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KURT LESLIE FREY (Name) for the MASTER OF SCIENCE (Degree)

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The dilute sulfuric acid hydrolysis of annual rye grass (Lolium multiflorum) straw was investigated. The straw was hydrolyzed in a stainless steel pressure bomb at 121° C, 135° C, and 150° C with 0.5 to 3.0 percent sulfuric acid for time periods up to 40 minutes. These conditions resulted in the hydrolysis of the hemicellulose portion and possibly some of the amorphous region of the alphacellulose.

The sugars in the hydrolyzates were identified and quantitated by gas-liquid chromatography. The predominate sugars were xylose with glucose and mannose following, respectively. These three sugars constituted approximately 80 to 90 percent of the total sugars. Galactose and arabinose were also recovered.

The results of the single-stage hydrolyses indicated that mannose was released at the milder hydrolytic conditions (121°C), and was destroyed as the conditions became more severe. Optimum yields of mannose were 2.5 to 3.2 grams per liter. Glucose yields

increased as the hydrolysis conditions increased in severity. Optimum yields of glucose were 3.5 to 4.3 grams per liter. Xylose yields increased as the conditions became more severe up to 150° C and then they started to decline. Optimum xylose yields were 12 to 16 grams per liter. The difference in mannose, glucose, and xylose release and destruction can be attributed to the total amounts of each sugar in the hemicellulose, relative stability to acid, number of sources, and accessibility of the sources.

A two-stage hydrolysis, undertaken to optimize hexose and total sugar yields, showed that a higher yield of hexose could be obtained in this manner.

Growth studies using Candida utilis (strain NRRL Y-1084) indicated that there was an increase in the inhibition of growth as the severity of the hydrolysis was increased. This was believed to be the result of increased degradation sugars to compounds toxic to the yeast.

Hydrolyses of Rye Grass Straw for Propagation of *Candida utilis*

by

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Redacted for privacy

Chairman of Department of Microbiology

Redacted for privacy

Dean of Graduate School

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INTRODUCTION

The Willamette Valley of Oregon is a major grass seed producing region. After harvesting the seed in July and August, the left-over straw and stubble, amounting to approximately 1.5 million tons, is burned to clear the fields. Open field burning has gained favor over the years as the principal means of removing straw and stubble because it is simple, inexpensive, and destroys many plant pathogens that would otherwise remain viable through the winter in the grass stubble. Due to the topography and meterological conditions, the Willamette Valley has little circulating air and, consequently, this practice produces excessive air pollution. In addition to being a potential public health hazard, air pollution decreases visibility which has hindered automobile and air traffic. Increased public opposition to burning has moved the legislature to ban open field burning after January 1, 1975.

The forthcoming ban on field burning means that alternative methods of disposing of the grass seed straw must be found. One potential method is to convert the celluloses in straw into their component monomeric sugars by dilute sulfuric acid hydrolysis. Food and fodder yeast with high protein content can then be grown on the

sugars. Dilute sulfuric acid hydrolysis of wood cellulose, developed in Germany and later perfected in the United States, has already been used to produce alcohol and food and fodder yeast (2, 22, 33).

The annual rye grass straw (Lolium multiflorum) has been hydrolyzed with dilute sulfuric acid in an autoclave at 121° C and 15 p. s. i. (20). The purpose of this study was to investigate the hydrolysis of annual rye grass straw at temperatures and pressures up to 150° C and 55 p. s. i. respectively. This procedure will hydrolyze only the hemicellulose fraction of straw and possibly some of the amorphous region of the alphacellulose.(6, 36, 42, p. 207). Higher temperatures are necessary to completely hydrolyze the remaining carbohydrates, particularly alphacellulose, but were not employed because of design limitations of the pressure bomb.

This study determined the optimal temperatures, acid concentration, and time of hydrolysis to obtain maximal yields of sugars from the hemicelluloses of annual rye grass straw. The sugars released from annual rye grass straw have been used to propagate Candida utilis, a food and fodder yeast (20). The project was designed to keep the costs as low as possible. The data may be useful in the development of a pilot plant for hydrolyzing straw and propagating yeast.

LITERATURE REVIEW

A literature review was conducted on the carbohydrate composition of wood and straw, the dilute sulfuric acid hydrolysis of the carbohydrates and wood, the potential microbial inhibitors in hydrolyzates, and the production of yeast. It should be noted that most of the previous work is concerned about the chemistry and hydrolysis of wood; many of the cited references deal therefore with wood rather than straw. This should pose no problem since the carbohydrate chemistry of straws and woods, in fact most plants, is very similar (38).

Carbohydrate Chemistry

The two major groups of chemical compounds in plants are the carbohydrates and lignins. The carbohydrates are long-chain polysaccharides which can be divided into three subgroups: celluloses, hemicelluloses, and pectins. The carbohydrates account for approximately 70 to 75 percent of wood and straw on a dry weight basis. The carbohydrate fraction of wood and straw is composed almost entirely of cellulose and hemicellulose (4, 6, 14, 17, 38).

Cellulose

Cellulose is the main structural component of the cell-wall of plants and accounts for approximately 40 to 50 percent of the dry

weight of most woods and straws (7, 19, 44). It is a polymer of several hundred to several thousand anhydroglucopyranose units linked by beta-1,4 glycosidic bonds (13, p. 11, 14). Cellulose is often called alphacellulose or "true" cellulose because it contains only glucose residues and is like the "ideal" alphacellulose, cotton (29, 36, 47). This definition is not entirely accurate for the vast majority of plants. In order to obtain alphacellulose, other carbohydrates and lignin must be extrated. It is difficult, however, to remove residual amounts of hemicellulose sugars and glucuronic acid from such extracts (49).

Several properties of alphacellulose distinguish it from other substances found in plants. It is insoluble in water or aqueous alkaline solutions, and resists dilute acid hydrolysis more than the other carbohydrates (8, 47). It differs from lignin in that it is soluble in 72 percent sulfuric acid (8, 47).

Alphacellulose is much more resistant to dilute sulfuric acid hydrolysis than would be expected of a beta-1,4 glycosidic bond. The increased resistance is believed to be related to the physical structure of the fibers in the cellulose (8, 13, p. 97, 19). Cellulose consists of densely packed fibers which, in turn, contain bundles of extremely long molecules. These molecules are in an orderly repeating arrangement that parallels the axis of the fiber. This nearly crystalline structure has been confirmed by x-ray diffraction studies (8, 19, 49).

The more highly ordered the crystalline region, the greater is the resistance to all types of degradation; such resistant areas are called nonaccessible regions. The crystalline structure also accounts for the solubility properties of alphacellulose (13, p. 58, 19). Other amorphous areas of cellulose, designated accessible regions, are much more easily hydrolyzed by dilute acids. The terms accessible and nonaccessible are relative; no sharp demarcation exists between amorphous and crystalline regions (8, 19, 49).

Hemicelluloses

The hemicelluloses constitute the other major carbohydrate component of plants. These polysaccharides of pentose and hexose sugars are intimately associated with the alphacellulose and lignin in the cell-walls. They constitute about 25 to 30 percent of the dry weight of annual rye grass straw and other grasses (3, 20, 28). Some may serve a structural function while others are food reserves (3, 30, 38, 47).

Hemicelluloses are by definition cell-wall polysaccharides that are easily extracted from plant material by either hot or cold dilute alkali and are released in the early states of hydrolysis by hot, dilute mineral acids. They are hydrolyzed by acids to the component sugars and sugar acids: D-xylose, L-arabinose, D-glucose, D-mannose, D-galactose, and D-glucuronic acid (8, 30, 38, 47).

The term hemicellulose and its definition are in error for two reasons: 1) The implied relationship between the hemicellulose polysaccharides and alphacellulose does not exist (30, 47). 2) Not all hemicelluloses are easily extracted. After extensive alkali extraction and acid degradation of alphacellulose, some nonglucose sugars (e.g., xylose, mannose) and sugar acids (e.g., glucuronic) remain in the residue (8, 13, p. 134). This evicence is cited by several authors as proof of the close physical association of hemicellulose with alphacellulose (30, 47). Whatever the failings, the term hemicellulose has become the accepted name for this definite group of polysaccharides.

The hemicelluloses of most land plants can be divided into three general groups: xylans, glucomannans, and arabinogalactans (8, 38, 45 p. 117). Xylan polysaccharides are considered to be the major hemicelluloses in angiosperms (i.e., grasses and hardwoods) (38). Xylans are linear or slightly branched polymers of beta-1,4 linked anhydroxylanopyranose units with sugars and sugars acids substituted at the two and three position of xylose (38, 45, p. 117). One of the most common xylan polymers is glucuronoxylan (38, 45, p. 117). Another xylan, arabinoxylan, is found primarily in grains and grasses (38). An arabinoglucuronoxylan is present in rye, wheat, flax, barley, and oat straws (4).

Glucomannan polymers are the major hemicelluloses of gymnosperms (i. e., conifers), and are found to a lesser extent in angiosperms (8, 45, p. 117). They exist as linear chains of beta-1,4 linked pyranose forms of mannose and glucose copolymers (45, p. 123).

The arabinogalactans have been reported in many species of plants including the grasses (38). They are highly branched structures, formed by galactopyranose units linked by beta-1,3 or beta-1,6 glycosidic bonds. Arabinose units are connected to the chain through the sixth position on a galactose unit (38, 45, p. 125).

Binger et al. (3) isolated hemicellulose from orchard grass, Dactylis glomerata, and demonstrated that xylose and glucose accounted for over 60 percent of the total sugar. Sullivan et al. (35, 41) extracted five species of grasses and found that xylose accounted for 56 to 73 percent of the total hemicellulose sugars. Myhre and Smith (27) isolated the hemicellulose of alfalfa, Medicago sativa, and found xylose and arabinose comprised 67 and 12 percent of the total sugar, respectively. Work done in this laboratory by Lekprayoon (20) on the hemicellulose component of annual rye grass straw indicated that xylose was the major sugar and accounted for approximately 70 percent of the total sugars.

Acid Hydrolysis of Carbohydrates

Alphacellulose

The kinetics of wood hydrolysis have been investigated extensively (13, p. 391-400, 14, 15, 36). Saeman (36) studied the effect of high temperature, dilute sulfuric acid, catalyzed hydrolysis of wood alphacellulose to determine rates of hydrolysis and the subsequent rates of sugar destruction. Previous to Saeman's work (36), hydrolysis research had been confined to lower temperatures with concentrated acids (13, p. 400, 36).

The important variables in the dilute sulfuric acid hydrolysis of wood material include the particle size, liquid to solid ratio, time period, acid concentration, and temperature (36). The optimum conditions for hydrolysis vary somewhat depending on the plant material; however, the general conditions necessary for maximum yields can be determined.

The hydrolysis rates of wood chips were found to be essentially equal for particle sizes of 20-mesh or less. Wood chips with larger particle sizes had slower rates of hydrolysis (36).

The liquid to solid ratios previously used to hydrolyze wood have been 2:1 (13, p. 400) and 3:1 (15) and 10:1 (12). A study at ratios of 5:1, 10:1, and 20:1 revealed that higher liquid to solid ratios resulted in faster rates of hydrolysis (36).

The effect of increasing the hydrolysis temperature, or acid concentration, or both on the hydrolysis of wood alphacellulose was reported by Saeman (36). He found that between 170 to 190° C and 0.4 to 1.6 percent sulfuric acid that sugar yields increased as the severity of the hydrolysis increased. Thus, as the hydrolysis becomes more extreme the efficiency of hydrolysis increases. He demonstrated that the rate of hydrolysis of alphacellulose was greater than the rate of destruction of glucose between 170 and 190° C (36). This agrees with the Harris et al. (14) review of dilute sulfuric acid hydrolysis of wood alphacellulose, wherein they concluded that hydrolysis with dilute acids occurs at a relatively slow rate at temperatures under 175° C.

There are practical limits to increasing the temperatures of hydrolysis. Meller (26) sets the limit at 200° C because of the cost of equipment, while Harris et al. (14) sets the limit at 185 to 190° C because the rapid rate of hydrolysis causes problems in extracting the hydrolyzate.

Hemicellulose

Hemicellulose sugars are released before the reaction mixture reaches 170° C. Time periods greater than those needed to reach 170° C, 1.5 minutes, cause degradation of the hemicellulose sugars (36). This is in agreement with the definition of hemicellulose as

being easily hydrolyzed (26, 30, 38), and also with the report that the hydrolysis of hemicellulose is an order of magnitude greater than alphacellulose (45, p. 170).

Sugar Degradation

Saeman's work on the decomposition of monomeric sugars confirmed that it was a first order reaction and that some sugars were cleaved to degradation products sooner than others (36). He subjected the five common wood sugars to temperatures of 180° C in 0.8 percent sulfuric acid for periods up to 40 minutes in a sealed pressure bomb. The half lives of the sugars were: D-glucose-28.6 minutes; D-galactose-26.4 minutes; D-mannose-19.4 minutes; L-arabinose-16.4 minutes; and D-xylose-9.6 minutes. From these results it is apparent that the hexose sugars are more resistant to degradation than pentose.

Monomeric sugars upon prolonged exposure to heat and acid undergo degradation and condensation to form insoluble resins. Hexose sugars characteristically decompose to form hydroxymethylfurfural, levulinic acid, and formic acid. Through loss of carbon dioxide, hexoses may be degraded to pentose sugars which, in turn, are degraded to furfural. The furfurals can then polymerize to form insoluble resins known as humic substances (13, p. 94, 26, 45, p. 229). Monomeric sugars may undergo reversion to oligosaccharides (45, p. 186).

The degradation of monomeric sugars and condensation of their decomposition products is detrimental from two points of view. First, there is a loss of a potential energy source for the yeast and secondly, many of the decomposition products are inhibitory to yeast growth (16, 21, 26). To avoid degradation of sugars, especially from the more easily hydrolyzed hemicelluloses (36, 45, p. 207), it is advisable to use a prehydrolysis of wood at 140 to 150° C (45, p. 207) prior to alphacellulose hydrolysis.

Wood Hydrolysis

Numerous methods have been used in the past to hydrolyze the carbohydrate fraction of plants. Wood hydrolysis has been more common than straw hydrolysis. The more popular methods have utilized concentrated hydrochloric, concentrated sulfuric, and dilute sulfuric acids (45, p. 182-213). The latter method has predominated, especially in the United States, because of its lower capital costs (26). The Scholler Process (12) and the Madison Wood Sugar Process (15), both of which use dilute sulfuric acid, are possible means for hydrolyzing annual rye grass straw.

