

LBL-7879  
c.2

RECEIVED  
LAWRENCE  
BERKELEY LABORATORY

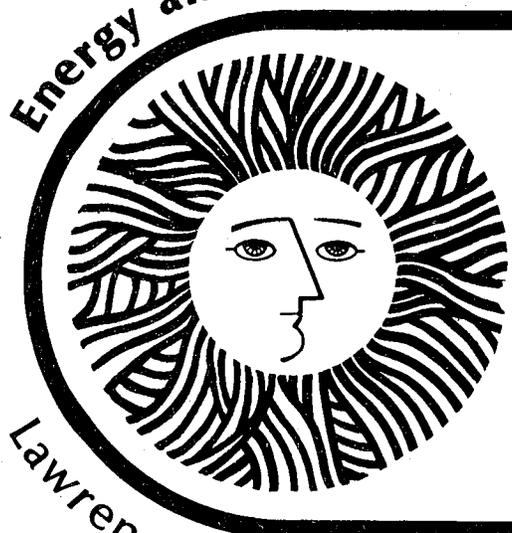
JAN 11 1979

LIBRARY AND  
DOCUMENTS SECTION

**TWO-WEEK LOAN COPY**

This is a Library Circulating Copy  
which may be borrowed for two weeks.  
For a personal retention copy, call  
Tech. Info. Division, Ext. 6782

**Energy and Environment Division**



**Effect of Nitrogen Oxide Pretreatments  
on Enzymatic Hydrolysis of Cellulose**

*Ronald Keith Borrevik, C. R. Wilke  
and D. L. Brink*

(M. S. Thesis)

September 1978

**Lawrence Berkeley Laboratory University of California/Berkeley**

Prepared for the U.S. Department of Energy under Contract No. W-7405-ENG-48

LBL-7879  
c.2

LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

EFFECT OF NITROGEN OXIDE PRETREATMENTS  
ON ENZYMATIC HYDROLYSIS OF CELLULOSE

Ronald Keith Borrevik, and C. R. Wilke

Energy and Environment Division, Lawrence Berkeley Laboratory  
and Department of Chemical Engineering

and

D. L. Brink

Forest Products Laboratory  
University of California  
Berkeley, California 94720



CONTENTS

1. INTRODUCTION . . . . .	1
2. OBJECTIVES . . . . .	5
3. PREVIOUS WORK . . . . .	6
3.1 Pretreatments for Enzymatic Hydrolysis . . . . .	6
3.1.1 Dilute Sulfuric Acid	
3.1.2 Chlorite-Acetic Acid	
3.1.3 Ethanol	
3.1.4 Ethylene Glycol	
3.1.5 Cadoxen	
3.2 Effects of Pretreatments on Rumen Digestion . . . . .	11
3.3 Chemical Methods of Obtaining Glucose . . . . .	11
3.4 Previous Work on Nitrogen Oxide Reactions . . . . .	12
Chapter 3 References . . . . .	14
4. THE CHEMISTRY OF LIGNOCELLULOSIC MATERIALS . . . . .	16
4.1 Chemical Composition . . . . .	16
4.1.1 Carbohydrates	
4.1.2 Lignins	
4.1.3 Extractives	
4.1.4 Inorganic Compounds	
4.2 Carbohydrate Reactions . . . . .	23
4.2.1 NO <sub>x</sub> Reactions	
4.2.2 Alkaline Reactions	
4.2.3 Acidic Reactions	
4.2.4 Enzymatic Hydrolysis	
4.3 Reactions of Nitrogen Oxides with Lignin . . . . .	30
Chapter 4 References . . . . .	32

5. EXPERIMENTAL PROCEDURES . . . . .	34
5.1 Overall Process Flow Scheme . . . . .	34
5.1.1 Reaction of Wheat Straw with Nitric Oxide and Air	
5.1.2 Aqueous Extraction Stage	
5.1.3 Enzymatic Hydrolysis	
5.2 Assay Procedures . . . . .	39
5.2.1 DNS Reducing Sugar Assay	
5.2.2 Filter Paper Activity	
5.2.3 Sugar Determination by Gas-Liquid Chromatography	
5.2.4 Solid Assay	
5.2.5 Off-Gas Analysis of NO <sub>x</sub> Stage	
Chapter 5 References . . . . .	47
6. RESULTS AND DISCUSSION . . . . .	49
6.1 Enzymatic Hydrolysis of Untreated Wheat Straw . . . . .	49
6.2 General Studies of the Extraction Stage . . . . .	50
6.2.1 Effect of pH	
6.2.2 Neutralization Versus Extraction	
6.3 The Use of Oxygen Versus Air in the Gas Phase Reaction. . . . .	63
6.4 Studies of Xylose During the Extraction Stage . . . . .	63
6.4.1 Rate of Formation	
6.4.2 Xylose Stability Experiment	
6.4.3 Conclusions of the Xylose Experiments	
6.5 The Effect of Varying Reaction Time of NO <sub>x</sub> Reaction at 25°C . . . . .	72
6.6 The Effect of Recycling the Extraction Liquor . . . . .	77
6.7 NO <sub>x</sub> Reactions at 80°C . . . . .	82
6.8 Composition of Off-Gas from NO <sub>x</sub> Stage . . . . .	83
Chapter 6 References . . . . .	87

