7	28.7
Z. mandschuricus:	Y-54	144	3.9	9.1	.4	15.6	.0	29.8	45.4
Z. mellis:	Y-10	72	4.2	5.4	.9	14.5	8.6	19.1	42.2
	Y-1053	72	5.4	4.7	.4	18.5	3.0	44.5	66.0
Z. nadsonii:	Y-1227	72	5.9	6.1	.3	16.5	5.6	15.7	37.8
Z. nussbaumeri:	Y-6	144	4.0	7.0	.4	9.7	7.5	24.0	41.2
	Y-996	72	4.6	5.9	.3	15.1	7.1	17.2	39.4
	Y-1242	72	4.6	4.7	1.6	19.5	6.7	13.0	39.2
Z. priorianus:	ZP-22	72	3.9	1.5	1.9	35.8	2.3	4.9	43.0
Z. richteri:	Y-998	72	4.7	4.9	.5	21.1	1.9	16.2	39.2
Z. rugosus:	Y-7	72	5.4	4.4	2.5	22.3	1.9	11.5	35.9
	Y-997	72	6.0	5.4	.4	25.6	5.0	14.6	45.2
Z. wyocena:	ZW-1	96	4.2	9.9	1.8	3.1	.1	37.2	40.4

[1] Initial glucose was 20 pct

Table 23.—Products and yields obtained in screening osmophilic isolates *(45)*

Culture	Fermentation time	Yeast volume	Initial glucose	Final glucose	Ethyl alcohol	Glycerol	Arabitol	Erythritol
	Hour				Pct			
I_2A	72	11.6	20.0	0.4	11.8	Trace	0.0	20.1
I_2B	96	12.6	20.0	.4	7.6	14.1	.0	.0
I_3B	48	5.3	11.3	.3	17.2	.0	7.4	.0
I_3C	72	12.2	20.0	.4	13.8	Trace	.0	19.9
I_3D	72	4.8	10.0	.2	12.0	20.8	6.7	.0
I_4B	72	6.1	11.5	.3	20.4	.0	16.1	.0
I_5	72	5.4	20.0	2.4	16.6	2.7	19.8	.0
I_6	72	5.0	20.0	3.1	16.8	2.6	16.5	.0
I_7	96	5.7	20.0	.6	12.2	Trace	19.3	.0
I_8	144	8.0	20.0	7.7	1.7	7.1	.0	15.2
I_9	96	3.9	10.0	5.0	10.0	Trace	Trace	Trace
I_{10}	96	5.2	10.0	3.7	15.2	.0	Trace	Trace
I_{11}	144	6.6	20.0	.4	5.1	Trace	53.3	.0
I_{12}	96	4.2	10.0	7.3	9.0	.0	.0	Trace
I_{13}	96	8.8	17.1	6.4	4.9	5.7	.0	Trace
I_{18}	72	6.9	20.0	.4	14.4	Trace	19.4	.0
I_{20}	72	7.5	17.1	.4	14.9	Trace	19.1	.0
I_{21}	72	6.2	11.4	.3	20.5	.0	17.9	.0
$I_{22}A$	72	14.0	15.9	.4	4.9	Trace	.0	20.8
$I_{22}B$	72	6.2	11.6	.3	19.4	.0	19.8	.0
I_{23}	72	7.1	20.0	.4	14.0	Trace	19.7	.0

Table 24.—Effect of temperature on fermentation of glucose by *Torulopsis magnoliae* I_2B *(46)*

Temperature	Final yeast volume	Glucose concentration (in hours)				Glycerol yield on sugar fermented (in hours)		
		0	24	48	72	24	48	72
°C				Pct				
30	7.5	11.9	7.9	3.2	0.3	31.7	33.1	31.7
35	6.5	10.0	4.2	.2	.2	29.8	36.3	20.9
42	1.6	10.3	10.2	7.6	7.6	.0	.0	16.7

Table 25.—Effect of yeast extract concentration upon yield of products by culture I_2B (medium, 10 percent glucose, 0.1 percent urea; 35° C) *(46)*