The Scholler Process as described by Wenzl (45, p. 203) and Faith (12) was based on three principles. 1) The hydrolysis of cellulose to glucose was independent of the degradation of glucose. 2) It was necessary to control the reaction variables in the hydrolysis

process to increase the efficiency. 3) To obtain optimum sugar yields, free sugars in the hydrolyzate must be removed quickly to prevent their degradation.

The Scholler Process requires the maintenance of a 0.4 percent sulfuric acid concentration at 170° C (115 p. s. i.) with a 10:1 liquid-solid ratio. The wood was prehydrolyzed at 140° C with 1.0 to 2.5 percent sulfuric acid in vertical percolation towers. The hydrolyzate was removed and neutralized to prevent the degradation of hemicelluloses. The wood was brought up to the desired temperature and acid injected at the top of the tower until the desired concentration was attained. The acid was allowed to percolate down through the wood. The wood was then treated by "intermittent percolation," in which the tower was drained of hydrolyzate and the pressure was maintained. The absorbed acid was allowed to hydrolyze the wood. A new volume of acid was then added to the tower and as it passed through the wood it removed the soluble sugars. This step was repeated 12 to 15 times. The hydrolyzate removed after each of these treatments contains approximately four percent sugar. Towards the last stages the sugar content dropped and essentially all that remains in the towers was lignin.

After removal, the hydrolyzate was cooled down and neutralized with calcium carbonate. It was filtered and fortified with inorganic nutrients essential for yeast growth.

The shortage of ethanol during World War Two prompted the investigation of the Scholler Process (12) by the Forest Products Laboratory at Madison, Wisconsin. Modifications and improvements of the process led to the development of the Madison Wood Sugar Process (15, 26, 40, 45, p. 205).

The Madison Process varied in several important aspects from the Scholler Process. Unlike the Scholler Process which used one batch of acid at a time, the Madison Process added acid and withdrew the resulting hydrolyzate on a continuous basis. As in the Scholler Process, the main hydrolysis was preceeded by a prehydrolysis at 150° C with 0.6 percent acid. The hydrolyzate withdrawn had between 5 and 15 percent sugar. The tower was then injected with an equal volume of acid at 150° C to make a final liquid-solid ratio of 3:1. The temperature was increased to 185° C (163 p. s. i.) at a rate of 0.5° C per minute. The sugar content was approximately five percent. Once it dropped below one percent, the hydrolysis was stopped. The hydrolyzate was neutralized and filtered in a method similar to the Scholler Process. The process also utilized heat exchangers to heat the dilute acid going into the tower with the hot hydrolyzate coming from the tower.

Harris and Belinger (15) compared the Scholler Process and the Madison Wood Sugar Process on the basis of efficiency and total yield. Even though the residual lignin contained about ten percent

carbohydrates (45, p. 207), the total yield of sugars were higher in the Madison Process. This was explained partly by the short residence time in the tower and quick cooling and neutralization, but also was due to the faster rate of hydrolysis at higher temperatures (i. e., 170 versus 185° C) as was previously suggested by Saeman (36).

The Madison Process was more efficient than the Scholler Process because it required less energy to run the operation. It took only 2.8 hours versus 13 to 20 hours for the Scholler Process and utilized heat exchangers for increased savings. Another advantage of the Madison Process was that the hydrolyzate was a better medium for yeast fermentation because fewer inhibitory compounds were present.

Inhibitors in Hydrolysis

The yeast fermentation of wood hydrolyzates is inhibited to some extent by the presence of various toxic factors (16, 21). They include the decomposition products of carbohydrates and lignin, wood extractives and the corrosion of equipment with the subsequent release of metal ions.

Among the carbohydrate decomposition products known to be inhibitory to yeast are the furfurals, formaldehyde, and formic acid (13, p. 429, 16, 45, p. 215). Other inhibitory organic substances are the extractives of wood, terpenes, and lignin decomposition products

such as polyhydroxyaromatics (21). The inhibitory properties of the various compounds are influenced by concentration, pH, temperature, and the age and volume of the inoculum (21).

Several techniques will enhance the growth of yeast on hydrolyzates. The removal of furfurals by steam distillation (37, 45, p. 234) improves the fermentation of hydrolyzates (16). Large inoculums of yeast (i. e., 16 to 18 percent) will reduce inhibitors, furfural, to nontoxic compounds (16, 21, 37). Additional methods used to improve hydrolyzates are acclimation, dilution of hydrolyzates, and adsorption of toxic factors on activated charcoal. Acclimation requires successive transfers of yeast in batches of hydrolyzate (30, 37). This method is not an improvement of the hydrolyzate, but rather, an improvement of the yeast's ability to grow in the hydrolyzate by selecting for resistant yeast. Dilution of the hydrolyzate increases the utilization of sugars and thereby, increases the yield (16, 20). The dilution of toxic substances may be responsible for the improved fermentation (16).

Yeast Production

The interest in propagating food and fodder yeast from cellulosic wastes began during World War One in Germany and the United States. Generally speaking, it has been limited to wartime because it is uneconomical in a normal economy (2, 5, 22, 33). Renewed interest in

the potential of yeast as a food source has developed because of the need for inexpensive protein in developing nations, especially those with large populations subsisting on carbohydrate diets (33, 39). In advanced nations it has been considered as a way of utilizing cellulosic wastes (20, 33, 34).

One of the most widely utilized species of food and fodder yeast has been Candida utilis primarily because of its ability to use a large variety of carbon and nitrogen sources (33). C. utilis was first isolated in the 1920's as a contaminate in several plants using wood hydrolyzates to propagate fodder yeast. It grew extremely well and was mistakenly identified as a member of the genus Torulopsis. Since that time, it has gone under the names of Torulopsis utilis, Torula utilis, and more commonly in industrial jargon as Torula (2, 33).

Authorities in yeast taxonomy (23, 43) now identify this organism as Candida utilis. It was placed in the genus Candida because it is anascosporogenous (i. e., non-ascospore forming) and produces pseudomycelium under anaerobic conditions. All other names should be considered incorrect; nevertheless, the name Torula has persisted, especially in the industrial literature (22, 32).

C. utilis has the ability to use a variety of carbon and nitrogen sources (22). It can utilize glucose, mannose, and xylose, the major sugars found in annual rye grass hydrolyzates (15). These sugars constitute 80 to 90 percent of the sugars released by dilute sulfuric

acid hydrolysis of rye grass straw at 121° C (20). C. utilis cannot use either galactose or arabinose (10, 15, 43). It can metabolize the disaccharides cellobiose, maltose, and sucrose (10, 15). Polysaccharides such as starch, hemicellulose, and alphacellulose must be hydrolyzed before they are utilized (10). Other usable carbon sources include acetaldehyde, glycerol, ethanol, lactic, pyruvic, and acetic acids (10).

C. utilis can use a variety of nitrogen sources. Among them are peptone, urea, asparagine, ammonium nitrate, potassium nitrate, and ammonia (10, 22).

Production of food yeast and the efficiency of growth can be increased by several methods. Vigorous aeration of the growth medium increases yeast cell yields and decreases alcohol production (31). This is desirable when one is growing food yeast because the carbohydrate source is incorporated into yeast protein rather than a by-product such as alcohol. The addition of a nitrogen source (e.g., ammonia, ammonium sulfate, and urea) to the growth medium has also greatly increased yields. The following minerals are also added in proportion to their content in yeast cells: phosphorous (e.g., phosphoric acid), potassium (e.g., potassium chloride), and magnesium (e.g., magnesium sulfate) (10, 18, 20).

C. utilis is one of the few common yeasts that can utilize pentose sugars (2, 10). For this reason it has been used in the fermentation

of deciduous wood hydrolyzates and sulfite waste liquor, which are high in pentose sugars (10, 45, p. 225). It has also been grown on other high pentose sources such as annual rye grass straw (20), and the pre-hydrolysis hydrolyzate of woods (2, 10, 45, p. 225).

The second major reason for the acceptability of C. utilis is its high protein content. C. utilis is approximately 49 to 53 percent crude protein on a dry weight basis when grown aerobically on annual rye grass straw hydrolyzate (20). When grown on sulfite waste liquor or molasses, it is 50 to 51 percent crude protein (33).

Approximately 80 percent of the crude protein nitrogen from yeast is derived from amino acids. Thus, about 40 percent of the total dry weight is true protein (33). Yeast protein is a good source of tryptophan, arginine, lysine, histidine, and threonine, but is a poor source of sulfur containing amino acids such as methionine and cystine (5, 10). This deficiency can be corrected by the addition of methionine and cystine which then makes a protein supplement that compares favorably with casein protein in growth studies with rats (5, 33). Methionine fortified yeast protein is used to improve cereal products, which are low in lysine and tryptophan (10, 22, 33).

Yeast protein has an advantage over soy protein because it is more digestible (10). The digestibility of yeast protein (87 percent) compares favorably to that of hen's egg protein (96 percent) (33).

A potential problem exists if humans consume food yeast as a significant part of their diet because of the relatively high levels of nucleic acids (i. e., purines) in yeasts. Specifically the problem is related to the accumulation and precipitation of uric acid in the joints and urinary tract (11). Maul et al. (25) reported that the nucleic acid content of Candida utilis can be reduced from 7.0 percent to 1.0 to 1.5 percent of the dry weight by a three-step heat-shocking procedure that does not affect the protein content.

Yeast are a rich source of the B-complex vitamins (e. g., riboflavin, thiamine, niacin, pyridoxine, folic acid, p-aminobenzoic acid, inositol, choline and biotin) with the exception of cobalmin (5, 33). C. utilis is the only common yeast that synthesizes biotin (9). Food yeasts are also good sources of the minerals: magnesium, calcium, and phosphorous (10).

A review of the current literature seems to project a good future for food and fodder yeast production. The latest available figures (i. e., 1966) indicate a world wide production of 180,000 metric tons of dried food and fodder yeast produced in some twenty countries. All estimates indicate that production will increase in the future (33).

MATERIALS AND METHODS

Hydrolysis Procedure

The dilute sulfuric acid hydrolysis of annual rye grass straw was investigated at temperatures and pressures of 121° C at 15 p. s. i.; 135° C at 31 p. s. i.; and 150° C at 55 p. s. i. Sulfuric acid concentrations (w/v) were 0.5 percent, 1.0 percent, and 2.0 percent. An additional series of hydrolyses were performed at 121° C with 3.0 percent acid. Hydrolysis time periods investigated ranged from zero time, the length of time required to reach the desired temperature, to 5, 10, 15, 20, 30, and 40 minutes.

The annual rye grass straw (Lolium multiflorum) used in this study has been described in a previous report (20). It was from a single source grown in the Willamette Valley and supplied by the Department of Farm Crops at Oregon State University. The straw was milled to 20-40 mesh.

The hydrolysis of annual rye grass straw was accomplished by heating the straw and acid in a stainless steel pressure bomb (Physics Shop, Department of Physics, Oregon State University). Figures 1 and 2 are photos of the pressure bomb. The bomb, when assembled was 15.5 inches in length. The chamber was 10 inches long and had an inside diameter of 2 inches. It was made of 1/8 inch stainless steel and was equipped with a pressure gauge (C), exhaust valve (D),

Figure 1. The Assembled Pressure Bomb.

A. Chamber
B. Top assembly
C. Pressure gauge
D. Exhaust valve
E. Safety valve set at 90 p. s. i.
F. Bolts and washers
G. 20 gauge thermocouple wire