7. PROCESS ECONOMICS AND EVALUATION . . . . .	88
7.1 Process Description . . . . .	88
7.1.1 Nitric Oxide Production	
7.1.2 NO <sub>x</sub> Reaction Stage	
7.1.3 Extraction	
7.1.4 Enzymatic Hydrolysis	
7.2 Cost Estimation Procedures . . . . .	93
7.3 Cost Estimation Results . . . . .	96
Chapter 7 References . . . . .	99
APPENDIX -- COST CALCULATIONS FOR NITRIC OXIDE PRODUCTION AND NO <sub>x</sub> REACTION STAGE . . . . .	100
Nitric Oxide Production . . . . .	100
NO <sub>x</sub> Reaction Stage . . . . .	102
Appendix References . . . . .	104



EFFECT OF NITROGEN OXIDE PRETREATMENTS  
ON ENZYMIC HYDROLYSIS OF CELLULOSE

Ronald Keith Borrevik\* and C.R. Wilke

Energy and Environment Division, Lawrence Berkeley Laboratory  
and Department of Chemical Engineering

and

D.L. Brink

Forest Products Laboratory  
University of California  
Berkeley, California 94720

ABSTRACT

In lignocellulosic materials cellulose is relatively inaccessible to enzymatic attack due to its crystalline structure and the presence of lignin and hemicelluloses. A low cost method of increasing accessibility would greatly improve the yields and economics of hydrolysis.

This work considers the effect of nitrogen oxide pretreatments on the subsequent enzymatic hydrolysis by Trichoderma viride cellulase of the cellulose occurring in wheat straw, Triticum Aestivum-L, em. Thell. In the pretreatment scheme the straw is first reacted with nitric oxide and air, and then extracted in aqueous solution. In this way, overall sugar yields increased from 17% for the case of no pretreatment to 70%. The glucose yield increased from 20 to 60%.

The yield of glucose during enzymatic hydrolysis is dependent on the reaction time of the gas phase reaction. For a 24 hour reaction the yield is 60%, but drops to 45% for a reaction time of 2 hours.

Xylose, a potentially valuable side product of the pretreatment,

is obtained by dilute acid hydrolysis during the extraction stage in yields of 90 to 96%. In acidic media, the kinetics of both the rate of formation and destruction of xylose were found to follow the first-order rate laws reported in the literature. These were determined to be  $4.5 \text{ (liter/gmole)(hr.}^{-1}\text{)}$  and  $0.03 \text{ hr.}^{-1}$ , respectively. However, the rate of formation is much greater ( $20.4 \text{ (liter/gmole)(hr.}^{-1}\text{)}$ ) when the extraction liquor is recycled. The most likely explanation for this is that the increased total acidity of the recycled liquor compensates for diffusional limitations.

A preliminary design and cost analysis of the pretreatment-hydrolysis scheme indicates that glucose can be produced at 10.86¢ per pound, exclusive of straw cost. The corresponding cost per pound of total sugars produced is 5.0¢. Sensitivity analyses indicate that 42% of the pretreatment cost (excluding hydrolysis) can be attributed to nitric oxide production, and the high yield of sugar obtained is advantageous when considering the cost of straw.

---

\* M.S. Thesis

This work was supported by the U.S. Department of Energy.

## 1. INTRODUCTION

The oil embargo of 1973-4 and rapidly dwindling world petroleum reserves serve as grim reminders of our technological dependence on petroleum as a fuel and chemical source. In the interest of society, it is imperative that new energy sources be developed. Because the automobile is the major mode of transportation, a large quantity of combustible liquid fuels is needed. Besides petroleum, the only sources from which combustible fuels can be derived are coal and living biomass. Much research is being done on converting these to suitable fuels.

This work is part of an overall process to develop the alternative liquid fuel, ethanol, from cellulosic materials. The process consists of enzymatically hydrolyzing, with Trichoderma viride cellulase, cellulose to the monomer sugar glucose, which is then fermented to ethanol. Two problems associated with this process stem from the following facts: 1) The rate of enzymatic hydrolysis of pure cellulose has been found to be low; and 2) Cellulose rarely occurs in a pure form in nature, but is in close association with lignin and hemicelluloses.