Yeast extract concentration	Fermentation time	Final pH	Yeast volume	Final glucose	Ethyl alcohol	Glycerol yield on total sugar	Glycerol yield on fermented sugar
Pct	Hour				Pct		
1.0	48	3.8	8.0	0.5	17.3	23.3	24.5
.5	48	2.9	6.2	.5	3.0	36.5	38.4
.25	72	3.9	4.0	4.8	Trace	22.7	42.4
.125	72	4.9	2.5	8.1	Trace	8.9	35.8

Table 26.—Effect of urea concentration upon fermentation with *Torulopsis magnoliae* I,B *(46)*[1]

Urea concentration	Fermentation time	Final pH	Yeast volume	Final glucose	Ethyl alcohol	Glycerol
Pct	Hour		———	Pct	———	
0.0	48	2.9	5.0	3.8	4.4	37.5
.0	72	2.9	5.3	1.0	4.4	36.4
.05	72	3.2	5.5	.2	5.5	38.8
.10	48	3.2	5.0	.3	5.8	41.4
.20	48	3.8	5.5	.4	5.4	41.6

[1] Medium: 10 percent glucose, 0.5 percent yeast extract, 35° C.

Table 27.—Effect of added phosphate on yields of products *(46)*[1]

KH_2PO_4 added	Time	pH	Yeast volume	Final sugar	Ethyl alcohol	Glycerol
g/100 ml	Hour		———	Pct	———	
0.0	72	2.9	6.0	0.2	3.8	38.9
.007	48	3.1	6.0	.2	7.0	33.4
.014	48	3.3	6.5	.2	9.0	28.0
.028	48	3.3	7.0	.2	10.2	19.0
.056	48	3.3	7.0	.2	12.3	16.0

[1] Medium: 10 percent glucose, 0.5 percent yeast extract, 0.1 percent urea, 35° C.

Table 28.—Effect of aeration and sugar concentration upon yields of glycerol by osmophilic culture I,B *(46)*

Sulfite-oxidation value	Time	Final pH	Yeast volume	Initial glucose	Final glucose	Glycerol
mM O_2/liter/hour	Hour		———	Pct	———	
0	72	4.7	2.5	9.8	0.2	12.1
42	48	3.7	5.0	9.4	.5	38.2
42	48	3.2	7.0	18.3	.4	27.7
42	96	3.5	9.2	30.0	.5	15.1
200	48	3.1	6.0	8.9	.2	40.9
200	72	2.9	11.0	18.4	.5	43.0
200	96	3.7	12.0	27.1	.9	35.6
360	72	3.4	5.7	11.1	.2	33.5
360	72	3.1	9.0	22.0	.5	31.0
360	96	3.4	12.0	32.0	.8	30.7

Table 29.—Fermentation of various substrates by *Torulopsis magnoliae* I_2B *(46)*

Substrate	Time	Final pH	Yeast volume	Concentration of substrate	Substrate fermented	Glycerol yield
	Hour		Pct	g/100 ml	Pct	Pct
L-Arabinose	96	5.8	3.7	11.8	18.2	0.0
D-Galactose	96	4.8	3.7	10.3	18.3	.0
D-Glucose	42	3.3	6.0	10.2	98.3	35.4
Glycerol	72	5.8	10.0	4.8	75.8	—
Lactose	72	7.3	.3	5.9	.0	.0
Levulose	48	2.9	6.3	11.0	99.5	31.3
Maltose	96	8.1	.3	3.8	.0	.0
D-Mannitol	96	5.6	5.5	8.0	24.1	.0
D-Mannose	48	3.3	6.0	9.0	96.1	44.4
D-Sorbitol	96	4.8	5.5	10.3	31.7	.0
Sucrose	48	3.0	5.5	11.2	99.2	34.5
D-Xylose	96	4.5	3.7	11.3	53.6	.0

[1] Medium contains 0.5 percent yeast extract, 0.1 percent urea.