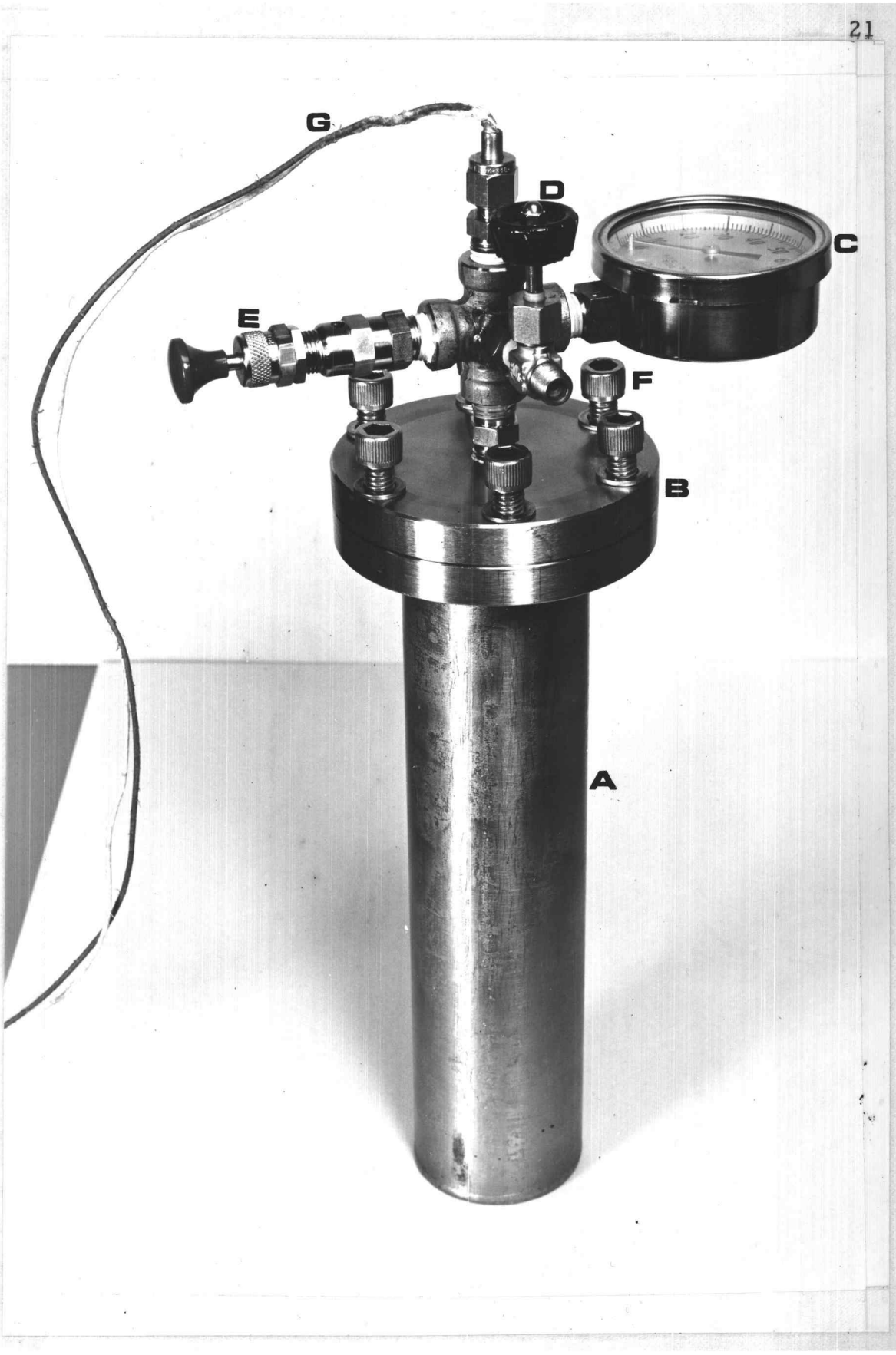
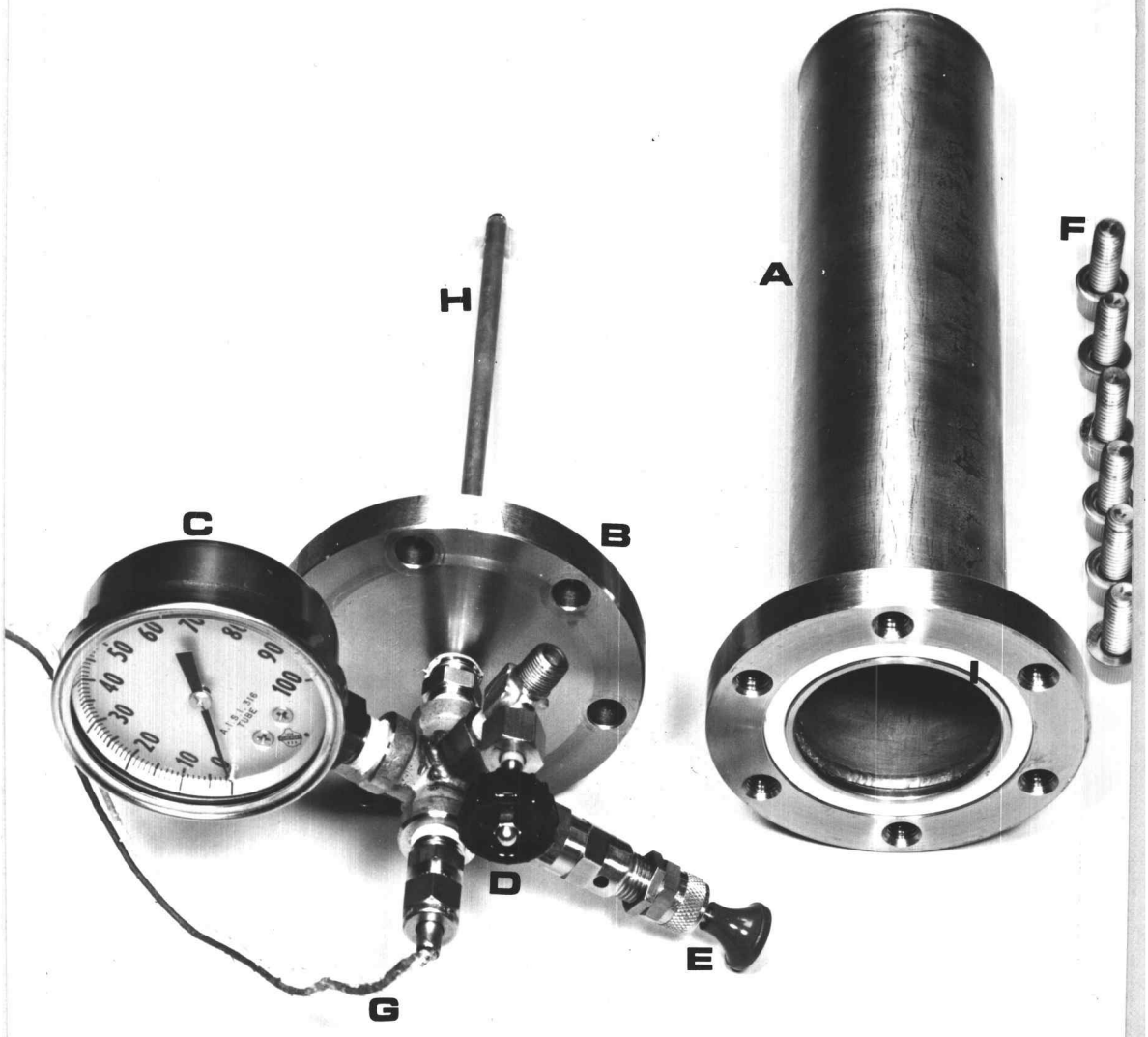

G
D
C
E
F
B
A

Figure 2. The Disassembled Pressure Bomb.

A. Chamber
B. Top assembly
C. Pressure gauge
D. Release valve
E. Safety valve set at 90 p. s. i.
F. Bolts and washers
G. 20 gauge thermocouple wire
H. Thermocouple probe
I. Teflon O-ring gasket


A
B
C
D
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I

safety valve (E), and a thermocouple probe (H). The thermocouple which measured the internal temperature of the bomb was connected to a Bristol Dynamaster Chart Recorder (Chart R-5073, Bristol Co., Waterbury, Conn.) by a 20 gauge thermocouple wire (G). The bomb was sealed by placing the top assembly (B) on the chamber (A) with a teflon O-ring gasket (I) in between, and then securing the bolts (F). Not shown in the figures was a handle that enabled the operator to lift the bomb by hand.

The pressure bomb was heated by immersing in a hot oil bath (Dow Corning 200 fluid, 350 cs., a dimethylpolysiloxane, Dow Corning, Midland, Mich.) which was maintained at 250° C with a hot plate. This bath was used to bring the contents of the bomb up to experimental temperatures and pressure levels as quickly as possible. A second hot oil bath, the hydrolysis bath, was maintained at approximately 12° C above the desired hydrolysis temperature (e.g., the 150° C hydrolysis bath was 150° C plus 12° C). This temperature setting produced the desired hydrolysis temperature within the chamber as indicated by the thermocouple read-out and pressure gauge. The oil baths were stirred constantly by an electrically driven impeller.

When the oil baths were at temperature, the thermocouple was calibrated. The thermocouple probe was immersed seven inches into a boiling water bath to establish the 100° C reading on the chart

recorder. The probe was then dried and plunged into the hydrolysis bath and the position of the chart recorder was noted. Calibration at both of these temperatures was repeated until consistent readings were obtained at each temperature. The equipment was calibrated before and after every experiment to insure accurate readings of the bomb's internal temperature.

A 20 percent (w/v) stock solution of technical grade sulfuric acid (Van Waters and Rogers, 94 percent, specific gravity 1.835) was used to make acid solutions of 0.5, 1.0, and 2.0 percent (w/v). The three percent (w/v) sulfuric acid solution was made from concentrated acid of the same type.

Twenty-five grams of annual rye grass straw were mixed with 100 ml of the desired acid solution. The straw and acid slurry was stirred with a glass rod to obtain thorough wetting of the straw. The slurry was placed in the pressure bomb's chamber. An additional 150 ml of acid was added to the chamber. The total of 250 ml of acid and 25 grams of straw gave a liquid-solid ratio of 10:1 (w/v).

The pressure bomb was sealed and then placed in the 250° C oil bath. When the bomb reached 20 p. s. i., it was exhausted for 30 seconds by opening the exhaust valve to expel trapped air and insure accurate pressure readings. In the 121° C hydrolysis the bomb was exhausted at 15 p. s. i.

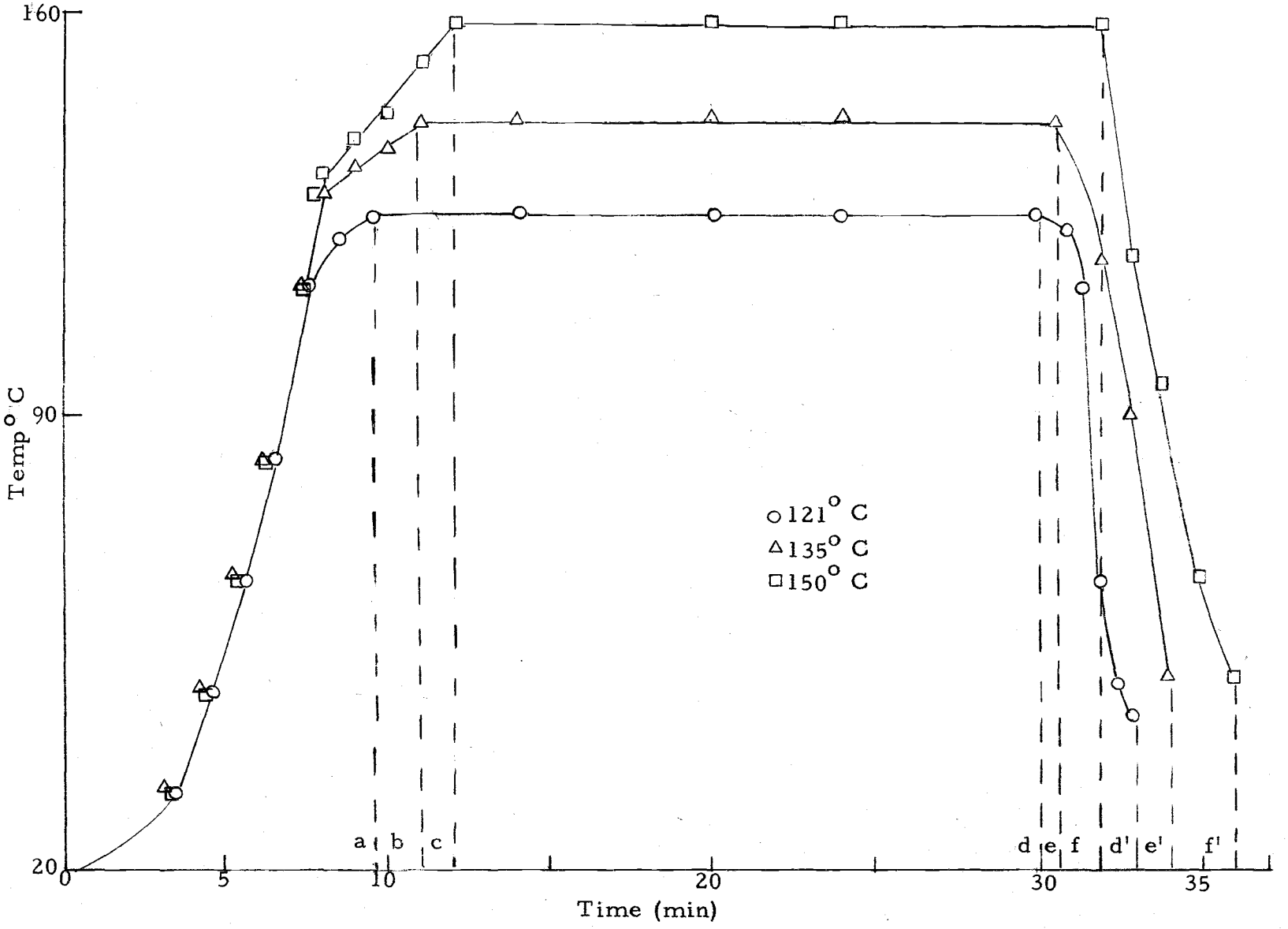
Figure 3 illustrates representative heating and cooling curves of 20 minute hydrolyses at 121, 135, and 150° C. The come-up-time, the time required to bring the bomb's contents from room to experimental temperature, was 9.5 minutes for 121 °C(a), 11.0 minutes for 135°C (b), and 12.0 minutes for 150°C (c). The come-up-times for all experiments at a given temperature were identical.

Once the desired temperature and pressure were reached, the bomb was removed from the 250° C oil bath to the hydrolysis bath. The internal temperature of the bomb was monitored throughout the hydrolysis by thermocouple read-out and the pressure was checked at regular intervals.

At the completion of the hydrolysis, the pressure bomb was drained for 30 seconds to remove the oil. It was then placed in a five gallon ice-water bath and whirled by hand. The rapid cooling slowed down further hydrolysis and sugar degradation. Figure 3 illustrates the cooling curve for each experimental temperature. The come-down-time, the time required to bring the bomb's contents from experimental to between 35 to 50° C was 3.0 minutes for 121° C (d→d'), 3.5 minutes for 135 C (e→e'), and 4.0 minutes for 150 C (f→f'). After cooling, the contents of the bomb were immediately removed and filtered using a Büchner funnel, suction flask, and no. 202 filter pad.

It was observed that between 50 to 70 ml of the original acid solution was absorbed by the straw residue. This volume was replaced

Figure 3. Heating and Cooling Curves for 20 min Hydrolyses.


160
90
20
Temp° C
0
5
10
20
30
35
Time (min)
a
b
c
d
e
f
d'
e'
f'
○121° C
△135° C
□150° C

by using 50 ml of tap water to rinse the chamber. The rinse water was then poured over the straw residue to remove any residual sugar in the chamber and to rinse the straw residue. The straw residue was pressed with a 250 ml beaker to expel most of the liquid.

The filtered hydrolyzate was immediately neutralized with calcium oxide (0.5 grams for 0.5 percent acid, 1.0 grams for 1.0 percent acid, and 3.0 grams for 2.0 percent acid). The neutralized hydrolyzate was filtered through a no. 40 Whatman filter pad, and adjusted to a pH of 5.5 to 6.0 with 4 N hydrochloric acid.

The hydrolyzate was measured and brought up to 250 ml by the addition of tap water. Ten ml of the hydrolyzate were refrigerated (5^{o} C) for sugar analysis by the alditol-acetate method to be described later. The remaining hydrolyzate was placed in a Whirl-Pak polyethylene bag (Van Waters and Rogers) and frozen at -20^{o} C.

Two-stage hydrolyses were performed to determine if sugar yields could be increased by hydrolyzing the straw twice. These experiments employed the same procedures.

Sugar Analysis

Gas-Liquid Chromatography

Gas-liquid chromatography was used to identify and quantitate the sugars present in the hydrolyzates. Gas chromatographic analyses

were performed using an F and M gas chromatograph, Model 402 (F and M Scientific Corporation, Avondale, Pa.), equipped with dual column and dual hydrogen flame ionization detector, and a Honeywell Strip Chart Recorder, Electronik 16 (Honeywell, Philadelphia, Pa.). Peak areas were measured by a Hewlett-Packard 3370 A integrator (Hewlett-Packard, Avondale, Pa.).

The temperature of the column was 180° C while the injection port and detection temperatures were 215° C and 210° C, respectively. Gas flow rates through the column were: helium, 70 ml per minute; hydrogen, 40 ml per minute; and air, 230 ml per minutes.

Preparation of Column

The column used in gas chromatographic analyses was developed by Maglothin (24). Two-hundred mg of XF 1150 (Applied Science Laboratories, State College, Pa.) and 100 mg ethylene glycol adipate (Applied Science Laboratories, State College, Pa.) were dissolved in 25 ml of acetone. One-hundred mg ethylene glycol succinate (F and M Scientific Corporation, Avondale, Pa.) were dissolved in 25 ml of chloroform. The above mixtures were poured together, mixed with 10 grams Gas Chrom P (Applied Science Laboratories, State College, Pa.), and stirred occasionally. After 30 minutes the mixture was poured into a fritted funnel and excess liquid was allowed to drain.

The column material was poured into a petri dish and dried at room temperature. Approximately 6 ml of this powder was packed by vibration into a 4 foot by 1/8 inch o.d. stainless steel column. The column was then equilibrated over-night at 180°C by purging with helium.

Alditol-Acetate Derivatives

Hydrolyzate sugars were converted to their alditol-acetate derivatives using a modification of the procedure of Albersheim et al. (1) as described by Lekprayoon (20). Lekprayoon encountered slight variation among replicate hydrolyzate samples using the modified method. However, the procedure was employed because it took approximately 4 hours compared to 8 hours for the method of Albersheim et al. (1). All hydrolyzates were subjected to duplicated sugar analyses for increased accuracy. The results listed in Appendix Tables are averages from the two analyses. Residual sulfate ion in the hydrolyzates, which inhibits the acetylation reaction, was removed by precipitation with 1.5 grams of barium chloride per 10 ml of straw hydrolyzate and stirring on a magnetic stirrer for 5 minutes. The sample was then adjusted to pH 5.0 to 5.5 with a saturated barium hydroxide solution or 1 N phosphoric acid. The insoluble precipitate which formed was removed by centrifuging for 15 minutes at 12,000 x g. After centrifugation, a 2.5 ml sample of clarified supernatant

fluid was transferred to a 250 ml round bottom flask, to which 1.0 ml of myo-inositol (10 mg per ml) was added as an internal standard. Approximately 0.15 grams of sodium borohydride was added to reduce the sugars to their respective alcohols. After 30 minutes of reaction time at room temperature, the excess sodium borohydride was decomposed by the dropwise addition of glacial acetic acid until the evolution of hydrogen gas ceased. The sample was then evaporated to dryness on a rotary evaporator "in vacuo" over a 65° C water bath. Boric acid formed during the decomposition of sodium borohydride complexes with alditols and hinders their acetylation. To prevent this, boric acid was removed by adding 10 ml of methanol and evaporating to dryness. This was done three times. The sample was then dried in an oven at 100 to 110° C for 10 minutes, removed, and allowed to cool. Ten ml of acetic anhydride and 0.5 grams of sodium acetate anhydrous, acting as the catalyst, were added. This mixture was refluxed at 140° C for 20 minutes, removed from the condensor, and evaporated to dryness on the rotary evaporator. Five ml of dichloromethane were added, the mixture stirred, and poured into a centrifuge tube. This was done three times. The sample was centrifuged at 12,000 x g for 30 minutes. Dichloromethane was decanted off into a test tube and evaporated to 1.0 ml by bubbling nitrogen through it. Two microliters of sample were injected into the gas chromatograph for analysis.

Growth Studies

Growth studies were undertaken to determine if any inhibitory substances were formed as a result of the hydrolytic conditions employed. The strain of Candida utilis (NRRL Y-1084) used in this study was obtained from the American Type Culture Collection (ATCC 9256).

The possible inhibitory action of the hydrolyzates was tested using two series of hydrolyzates as growth media. Series A consisted of 10 minutes hydrolyzates from 121° C at 1.0 percent, 2.0 percent, and 3.0 percent acid, and 2.0 percent acid at 135° C and 150° C. Series B consisted of 30 minutes hydrolyzates from 121° C at 1.0 percent, 2.0 percent, and 3.0 percent acid, and 1.0 percent acid at 135° C and 150° C. Thus, there were five hydrolyzates investigated in each series. Each series increased in temperature at one acid concentration and increased in acid concentration at one temperature. This would show if there was a relationship between increasing the severity of the hydrolysis conditions and the formation of substances inhibitory to yeast growth. Only Series A and B were investigated due to the shortage of equipment, time, and loss of some samples.

The frozen hydrolyzates were thawed and 100 ml portions of each sample were removed for growth studies. To insure that inorganics were not limiting, the hydrolyzates were fortified with essential minerals. The hydrolyzates were adjusted so they contained

equal quantities of xylose, glucose, and mannose. The adjustment of the various sugar concentrations was accomplished by using 10 percent solutions of D-mannose, D-glucose, and D-xylose. Corrections for L-arbinose and D-galactose were not made because these sugars are not utilized by Candida utilis (43). These procedures insured that any differences in the growth of the yeast culture would be due to the presence of toxic compounds and would not be attributable to limiting minerals or sugar concentration.

According to Harrison (18) the composition of C. utilis consists of 8.5 percent nitrogen, 1.1 percent phosphorous, and 2.2 percent potassium. The maximum dry weight yield of C. utilis under optimum growth conditions is equivalent to 50 percent of the assimible sugars present (20). The maximum yield of sugars was approximately 21 grams per liter of straw hydrolyzate. Thus, 21 grams of straw sugar would theoretically yield 10.5 grams per liter of yeast cells on a dry weight basis. Based on this cell yield the amount of additives needed was calculated to be 0.89 gram of nitrogen, 0.115 gram of phosphorous, and 0.23 gram of potassium per liter (20). Nitrogen was added as ammonium hydroxide; phosphorous as phosphoric acid; and potassium as potassium chloride. Calculated on the elemental basis the additions were 4.2 ml of 28.5 percent ammonium hydroxide, 0.25 ml of 85 percent phosphoric acid, and 0.44 gram of potassium chloride per

liter of hydrolyzate. To insure that magnesium was not limiting, 0.10 gram of magnesium sulfate was added per liter.

After adjusting to pH 4.0 with concentrated hydrochloric acid, each hydrolyzate was measured in a graduate cylinder and brought up to a standard volume of 120 ml with tap water. The hydrolyzates were clarified and sterilized by Seitz filtration using Hercules filters (Republic Seitz Filter Corporation, Milldale, Conn.) and stored at 5^{o} C in sterile flasks.

The growth studies were performed in matched 300 ml sidearm flasks. Twenty-five ml aliquots were used for the uninoculated controls and 50 ml aliquots for inoculated experimentals. The hydrolyzates were inoculated with actively growing, 18 hour cultures of C. utilis. Series A Hydrolyzates (Figure 11) were inoculated with approximately 1×10^{5} cells and Series B Hydrolyzates (Figure 12) with 1×10^{7} cells. The approximate inoculum was determined by plating the 18 hour cultures.

Control and experimental side-arm flasks were placed on a shaker and incubated at 24^{o}C. The growth was monitored using a Klett-Summerson Photoelectric Colorimeter (Klett Manufacturing Corporation, N. Y., N. Y.) at 600 nm. Series A hydrolyzates were monitored for 78 hours and Series B Hydrolyzates for 98 hours.

RESULTS AND DISCUSSION

The hydrolysis procedures used in this study were only adequate for the hydrolysis of the hemicellulose fraction and possibly some of the amorphous region of the alphacellulose (6, 38, 45, p. 207). Table 1 shows the hemicelluloses comprise approximately 26.8 percent of annual rye grass straw. Previous reports indicated that the hemicelluloses constituted 25 to 30 percent of annual rye grass straw (20, 28). The major sugars recovered were: mannose, glucose, and xylose (Table 1).

Hexose Sugars

Mannose and glucose were the major hexose sugars recovered from the hydrolysis of annual rye grass straw (Table 1). Minor amounts of galactose were also recovered. The results and discussion of hexose sugars will be limited to mannose and glucose because galactose is not assimilated by Candida utilis (43).

Mannose

The high mannose yields ranged from 2.5 to 3.2 grams per liter (Appendix Table 4). The maximum yield represents approximately 3.2 percent of annual rye grass straw (Table 1).

Table 1. Potential Hemicellulose Sugars Present in Annual Rye Grass Straw.

Sugars	Arabinose	Xylose	Total Pentoses	Mannose	Galactose	Glucose	Total Hexoses	Total Sugars
Maximum Yield * grams/ liter or Percent	2.3	16.1	18.4	3.2	0.9	4.3	8.4	26.8

*These values represent the maximum yield of each sugar recovered from annual rye grass hydrolyzates. Since these values represent the grams of sugar recovered per 100 grams of straw they are also the approximate percentage of each sugar in annual rye grass straw.

Table 2. Hot-Water Extraction at 121°C, 15 p. s. i. for 15 min.

Sugars	Arabinose	Xylose	Total Pentoses	Mannose	Galactose	Glucose	Total Hexoses	Total Sugars
grams/liter	0.1	--	0.1	0.8	--	1.6	2.4	2.5

The release of mannose is illustrated in Figures 5 and 6. Mannose was released in maximum yields at the milder hydrolysis conditions as shown in Figure 5 at 121° C and Figure 6 at 0.5 percent acid. It decreased in concentration due to degradation at the more severe hydrolyses as shown in Figure 5 at 135 and 150° C, and in Figure 6 at 1.0 and 2.0 percent acid. The decrease in hexose sugars observed in Figure 4 at 150° C paralleled the destruction of mannose in Figure 5.

The early release of mannose implies that its source(s) was monomeric sugar or a very accessible hemicellulose. The straw was subjected to a hot-water extraction (121° C) to determine if water and heat would remove sugar. Mannose (0.8 gram per liter) was released without acid hydrolysis (Table 2). This may indicate that some mannose was present as monomeric sugar.

Bishop (4) reported that arabinoglucuronoxylan present in five common grasses, including rye grass, was hydrolyzed with water at 120° C. It seems possible that other hemicelluloses could be hydrolyzed under similar conditions. A mannose source (e.g., glucomannan) could undergo a partial hydrolysis to yield glucose and mannose. The presence of a glucomannan was suggested by Figures 5 and 7 at 121° C. Since the yields of glucose and mannose parallel one another at 121° C, this may indicate hydrolysis of a glucomannan.

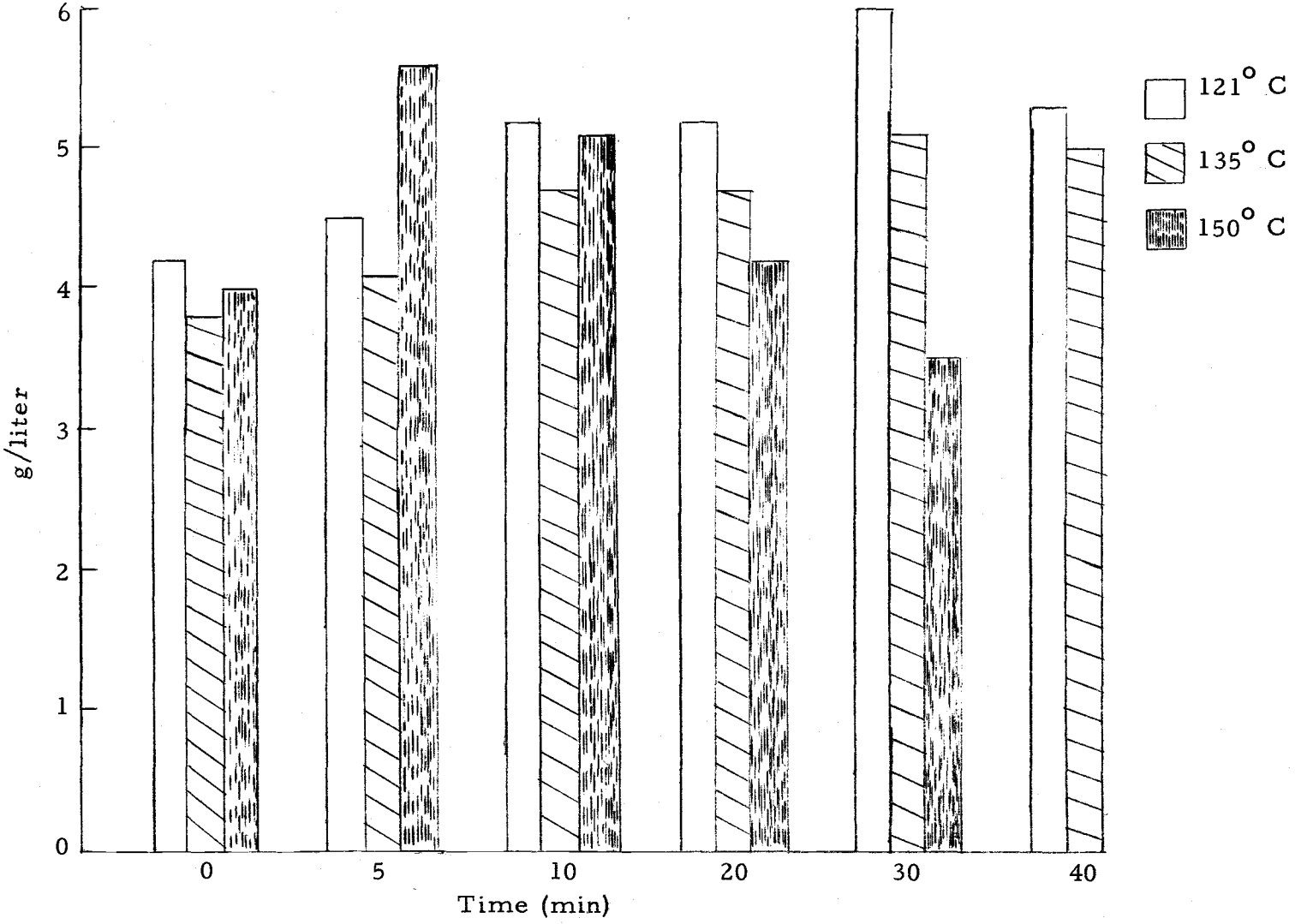


Figure 4. Hexose Yields at 1.0% Sulfuric Acid.