Table 30.—Distribution of carbon in a fermentation by *Torulopsis magnoliae* I_2B *(46)*

Carbon constituents	Weight of constituent	Carbon	Total carbon
	mg/100 ml	mg/100 ml	Pct
I. Carbon in unfermented medium:			
1. Glucose	10,000	4,000	93.5
2. Yeast extract[1]	500	230	5.4
3. Urea	100	20	.5
4. Inoculum[1]	60	28	.6
5. Total, 1 through 4	—	4,278	100.0
II. Carbon in products:			
6. Carbon dioxide	3,593	980	22.9
7. Yeast cells[1]	1,200	552	12.9
8. Glucose, unused	190	76	1.8
9. Soluble nitrogen constituents[2]	357	189	4.4
10. Glycerol	5,101	1,996	46.6
11. Ethanol	339	177	4.1
12. Acetate	170	68	1.6
13. Total, 6 through 13	—	4,038	94.4
14. Unaccounted for, 5 minus 13	—	240	5.6
III. Carbon in cell-free beer:			
15. Direct determination	—	2,610	61.0
16. Total products, 8 through 12	—	2,506	58.6
17. Unaccounted for in beer, 15 minus 16	—	104	2.4
18. Lost as ethanol and other volatile products, 14 minus 17	—	136	3.2

[1] Both yeast cells and yeast extract were assumed to contain 46 percent carbon.
[2] Calculated on the carbon nitrogen ratio of protein, 3.3.

Table 31.—Synthetic medium for *Torulopsis magnoliae* fermentation *(45)*

Constituent	Quantity	Constituent	Quantity
	g/liter		mg/liter
Glucose	100.0	$ZnSO_4 \cdot 7H_2O$	1.75
Urea	1.0	$FeSO_4(NH_4)_2\ SO_4 \cdot 6H_2O$	1.05
Ammonium lactate	1.0	$CuSO_4 \cdot 5H_2O$	.096
KH_2PO_4	.3	$MnSO_4 \cdot H_2O$	.005
KC1	.5	Biotin	.01
$MgSO_4 \cdot 7H_2O$	.25	Thiamin•HC1	1.0

Table 32.—Twenty-gallon stirred aerated arabitol fermentation *(44)*

Fermentation time	pH	Cell volume	Glucose	Arabitol concentration	Arabitol yield on glucose used
Hour		Pct	g/100 ml	g/100 ml	Pct
0	6.1	0.5	13.5	0.0	0.0
17	6.2	5.0	11.3	1.0	44.0
24	6.4	6.0	10.1	1.6	45.8
48	6.3	6.5	7.6	3.3	56.1
72	6.5	7.0	5.4	4.4	53.8
100	6.7	7.5	3.3	5.2	50.9
122	6.8	7.5	2.0	5.5	47.6
144	6.8	7.5	.9	5.8	46.2
168	6.9	7.5	.5	5.7	43.4

[1] Medium: 10 percent glucose, 10 percent blackstrap molasses, 0.1 percent urea; temperature 30° C; aeration 46 mM O_2/liter/hr.

Table 33.—Survey of erythritol-producing isolates *(47)*

Isolate	Fermentation time	Yeast volume	Glucose		Yield based on glucose used		
			Initial	Final	Erythritol	Glycerol	Arabitol
	Hour				Pct		
I_2A	72	10.0	10.6	0.2	37.0	0.0	0.0
I_3C	48	10.0	10.2	.7	*37.0*	*Trace*	*Trace*
$I_{22}A$	72	9.4	10.2	.1	30.0	Trace	Trace
I_8	96	5.0	10.2	5.5	17.0	15.9	0.0

[1] Medium: 10 pct glucose, 0.5 pct yeast extract, 0.1 pct urea; temperature 30° C; aeration 42 mM O_2/liter/hr.

Table 34.—Effect of sugar concentration and nutrient composition upon yields of erythritol *(47)*[1]

Medium							
Initial glucose	Corn steep liquor	Urea	Nitrogen in medium	Final glucose	Fermentation time	Erythritol in beer	Erythritol yield on glucose used
		Pct			Hour	Pct	
5.96	1.0	0.03	0.054	0.26	42	1.88	33.0
5.70	.5	.07	.053	.46	42	1.60	30.5
10.53	1.0	.0	.04	1.05	86	3.65	38.5
10.25	1.0	.1	.086	.28	72	3.84	38.5
17.68	1.0	.17	.119	.48	115	5.61	32.6
26.00	1.0	.17	.119	1.00	112	8.65	34.6
35.70	.5	.21	.118	13.80	168	8.7	39.7
35.70	1.0	.17	.119	7.20	168	13.0	45.6
35.70	2.0	.09	.122	4.80	168	13.3	43.0

[1] Temperature 30° C; aeration 42 mM O_2/liter/hr.