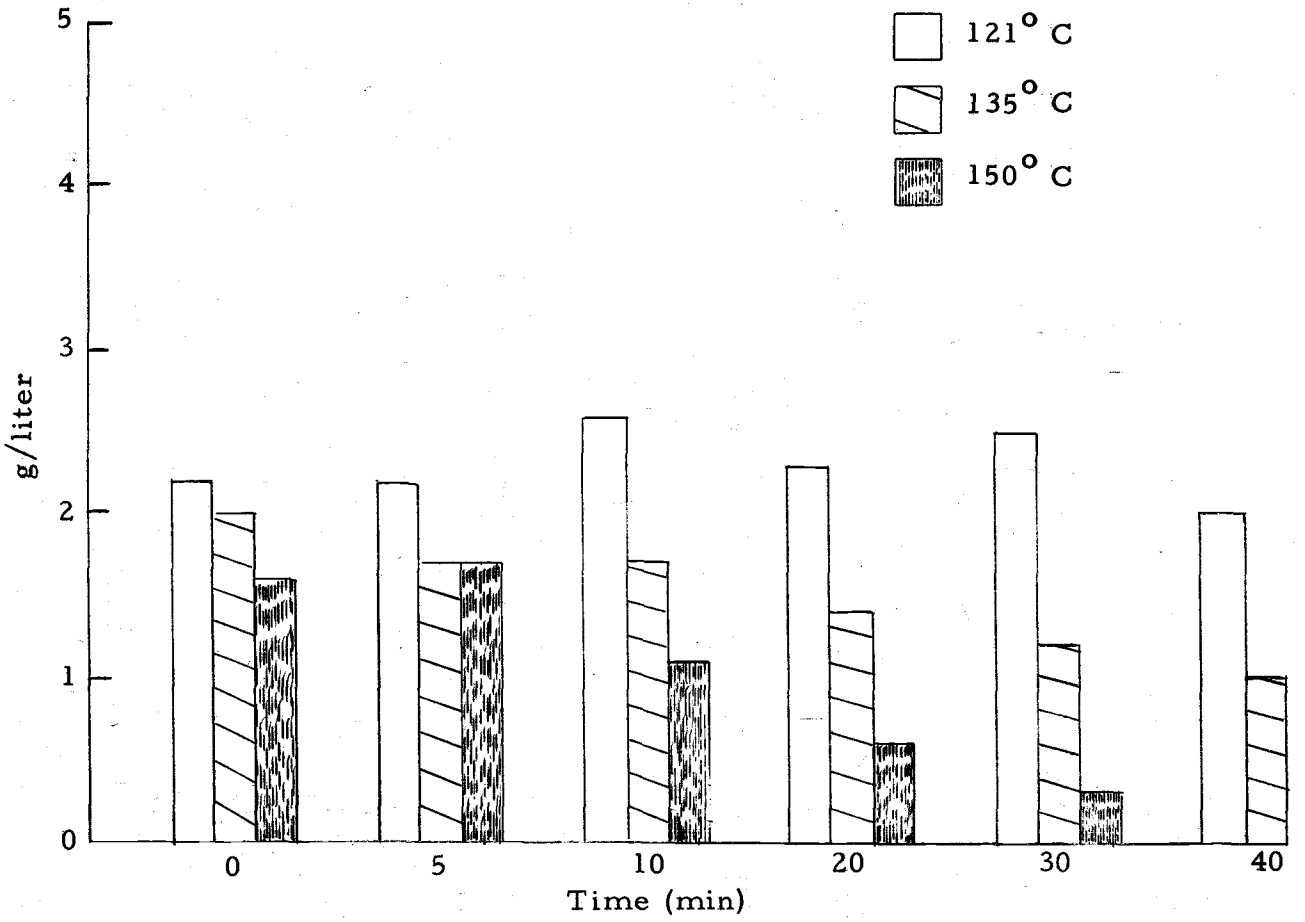


Figure 5. Mannose Yields at 1.0% Sulfuric Acid.

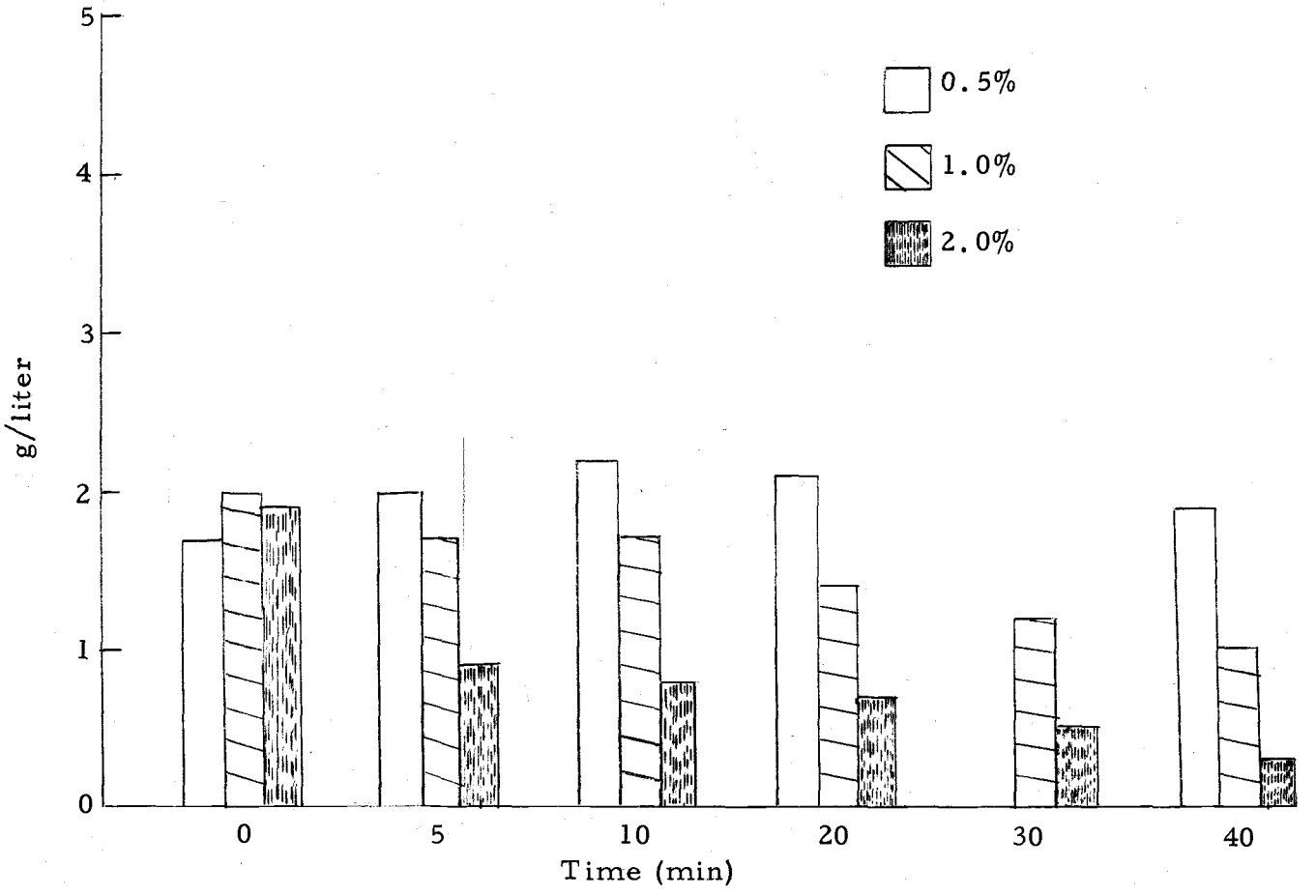


Figure 6. Mannose Yields at 135° C.

Glucose

Glucose was the predominate hexose sugar recovered in the hydrolyzates. The high glucose yields ranged from 3.5 to 4.3 grams per liter (Appendix Table 3). Glucose from the hemicelluloses constitutes approximately 4.3 percent of annual rye grass straw (Table 1).

The release of glucose is illustrated in Figures 7 and 8. Both figures demonstrate that the glucose yield, for the most part, increased as the severity of the hydrolysis increased. This can be observed in Figure 7 at 121 and 135° C. The 150° C hydrolyses reached a maximum yield in 10 minutes, and then decreased at 20 and 30 minutes. Figure 8 illustrates similar conditions to Figure 7 except the acid concentration was 2.0 percent. The trend towards increasing yields was observed at 121 and 150° C with no decrease at 10 minutes. The glucose yields of the 135° C hydrolysis were erratic between 5 and 30 minutes, but increased at 40 minutes.

The release of glucose in good yields at both mild and severe hydrolytic conditions may result from the hydrolysis of more than one source. Table 2 shows that 1.6 grams per liter of glucose was released during the hot-water extraction of straw. This source was easily extracted or hydrolyzed and could be either a monomeric sugar or a glucomannan as discussed earlier. The glucose observed at the more severe conditions could be a glucoglucuronoxylan (3). Figures

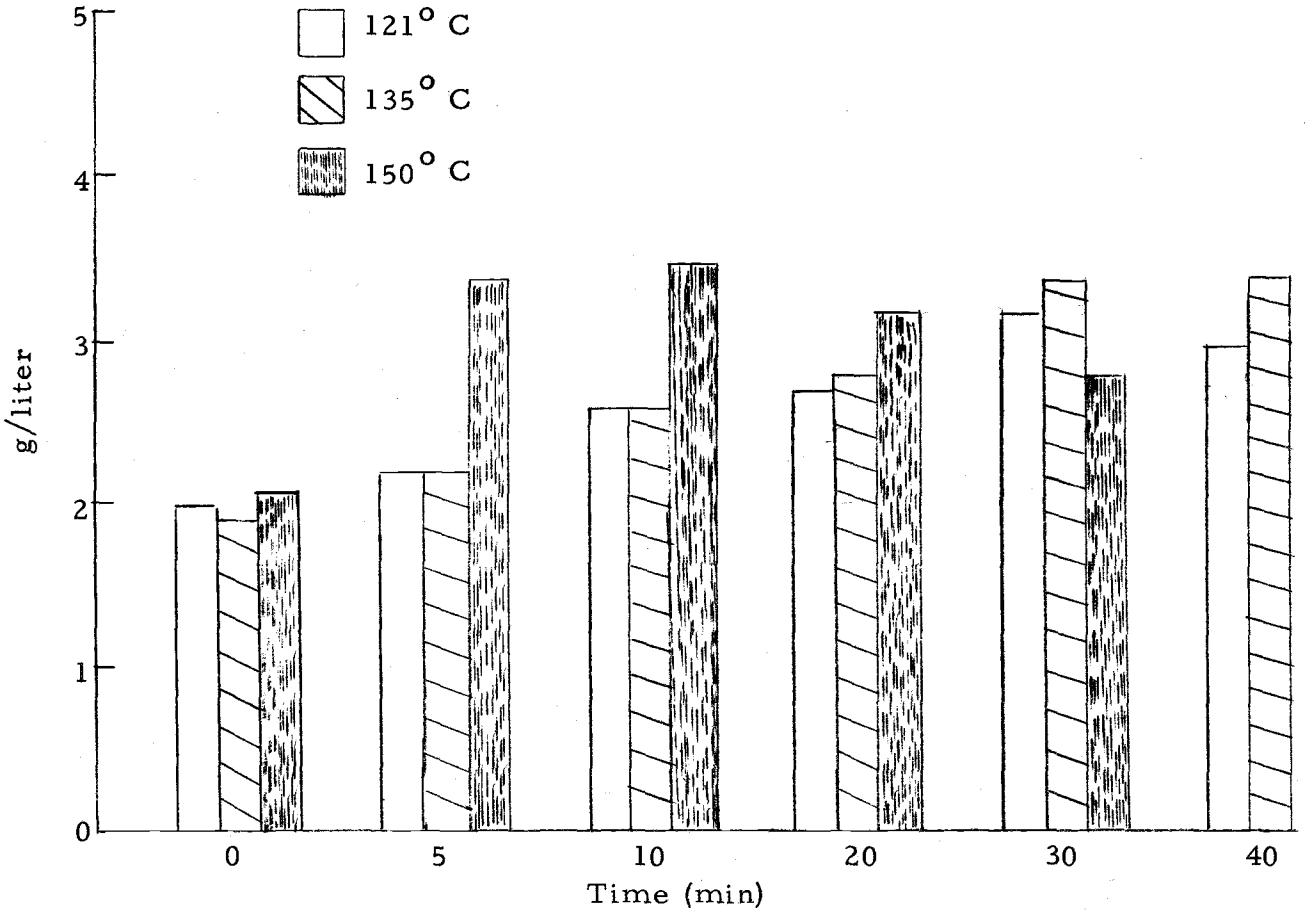


Figure 7. Glucose Yields at 1.0% Sulfuric Acid.

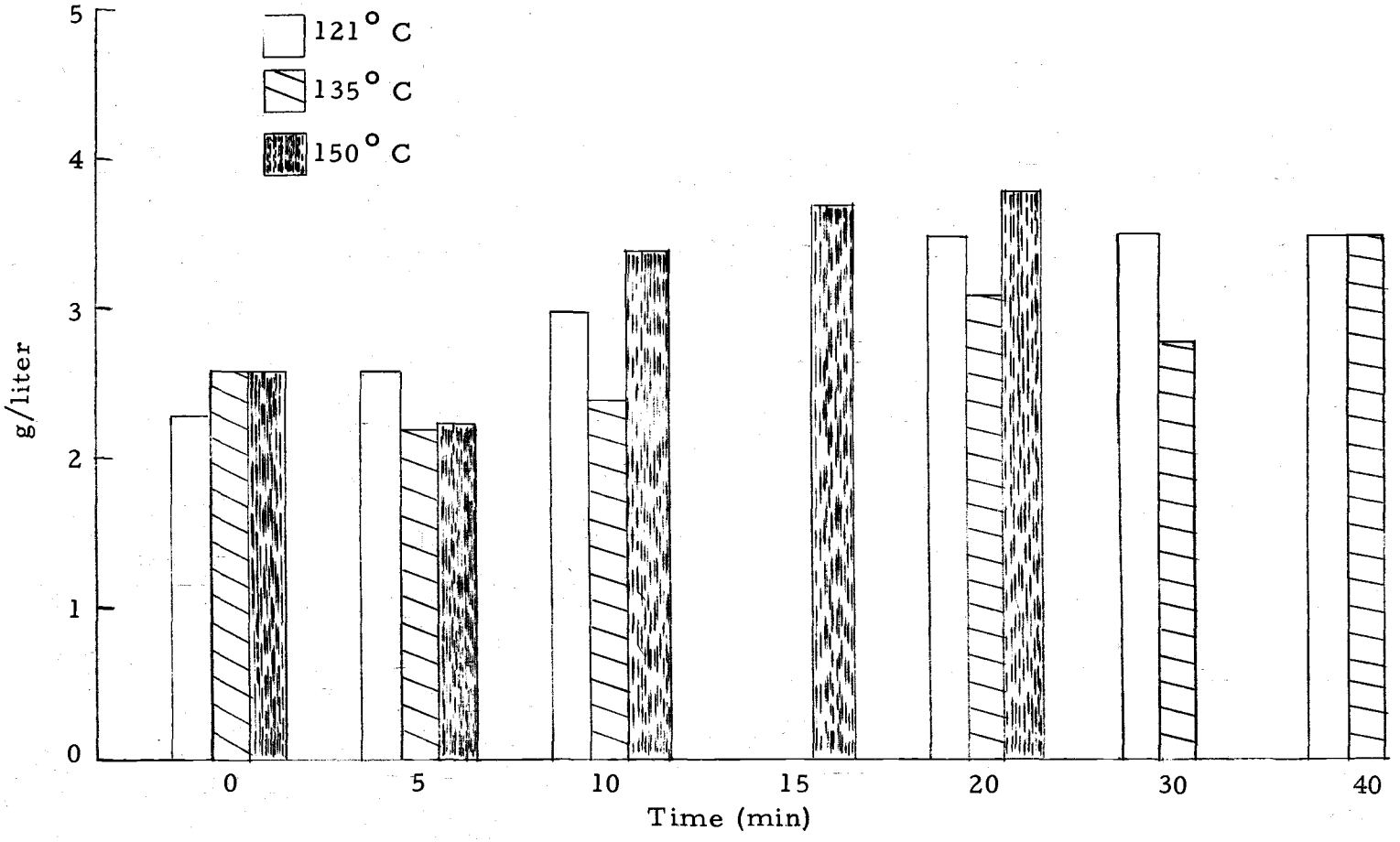


Figure 8. Glucose Yields at 2.0% Sulfuric Acid.

7 and 9 show that both glucose and xylose increase as the conditions become more severe, suggesting they are released from the same hemicellulose. Another potential source could be the amorphous alphacellulose. Those areas of the amorphous alphacellulose that are not protected by crystalline alphacellulose are labile to the hydrolytic conditions used in this study (6, 8, 19, 49). In addition, the increase in glucose at the more severe hydrolyses could be due to a greater increase in the rate of hydrolysis relative to the rate of destruction. This was shown to be true for the hydrolysis of alphacellulose at 170 to 190° C with dilute sulfuric acid (36).

Pentose Sugars

Xylose and arabinose were the predominate pentose sugars recovered from annual rye grass straw (Table 1). The results and discussion of pentoses will be restricted to xylose because arabinose is not assimilated by Candida utilis (43). Furthermore, the release of arabinose parallels xylose (Appendix Tables 1, 5, and 6), so discussion would serve no useful purpose.

Xylose

Xylose is the major hemicellulose sugar recovered from the hydrolysis of annual rye grass straw. This agrees with previous reports for rye and other common grasses (3, 4, 20, 27, 38, 41). It

constitutes 16.1 percent of annual rye grass straw (Table 1). The high xylose yields ranged from 12 to 16 grams per liter (Appendix Table 5).

The release of xylose is illustrated in Figures 9 and 10. Xylose was released in maximum yields during the more severe hydrolysis at 150° C as shown in Figure 9 and at 2.0 percent acid in Figure 10. It began to decrease at 150° C after 10 minutes of hydrolysis (Figures 9 and 10).

Xylose is the most heat and acid labile of the sugars recovered in the hydrolysis of annual rye grass straw (36, 46, 48). Thus, the increase in xylose yields at the more severe hydrolyses was perplexing in view of the degradation of mannose under the same conditions. The reason may be that the xylan was less accessible than the mannan. This agrees with two papers on the hydrolysis of five common grasses (35, 41). They reported that xylose was released more slowly than other sugars with the exception of glucose. Thus, the majority of the xylose was released later in the hydrolysis and, therefore, was exposed to heat and acid for a shorter period of time (Figure 9). Mannose was released early in the hydrolysis and exposed to degrative conditions for longer periods of time (Figure 5). In addition the increase in xylose yields under more severe conditions could be due to a

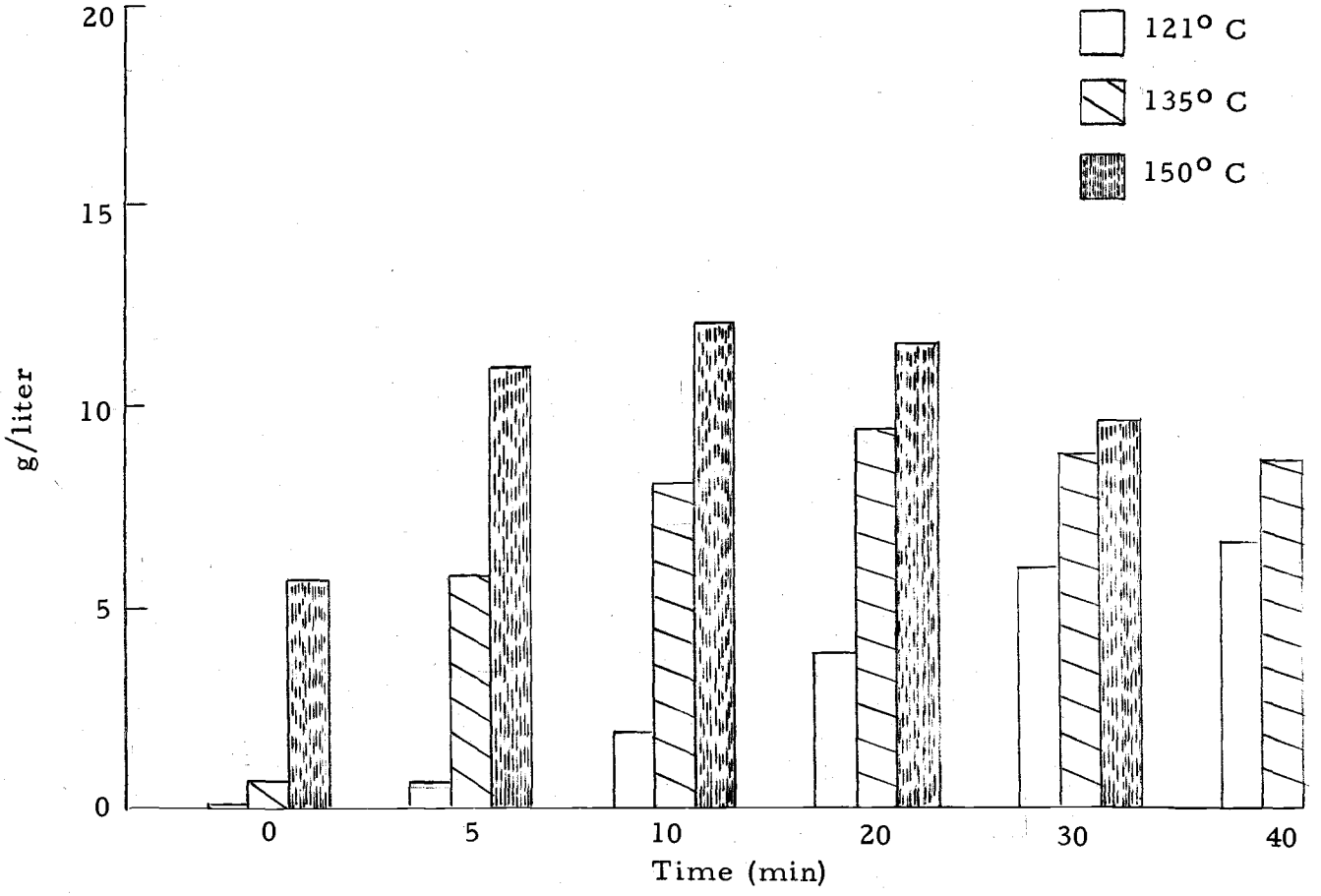


Figure 9. Xylose Yields at 1.0% Sulfuric Acid.

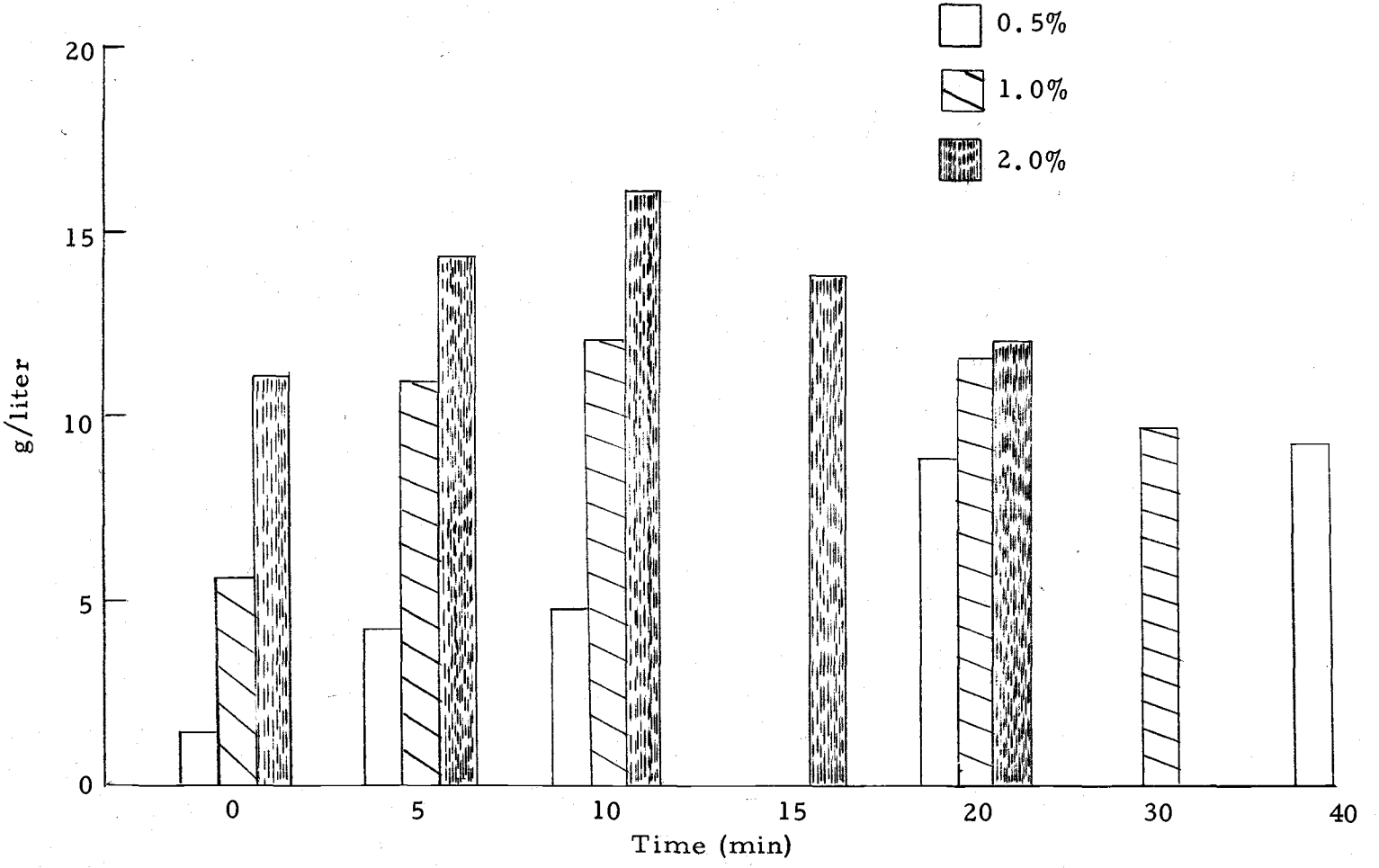


Figure 10. Xylose Yields at 150° C.

to a greater increase in the rate of hydrolysis compared to the rate of destruction. This was shown to be true for glucose (36).

The decrease in xylose at 150° C (Figures 9 and 10) was probably due to the hydrolysis of most of the xylan hemicellulose initially present. Continued exposure to heat and acid only degraded the monomeric xylose. Annual rye grass straw is 25 percent pentose sugar (20), of which 87 percent is xylose (Table 1). The maximum pentose recovered was 18.4 percent of the straw (Table 1 and Appendix Table 1). Thus, approximately 6.6 percent of the pentose was unaccounted for and probably represented sugar destroyed.

Reports indicate that the sources of xylose in grasses are: arabinoxylan, arabinoglucuronoxylan, glucoglcuronoxylan, and glucuronoxylan (3, 4, 38).

Two-Stage Hydrolysis

The decrease in hexose sugars observed at the more severe conditions of hydrolysis is undesirable (Figure 4 and Appendix Table 2). Candida utilis, the yeast propagated on annual rye grass straw hydrolyzate has been shown to preferentially use hexose sugars (20). In addition, hexoses contribute more to the dry weight cell yield than do pentoses (20).

The loss of hexose sugars was due principally to mannose degradation at hydrolysis conditions that produced high xylose and

glucose yields. A two-stage hydrolysis was designed to avoid mannose degradation and also to obtain optimum yields of the other sugars. The first stage was operated at milder hydrolysis conditions, 121° C with 1.0 percent acid for 10 minutes, for the purpose of removing most of the mannose and some of the glucose (Appendix Table 4). A second and more severe hydrolysis, 150° C with 2.0 percent acid for 15 minutes, of the straw residue was used to obtain optimum yields of xylose and glucose (Appendix Tables 3 and 5).