Table 35.—Products of fermentation by *Clostridium felsineum* and *Clostridium butylicum (153)*

Culture	Carbohydrate source	Sugar fermented	Yield of products[1]	Distribution of products			
				Butyl alcohol	Ethyl alcohol	Acetone	Isopropyl alcohol
				Pct			
Clostridium felsineum	hemlock	92	34.0	59	17	24	0
Clostridium felsineum	beech	88	31.0	56	18	26	0
Clostridium felsineum	glucose	97	35.6	58	20	22	0
Clostridium butylicum	hemlock	92	34.6	59	9	4	28
Clostridium butylicum	beech	90	33.2	53	8	4	35
Clostridium butylicum	glucose	99	29.8	70	7	3	20

[1] Yield based on sugar fermented.

Table 36.—Products of fermentation by *Clostridium butylicum (77)*

Carbohydrate source	Sugar fermented	Yield of products[1]	Distribution of products		
			Butanol	Ethanol	Acetone
			Pct		
Maple	85	33	67	3	30
Spruce	64	35	—	—	—
Oak	91	19	74	4	22
Douglas-fir	83	25	67	4	29

[1] Yield based on sugar fermented.

Table 37.—Yields of 2,3-butylene glycol from wood sugars *(113)*

Wood species	Initial sugar	Sugar fermented	Fermentation time	Yield of 2,3-butylene glycol
	g/100 ml	Pct	Hour	Pct
Southern yellow pine	4.3	90.1	18	38.8
	8.5	89.6	38	37.2
Southern red oak	10.0	90.5	36	26.6
White spruce	5.9	87.6	48	31.7
	17.8	88.0	75	37.7

Table 38.—Growth of various yeast cultures in wood hydrolyzate in 24 hours *(50)*

Strain	Initial sugar utilized[1]		Yield of yeast[1]	
	First transfer	Twelfth to fifteenth transfer	First transfer	Twelfth transfer
	Pct			
Torula utilis major	78	82	25	42
Torula utilis thermophilis	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
Mycotorula lipolytica	10	84	5	36
Hansenula anomala	50	80	21	33
Hansenula suaveolens	25	82	11	34
Saccharomyces ananensis	75	84	30	36
Saccharomyces cerevisae 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.

Table 39.—Nitrogen requirements for yeast growth *(50)*[1]

Nitrogen[2] per 100 lb of reducing sugar in feed[3]	Nitrogen content of yeast	Nitrogen recovered in yeast	pH		Reducing sugar utilized
			Feed	Propagator	
Lb	Pct				Pct
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 lb; potassium chloride, 1.6 lb; and magnesium sulfate as 1 lb/100 lb of sugar in feed.
[2] Nitrogen was introduced as urea.
[3] Douglas-fir hydrolyzate reducing sugar content 4.6 percent.

Table 40.—Phosphate requirement for yeast growth *(50)*[1]

Phosphate[2] (P_2O_5) per 100 lb of reducing sugar in feed[3]	Duration of test	Yeast yield based on sugar used	Protein content of yeast	Reducing sugar utilized
Lb	Hour	Pct		
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 at: Nitrogen, 3.4 lb; potassium chloride, 1.6 lb; magnesium sulfate, 1.5 lb/100 lb of sugar in feed.
[2] Phosphate (P_2O_5) was added as $(NH_4)_2HPO_4$.
[3] Douglas-fir hydrolyzate with reducing sugar content 4.5 percent. Feed rate was 3 liters/hr into a tank with 11.5 liters operating capacity.

Table 41.—Potassium requirements for yeast growth *(50)*[1]

Potassium chloride per 100 lb of reducing sugar[2] in feed	Duration of test	Yeast yield based on sugar used	Protein content of yeast	Reducing sugar utilized
Lb	Hour	Pct		
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 lb, magnesium sulfate, 1.5 lb; phosphoric pentoxide, 1.6 lb/100 lb of sugar in feed.
[2] Douglas-fir hydrolyzate with reducing sugar content 4.5 percent. The feed rate was 3 liters/hr into a tank with 11.2 liters operating capacity. Air was introduced at 0.5 cubic foot per minute.