The two-stage hydrolysis results are presented in Table 3. Mannose yield was higher in the two-stage hydrolysis (3.2 grams per liter) than either single-stage (2.6 and 0.1 grams per liter). The same was true for glucose (4.2 grams per liter) versus either single-stage (2.6 and 3.7 grams per liter). Thus, the combined yields of mannose and glucose (7.4 grams per liter) from the two-stage hydrolysis were greater than either single-stage hydrolysis (5.2 and 3.8 grams per liter). The combined yield also compared favorably to the combined maximum yields of mannose and glucose (7.5 grams per liter) as shown in Table 1.

Xylose yields were lower than expected in the two-stage hydrolysis (Table 3). The xylose yield was 8.7 grams per liter compared to the single-stage hydrolysis high of 13.8 grams per liter. This was difficult to explain because results from the single-stage hydrolyses indicated that the yield should have been 12 to 16 grams per liter

Table 3. Sugar Yields of Two-Stage Hydrolysis vs. Single-Stage Hydrolyses.

Single-Stage Hydrolyses	Mannose	Glucose	Totals of Mannose and Glucose	Xylose
121°C, 1.0% acid, 10 min	2.6	2.6	5.2	1.9
150°C, 2.0% acid, 15 min	0.1	3.7	3.8	13.8
Two-Stage Hydrolysis				
121°C, 1.0% acid, 10 min	3.2	2.6	5.8	1.5
150°C, 2.0% acid, 15 min	---	1.6	1.6	7.2
Total of Two-Stage	3.2	4.2	7.4	8.7

(Appendix Table 5). The lower yields could be due to xylose degradation resulting from the two consecutive hydrolyses. The first hydrolysis might have made the xylan hemicellulose more accessible to acid, thus, resulting in rapid xylose release during the second hydrolysis. Xylose would then be degraded more rapidly than at the corresponding single-stage hydrolysis.

The results indicate that the two-stage hydrolysis recovered more hexose sugar than any of the single-stage hydrolyses. Lekprayoon's report (20) suggests that the combined hydrolyzate from the two-stage hydrolysis would result in higher dry weight cell yield because C. utilis can assimilate mannose and glucose more efficiently than xylose.

Growth Studies

Hot dilute sulfuric acid hydrolysis of wood results not only in release of sugars, but also degradation of the released sugars to various toxic compounds that inhibit yeast growth (16, 21, 37). Among these inhibitory compounds are furfurals, formaldehyde, and formic acid (16, 21, 45, p. 215). The hydrolysis of annual rye grass could produce similar inhibitory products. This was investigated by propagating Candida utilis on the annual rye grass hydrolyzates.

Figures 11 and 12 represent the growth curves of C. utilis from Series A Hydrolyzates and Series B Hydrolyzates, respectively. The figures have an ordinate that begins at 10 Klett units. This was done because of fluctuations in readings below this value that were probably caused by variations in the electrical current. The data show that the lag phase of the yeast culture increased as the acid concentration was increased at one temperature of hydrolysis. The same trend was observed with increasing temperature at one acid concentration. Table 4 shows the approximate times that the yeast cultures in Figures 11 and 12 came out of lag phase. It appears that increasing the temperature at a specific acid concentration results in longer lag phase than increasing the acid concentration at a specific temperature.

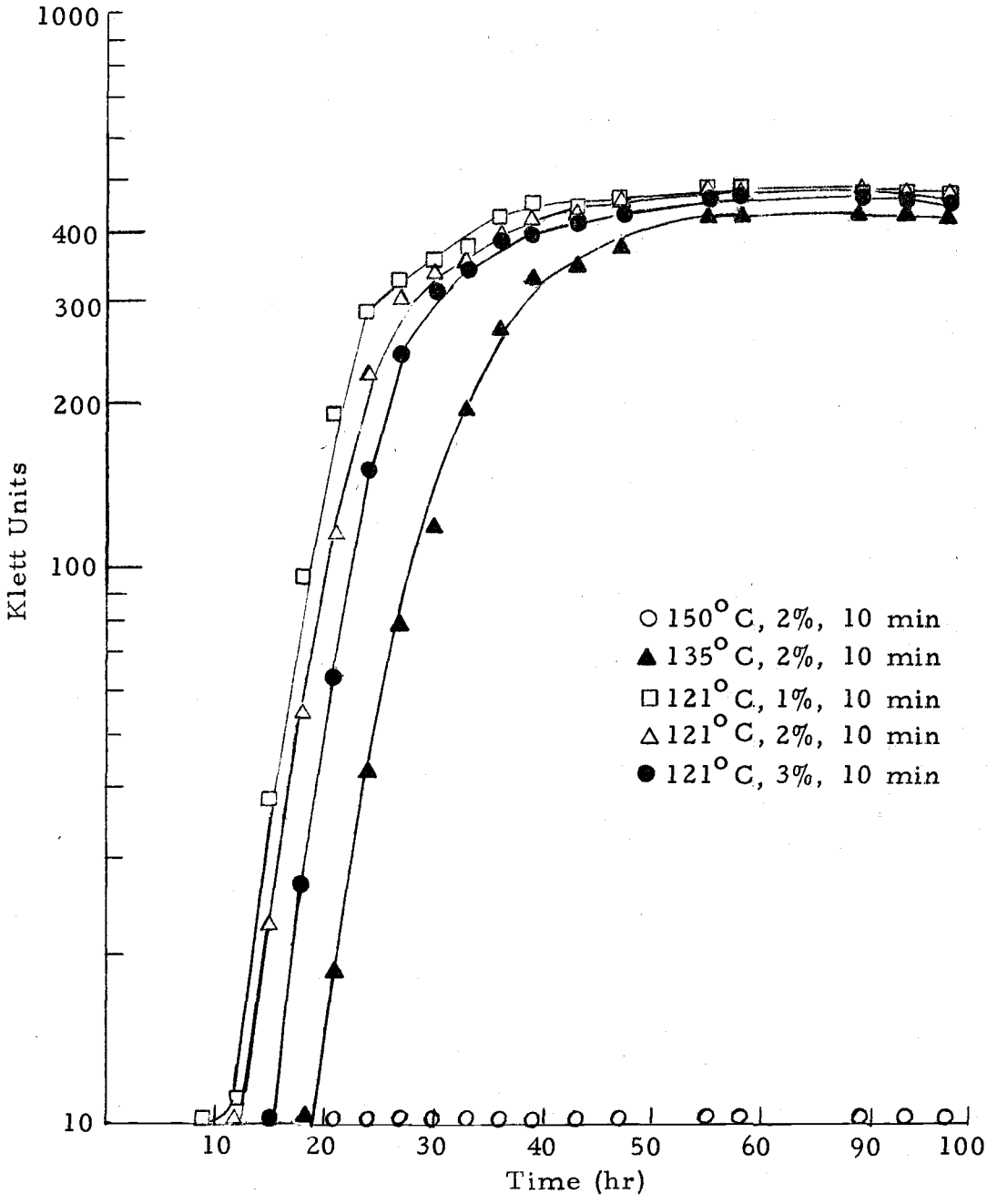


Figure 11. Growth Studies of Series A Hydrolyzates.

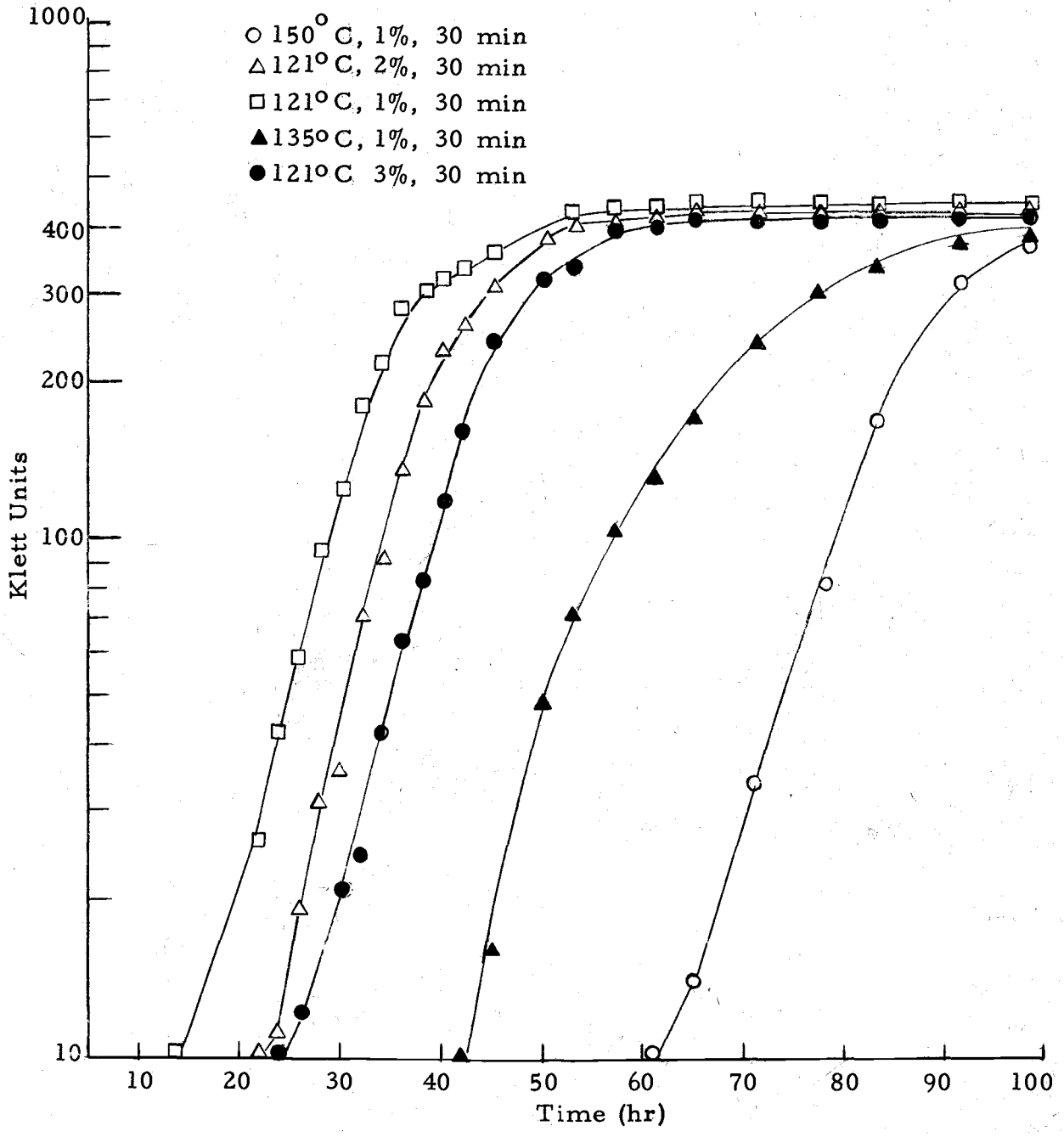


Figure 12. Growth Studies of Series B Hydrolyzates.

Table 4. Lag Phases of Yeast Cultures in the Various Hydrolyzate Media.

Series A Hydrolyzates Figure 11	Lag Phase (hr)	Series B Hydrolyzates Figure 12	Lag Phase (hr)
121° C, 1%, 10 min	9	121° C, 1%, 30 min	14
121° C, 2%, 10 min	12	121° C, 2%, 30 min	22
121° C, 3%, 10 min	15	121° C, 3%, 30 min	24
135° C, 2%, 10 min	18	135° C, 1%, 30 min	42
150° C, 2%, 10 min	N. G.	150° C, 1%, 30 min	61

N. G. = No growth

The trend towards increasing lag phase, observed as the conditions of hydrolysis became more severe, can be explained by increased levels of toxic compounds that were inhibitory to yeast growth. Table 5 shows the mannose present in the hydrolyzates used for the growth studies. A definite trend was observed between the decrease in mannose content and the increase in lag phase as the severity of the hydrolysis conditions increased. This suggests that mannose degradation resulted in the formation of inhibitory compounds. In addition, glucose and xylose were probably undergoing some degradation even though their yields were increasing as the hydrolysis conditions become more severe (Appendix Tables 3 and 5). Xylose is more labile to dilute sulfuric acid than is mannose (36, 48). Glucose is also labile, but not to the extent of xylose and mannose (36, 44). The reason that xylose and glucose concentrations tend to increase has been discussed previously.

Table 5. Decreasing Mannose Yields in Series A and Series B Hydrolyzate Media.

Hydrolyzate Media	Mannose Yields (g/liter)
Series A:	
121° C, 1%, 10 min	2.6
121° C, 2%, 10 min	2.6
121° C, 3%, 10 min	1.7
135° C, 2%, 10 min	0.8
150° C, 2%, 10 min	0.2
Series B:	
121° C, 1%, 30 min	2.5
121° C, 2%, 30 min	1.9
121° C, 3%, 30 min	0.9
135° C, 1%, 30 min	1.2
150° C, 1%, 30 min	0.3

The growth studies indicated that the formation of toxic compounds during the hydrolysis of annual rye grass straw must be considered when the hydrolyzate is used to propagate C. utilis.

SUMMARY

The dilute sulfuric acid hydrolysis of the hemicellulose portion of annual rye grass straw indicated that most of the hemicellulose sugars can be recovered under the conditions employed in this study. There was a significant decline in the mannose concentration and increases in the xylose and glucose concentrations as the hydrolysis conditions became more severe. The two-stage hydrolysis results demonstrated that increased levels of hexose sugar can be obtained by removing the sugars released at the milder hydrolysis, thus preventing their degradation. Growth studies using C. utilis showed that there was a relationship between increased inhibition of yeast growth and the severity of the hydrolysis procedure. This was probably the result of increased degradation of sugars to compounds toxic to C. utilis.

Recommendations from this study would include research into the hydrolysis of the alphacellulose portion of annual rye grass straw. Alphacellulose accounts for 46 percent of the dry weight of annual rye grass straw which was essentially untouched by the procedures used in this study (20, 36). In order to hydrolyze this potential sugar source, it would be necessary to conduct the hydrolysis at 180 to 190°C (14, 36). Further research would seem necessary to determine the identity and concentration of inhibitors in the hydrolyzates of annual rye grass straw.

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APPENDIX

Appendix Table 1. Hydrolysis Yields of Pentoses.

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Pentose Released (g/liter)	Percent Pentose[a]
121	0.5	0	4.3	0.3	7.0
		5	4.1	0.7	17.1
		10	5.0	0.9	18.0
		20	5.8	1.4	24.1
		30	6.8	1.7	25.0
		40	6.5	2.2	33.9
121	1.0	0	5.0	0.8	16.0
		5	6.3	1.9	30.2
		10	8.6	3.4	39.5
		20	10.8	5.5	51.4
		30	13.8	7.8	56.5
		40	13.7	8.4	61.3
121	2.0	0	6.6	1.6	24.2
		5	11.1	5.7	51.3
		10	13.2	7.6	57.6
		20	15.9	9.7	61.0
		30	16.2	10.3	63.6
		40	16.0	10.6	66.2
121	3.0	0	4.8	1.7	35.4
		5	11.6	7.2	62.1
		10	15.4	9.8	63.6
		20	15.2	9.9	65.1
		30	13.9	9.6	69.1
		40	14.7	10.4	70.8
135	0.5	0	3.9	0.5	12.8
		5	5.3	1.2	22.6
		10	6.5	2.0	30.7
		20	7.4	3.3	44.6
		40	8.3	4.1	48.4

Appendix Table 1. (Continued)

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Pentose Released (g/liter)	Percent Pentose [a]
135	1.0	0	5.7	1.9	33.3
		5	11.6	7.5	64.7
		10	14.5	9.8	67.6
		20	16.1	11.4	70.8
		30	15.9	10.8	67.9
		40	15.3	10.3	67.3
135	2.0	0	11.4	6.6	57.9
		5	12.4	8.9	71.8
		10	14.1	10.4	73.8
		20	17.3	12.6	72.8
		30	14.4	10.4	72.2
		40	16.4	12.1	73.8
150	0.5	0	5.3	1.1	20.8
		5	9.1	4.3	47.3
		10	11.0	6.6	60.0
		20	17.3	10.8	62.4
		40	16.8	10.8	64.3
150	1.0	0	11.5	7.5	65.2
		5	18.6	13.0	69.9
		10	19.3	14.2	73.6
		20	17.6	13.4	76.1
		30	14.9	11.4	76.5
150	2.0	0	16.6	12.9	77.7
		5	19.5	16.6	85.1
		10	22.2	18.2	82.0
		15	20.3	16.1	79.3
		20	18.4	14.1	76.6

[a] g/liter pentose/g of total sugar X 100

Appendix Table 2. Hydrolysis Yields of Hexoses.

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Hexose Released (g/liter)	Percent Hexose [a]
121	0.5	0	4.3	4.0	93.0
		5	4.1	3.4	82.9
		10	5.0	4.1	82.0
		20	5.8	4.4	75.9
		30	6.8	5.1	75.0
		40	6.5	4.3	66.2
121	1.0	0	5.0	4.2	84.0
		5	6.3	4.4	69.8
		10	8.6	5.2	60.5
		20	10.8	5.2	48.6
		30	13.8	6.0	43.5
		40	13.7	5.3	38.7
121	2.0	0	6.6	5.0	75.8
		5	11.1	5.4	48.7
		10	13.2	5.6	42.4
		20	15.9	6.2	39.0
		30	16.2	5.9	36.4
		40	16.0	5.4	33.8
121	3.0	0	4.8	3.1	64.6
		5	11.6	4.4	37.9
		10	15.4	5.6	36.4
		20	15.2	5.3	34.9
		30	13.9	4.3	30.9
		40	14.7	4.3	29.3
135	0.5	0	3.9	3.4	87.2
		5	5.3	4.1	77.4
		10	6.5	4.5	69.3
		20	7.4	4.1	55.4
		40	8.3	4.2	50.6

Appendix Table 2. (Continued)

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Hexose Released (g/liter)	Percent Hexose [a]
135	1.0	0	5.7	3.8	66.7
		5	11.6	4.1	35.3
		10	14.5	4.7	32.4
		20	16.1	4.7	29.2
		30	15.9	5.1	32.1
		40	15.3	5.0	32.7
135	2.0	0	11.4	4.8	42.1
		5	12.2	3.5	28.2
		10	14.1	3.7	26.2
		20	17.3	4.7	27.2
		30	14.4	4.0	27.8
		40	16.4	4.3	26.2
150	0.5	0	5.3	4.2	79.3
		5	9.1	4.8	52.8
		10	11.0	4.4	40.0
		20	17.3	6.5	37.6
		40	16.8	6.0	35.7
150	1.0	0	11.5	4.0	34.8
		5	18.6	5.6	30.1
		10	19.3	5.1	26.4
		20	17.6	4.2	23.9
		30	14.9	3.5	23.5
150	2.0	0	16.6	3.7	22.3
		5	19.5	2.9	14.9
		10	22.2	4.0	18.0
		15	20.3	4.2	20.7
		20	18.4	4.3	23.4

[a] g/liter hexose/g of total sugar X 100

Appendix Table 3. Hydrolysis Yields of Glucose.

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Glucose Released (g/liter)	Percent[a] Glucose
121	0.5	0	4.3	2.0	46.5
		5	4.1	1.8	43.9
		10	5.0	2.0	40.0
		20	5.8	2.1	36.2
		30	6.8	2.5	36.8
		40	6.5	2.3	35.4
121	1.0	0	5.0	2.0	40.0
		5	6.3	2.2	34.9
		10	8.6	2.6	30.2
		20	10.8	2.7	25.2
		30	13.8	3.2	23.2
		40	13.7	3.0	21.9
121	2.0	0	6.6	2.3	34.9
		5	11.1	2.6	23.4
		10	13.2	3.0	22.7
		20	15.9	3.5	22.0
		30	16.2	3.5	21.6
		40	16.0	3.5	21.9
121	3.0	0	4.8	1.6	33.3
		5	11.6	2.5	21.6
		10	15.4	3.6	23.4
		20	15.2	3.6	23.7
		30	13.4	3.0	21.6
		40	14.7	3.2	21.8
135	0.5	0	3.9	1.7	43.6
		5	5.3	2.1	39.6
		10	6.5	2.3	35.4
		20	7.4	2.0	27.0
		40	8.3	2.2	26.5

Appendix Table 3. (Continued)

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Glucose Released (g/liter)	Percent Glucose[a]
135	1.0	0	5.7	1.9	33.3
		5	11.6	2.2	19.0
		10	14.5	2.6	17.9
		20	16.1	2.8	17.4
		30	15.9	3.4	21.4
		40	15.3	3.4	22.2
135	2.0	0	11.4	2.6	22.8
		5	12.4	2.2	17.7
		10	14.1	2.4	17.0
		20	17.3	3.1	17.9
		30	14.4	2.8	19.4
		40	16.4	3.5	21.3
150	0.5	0	5.3	1.9	35.9
		5	9.1	2.6	28.6
		10	11.0	2.4	21.8
		20	17.3	4.3	24.9
		40	16.8	3.9	23.2
150	1.0	0	11.5	2.1	18.3
		5	18.6	3.4	18.3
		10	19.3	3.5	18.1
		20	17.6	3.2	18.2
		30	14.9	2.8	18.8
150	2.0	0	16.6	2.6	15.7
		5	19.5	2.5	12.8
		10	22.2	3.4	15.3
		15	20.3	3.7	18.2
		20	18.4	3.8	20.7

[a] g/liter glucose/g of total sugar X 100

Appendix Table 4. Hydrolysis Yields of Mannose.

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Mannose Released (g/liter)	Percent Mannose [a]
121	0.5	0	4.3	2.0	46.5
		5	4.1	1.6	39.0
		10	5.0	2.1	42.0
		20	5.8	2.3	39.9
		30	6.8	2.6	38.2
		40	6.5	2.0	30.8
121	1.0	0	5.0	2.2	44.0
		5	6.3	2.2	34.9
		10	8.6	2.6	30.2
		20	10.8	2.3	21.5
		30	13.8	2.5	18.1
		40	13.7	2.0	14.6
121	2.0	0	6.6	2.7	40.9
		5	11.1	2.8	25.2
		10	13.2	2.6	19.7
		20	15.9	2.3	14.5
		30	16.2	1.9	11.7
		40	16.0	1.4	8.8
121	3.0	0	4.8	1.5	31.3
		5	11.6	1.6	13.8
		10	15.4	1.7	11.0
		20	15.2	1.2	7.9
		30	13.9	0.9	6.5
		40	14.7	0.7	4.8
135	0.5	0	3.9	1.7	43.6
		5	5.3	2.0	37.7
		10	6.5	2.2	33.9
		20	7.4	2.1	28.4
		40	8.3	1.9	22.9

Appendix Table 4. (Continued)

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Mannose Released (g/liter)	Percent [a] Mannose
135	1.0	0	5.7	2.0	35.1
		5	11.6	1.7	14.7
		10	14.5	1.7	11.7
		20	16.1	1.4	8.7
		30	15.9	1.2	7.6
		40	15.3	1.0	6.5
135	2.0	0	11.4	1.9	16.7
		5	12.4	0.9	7.3
		10	14.1	0.8	5.7
		20	17.3	0.7	4.1
		30	14.4	0.5	3.5
		40	16.4	0.3	1.8
150	0.5	0	5.3	2.3	43.4
		5	9.1	2.2	24.2
		10	11.0	1.8	16.4
		20	17.3	1.7	9.8
		40	16.8	1.6	9.5
150	1.0	0	11.5	11.6	13.9
		5	18.6	1.7	9.1
		10	19.3	1.1	5.7
		20	17.6	0.6	3.4
		30	14.9	0.3	2.0
150	2.0	0	16.6	0.9	5.4
		5	19.5	0.2	1.0
		10	22.2	0.2	0.9
		15	20.3	0.1	0.5
		20	18.4	0.1	0.5

[a] g/liter mannose/g of total sugar X 100

Appendix Table 5. Hydrolysis Yields of Xylose.

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Xylose Released (g/liter)	Percent Xylose [a]
121	0.5	0	4.3	0	0
		5	4.1	0.1	2.4
		10	5.0	0.1	2.0
		20	5.8	0.2	3.5
		30	6.8	0.4	5.9
		40	6.5	0.6	9.2
121	1.0	0	5.0	0.1	2.0
		5	6.3	0.6	9.5
		10	8.6	1.9	22.1
		20	10.8	3.9	36.5
		30	13.8	6.0	43.5
		40	13.7	6.7	48.9
121	2.0	0	6.6	0.5	7.6
		5	11.1	4.2	37.8
		10	13.2	6.0	45.5
		20	15.9	8.0	50.3
		30	16.2	8.6	53.1
		40	16.0	8.9	55.6
121	3.0	0	4.8	0.6	12.5
		5	11.6	5.7	49.1
		10	15.4	8.1	52.6
		20	15.2	8.3	54.6
		30	13.9	8.1	58.3
		40	14.7	8.8	59.9
135	0.5	0	3.9	0	0
		5	5.3	0.2	3.8
		10	6.5	0.6	9.2
		20	7.4	1.8	24.3
		40	8.3	2.7	32.5

Appendix Table 5. (Continued)

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Xylose Released (g/liter)	Percent[a] Xylose
135	1.0	0	5.7	0.7	12.3
		5	11.6	5.8	50.0
		10	14.5	8.1	55.9
		20	16.1	9.5	59.0
		30	15.9	8.9	56.0
		40	15.3	8.7	56.9
135	2.0	0	11.4	5.0	43.9
		5	12.4	7.3	58.9
		10	14.1	8.9	63.1
		20	17.3	10.8	62.4
		30	14.4	8.6	59.7
		40	16.4	10.3	62.8
150	0.5	0	5.3	1.1	20.8
		5	9.1	4.3	47.3
		10	11.0	4.8	43.6
		20	17.3	8.9	51.4
		40	16.8	9.3	55.4
150	1.0	0	11.5	5.7	49.6
		5	18.6	11.0	59.1
		10	19.3	12.1	62.7
		20	17.6	11.6	65.9
		30	14.9	9.7	65.1
150	2.0	0	16.6	11.2	67.5
		5	19.5	14.4	73.9
		10	22.2	16.1	72.5
		15	20.3	13.8	68.0
		20	18.4	12.0	65.2

[a] g/liter xylose/g of total sugar X 100

Appendix Table 6. Hydrolysis Yields of Arabinose.

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Arabinose Released (g/liter)	Percent [a] Arabinose
121	0.5	0	4.3	0.3	7.0
		5	4.1	0.6	14.6
		10	5.0	0.8	16.0
		20	5.8	1.2	20.7
		30	6.8	1.3	19.1
		40	6.5	1.6	24.6
121	1.0	0	5.0	0.7	14.0
		5	6.3	1.3	20.6
		10	8.6	1.5	17.4
		20	10.8	1.6	15.0
		30	13.8	1.8	13.0
		40	13.7	1.7	12.4
121	2.0	0	6.6	1.1	16.7
		5	11.1	1.5	13.5
		10	13.2	1.6	12.1
		20	15.9	1.7	10.7
		30	16.2	1.7	10.5
		40	16.0	1.7	10.6
121	3.0	0	4.8	1.1	22.9
		5	11.6	1.5	12.9
		10	15.4	1.7	11.0
		20	15.2	1.6	10.5
		30	13.9	1.5	10.8
		40	14.7	1.6	10.9
135	0.5	0	3.9	0.5	12.8
		5	5.3	1.0	18.9
		10	6.5	1.4	21.5
		20	7.4	1.5	20.3
		40	8.3	1.4	16.9

Appendix Table 6. (Continued)

Temp °C	Percent Acid (w/v)	Time (min)	Total Sugar Released (g/liter)	Total Arabinose Released (g/liter)	Percent [a] Arabinose
135	1.0	0	5.7	1.2	21.1
		5	11.6	1.7	14.7
		10	14.5	1.7	11.7
		20	16.1	1.9	11.8
		30	15.9	1.9	12.0
		40	15.3	1.6	10.5
135	2.0	0	11.4	1.6	14.0
		5	12.4	1.6	12.9
		10	14.1	1.5	10.6
		20	17.3	1.8	10.4
		30	14.4	1.8	12.5
		40	16.4	1.8	11.0
150	0.5	0	5.3	0.9	17.0
		5	9.1	1.6	17.6
		10	11.0	1.8	16.4
		20	17.3	1.9	11.0
		40	16.8	1.5	8.9
150	1.0	0	11.5	1.8	15.7
		5	18.6	2.0	10.8
		10	19.3	2.1	10.9
		20	17.6	1.8	10.2
		30	14.9	1.7	11.4
150	2.0	0	16.6	1.7	10.2
		5	19.5	2.2	11.3
		10	22.2	2.1	9.5
		15	20.3	2.3	11.3
		20	18.4	2.1	11.4

[a] g/liter arabinose/g of total sugar X 100