Table 42.—Effect of sugar concentration on yeast growth *(50)*

Concentration of sugar in feed[1]	pH	Dry yeast on sugar used	Protein content	Sugar utilized
Pct		Pct		
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/hr. Air was introduced at a rate of 0.5 cubic foot per minute.
[2] Air rate 0.75 cubic foot per minute.

Table 43.—Growth of yeast on various hydrolyzates *(50)*

Hydrolyzate	Rate of feed per hour	Acidity	Yeast yield	Protein content	Sugar used
	Liters	pH	Pct		
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

[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 44.—Composition of *Torula utilis* grown on sugars from various sources *(160)*[1]

Components	Source of sugar		
	Pulp liquor	Wood hydrolysis	Blackstrap molasses
Crude protein, percent	50.4	54.2	46.7
Crude fat, percent	5.14	3.76	5.82
Ash, percent	9.53	5.56	7.66
Phosphorous, percent	2.08	1.19	1.81
Calcium, percent	.90	.14	.13
Thiamin, mcg/g[2]	5.6	6.9	9.9
Riboflavin, mcg/g	47.9	80.8	39.8
Biotin, mcg/g	2.4	2.3	3.4
Niacin, mcg/g	443.0	450.0	402.0
Pantothenic acid, mcg/g	41.7	134.4	50.8
Pyridoxine, mcg/g	35.5	38.3	43.3

[1] Data of A. J. Wiley, Sulfite Pulp Manufacturers' Research League, Appleton, Wis.
[2] Micrograms per gram.

Table 45.—Amino acid composition of *Torula* yeast and casein

Amino acid	Yeast *(111)*	Casein *(115)*
	g/100 g protein	g/100 g protein
Alanine	3.4	3.5
Arginine	5.4	4.0
Aspartic acid	4.7	7.2
Cystine	.7	.3
Glutamic acid	15.0	22.0
Glycine	4.8	1.9
Histidine	1.9	3.2
Isoleucine	5.3	7.6
Leucine	7.0	10.3
Lysine	6.7	8.2
Methionine	1.2	3.1
Phenylalanine	4.3	5.5
Proline	3.5	11.6
Serine	5.5	5.9
Threonine	5.5	4.5
Tryptophan	1.2	1.2
Tyrosine	3.3	6.1
Valine	6.3	7.2

Table 46.—Yield and protein content of six strains of yeast grown on aspen wood sugars *(54)*

Strain of yeast	Rate of feed	Yeast		
		Sugar utilized	Yield	Protein content
	Liters/hr	Pct		
Torula utilis	18	95.0	46	50.7
Candida krusoides	18	95.0	45	45.4
Mycotorula lipolytica	16	94.0	51	46.1
Torula utilis thermophilis	16	94.8	50	47.6
Candida albicans	12	93.0	49	47.2
Saccharomyces cerevisiae	14	96.0	42	50.7

Table 47.—Evaluation of protein from various feed yeasts compared to casein (54)

Source of protein	Run No.		
	1	2	[1]3
	Gain/rat/day, g		
Torula utilis	3.1	3.1	6.2
Candida krusoides	3.2	2.8	5.9
Mycotorula lipolytica	3.7	3.4	6.6
Torula utilis thermophilis	3.0	3.1	6.2
Candida albicans	4.2	3.5	6.6
Saccharomyces cerevisiae	3.6	3.7	6.1
Sulfite yeast	3.1	3.5	5.8
Casein	5.0	5.5	5.6

[1] With methionine.

Table 48.—Composition of wood molasses (168)[1]

Constituent	Content
	Pct
Total dry matter	60-62
Reducing substances (as glucose)	48-50
Polysaccharides	0.5-1.5
Nonsugar organic matter	6.0-8.0
Ash	2.0-3.0
Nitrogen	0.065
Volatile organic acids	1.0-2.0
Insoluble fiber	None

[1] Average for various species.

Table 49.—Feeding trials of wood molasses and corn at Mississippi State College *(10, 168)*

Test factors	Lot I ration cottonseed meal, hay, corn	Lot II ration cottonseed meal, hay, three-fourths corn one-fourth molasses	Lot III ration cottonseed meal, hay, three-fourths corn
Number of steers	4	4	4
Length of trial, days	120	120	120
Average initial weight, lb	474	474	474
Average final weight, lb	693	708	655
Average gain, lb	219	234	181
Average daily gain, lb	1.83	1.95	1.51
Feed per steer, lb			
Corn	[1]1,287	[1]952	[1]952
Wood molasses		[1]290	
Cottonseed meal	257	257	257
Hay	389	389	389
Lb feed/lb gain	8.8	8.1	8.8

[1] Dry matter basis.

Table 50.—Reversal feeding trial of wood molasses and corn *(10, 168)*

Test factors	Lot I ration cottonseed meal, hay, corn	Lot II ration cottonseed meal, hay, three-fourths corn	Lot III ration cottonseed meal, hay, three-fourths corn one-fourth molasses
Number of steers	4	4	4
Length of trial, days	30	30	30
Average initial weight, lb	693	708	655
Average final weight, lb	749	754	729
Average gain, lb	56	46	74
Average daily gain, lb	1.87	1.53	2.47
Feed per steer, lb			
Corn	[1]404	[1]303	[1]303
Wood molasses			[1]87
Cottonseed meal	81	81	81
Hay	90	90	90
Lb feed/lb gain	10.3	10.5	7.6

[1] Dry matter basis.

Table 51.—Effects of wood molasses in egg-laying tests with poultry at Oregon State College *(20)*

Year	Ration	Hens started on test	Egg production	Feed consumption per dozen eggs	Mortality
			Pct	Lb	Pct
[1]1948-49	Control	24	62.2	5.2	45.8
	7.5 pct Wood molasses	24	66.4	5.9	29.2
	15 pct Wood molasses	24	57.0	6.6	20.8
	7.5 pct Wood molasses; 7.5 pct beet pulp	24	52.8	6.3	37.5
[2]1949-50	Control	36	57.7	5.7	38.9
	7.5 pct Wood molasses	36	63.6	5.7	22.2
	15 pct Wood molasses	36	53.8	6.9	27.9
	7.5 pct Wood molasses; 7.5 pct beet pulp	36	58.4	6.8	19.4

[1] Test period of 304 days.
[2] Test period of 273 days.

Table 52.—*In vitro* digestibility of various woods and barks *(91)*

Species	Digestibility	
	Wood	Bark
	Pct	
HARDWOODS		
Trembling aspen	33	30
Soft maple	20	—
Black ash	17	45
Sugar maple	7	14
White birch	8	—
Yellow birch	6	16
American elm	8	27
Shagbark hickory	5	—
Eastern cottonwood	4	—
White oak	4	—
Red oak	3	—
SOFTWOODS		
Douglas-fir	5	—
Western hemlock	0	—
Western larch	3	7
Lodgepole pine	0	—
Ponderosa pine	4	—
Slash pine	0	—
Redwood	3	—
Sitka spruce	1	—
White spruce	0	—

Table 53.—Composition and cellulase digestion of various woods before and after SO_2 treatment *(9)*

Species	Lignin		Carbohydrate		Digestibility[1]	
	Before	After	Before	After	Before	After
	Pct					
Quaking aspen	20	7	70	71	9	63
Yellow birch	23	9	66	67	4	65
Sweetgum	20	5	66	64	2	67
Red oak	26	8	62	60	1	60
Douglas-fir	30	24	65	63	0	46
Ponderosa pine	31	19	59	58	0	50
Alfalfa	17	—	51	—	25	—

[1] Cellulase digestion method.

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U.S. Forest Products Laboratory.

Biological utilization of wood for production of chemicals and foodstuffs, by George J. Hajny, Madison, Wis., 1981.

65p. (USDA For Serv. Res. Pap. FPL 385).

Reviews work the Forest Products Laboratory has done over the past 70 years to produce chemicals and foodstuffs from wood residues.

KEYWORDS: Cellulose, hemicellulose, saccharification, fermentation, fodder yeast, wood molasses.

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