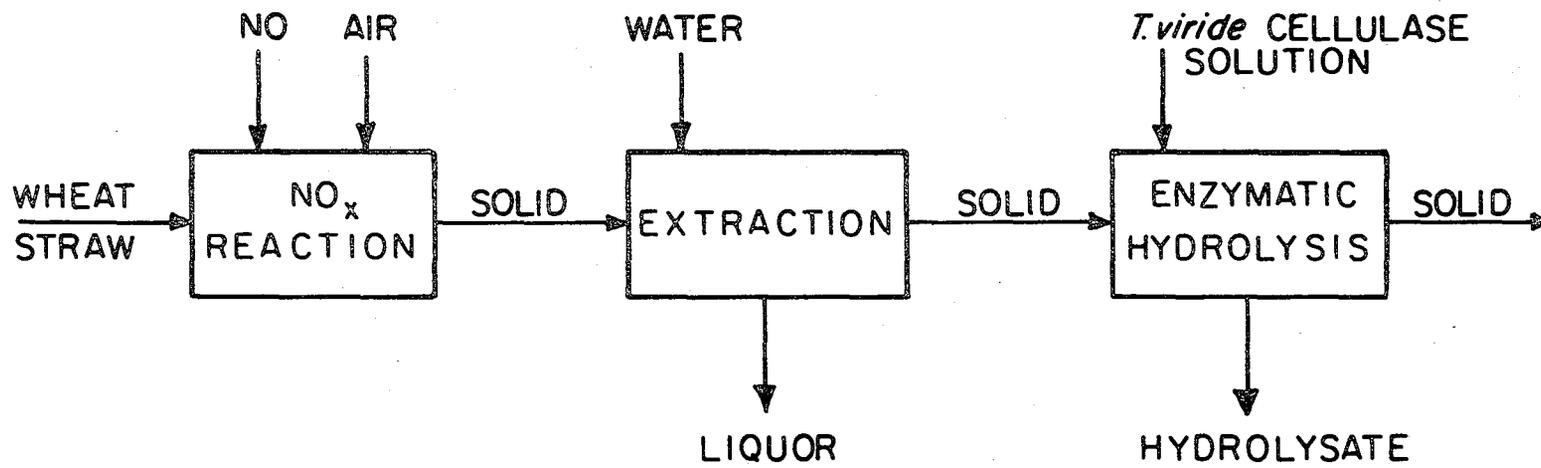
The fact that pure cellulose hydrolyzes at a very low rate can be attributed to the heterogeneous nature of the reaction caused by the crystalline structure of the cellulose microfibril. To aggravate matters, the protective lignin sheath also substantially hinders reaction.

Much research concerning the effect of various pretreatments on subsequent enzymatic hydrolysis has been done. Of these past studies, the majority have focused on delignification rather than physical

alteration of the cellulose fine structure. However, it is well established that the latter factor is a more dominant obstacle with regard to enzyme attack. The reason most pretreatments have dealt with delignification is largely economic. The fact remains that an extensive changing of the cellulose physical structure is still an expensive proposition -- regardless of the method employed.

A pretreatment which has received much study in this laboratory involves contacting of lignocellulosic materials with dilute boiling sulfuric acid. It is especially attractive for use with materials of high hemicellulose content, such as agricultural residues. In the case of agricultural residues, the pretreatment liquor is rich in xylose -- a versatile chemical which can be converted to furfural or ethanol. The residual solid from pretreatment is subjected to enzymatic hydrolysis. An approximate doubling of the overall sugar yield over that obtained without pretreatment is obtained in this way.

Although the results of the dilute acid pretreatment scheme are promising, it does not accomplish a vital objective -- delignification. A process which can combine delignification and hemicellulose removal would appear, a priori, to have a distinct advantage over the dilute acid process. A processing scheme which meets this criterion is the nitrogen oxide pretreatment (Figure 1.1). In this process, the material is first reacted with nitric oxide and air at atmospheric pressure. The gas treated material is then extracted in water. As in the dilute acid process, the extraction liquor is rich in xylose. However, this liquor also contains a sizable fraction of the lignin.



XBL 789-5768

Fig. 1.1 Overall process flow diagram for enzymatic hydrolysis of lignocellulose employing a  $\text{NO}_x$  pretreatment step.

In the present investigation, the use of nitrogen oxides as a pretreatment method has been investigated. The effect of the pretreatments on overall sugar yield was determined for a wide range of experimental conditions. The basic data acquired were then used to perform a preliminary process design and evaluation.

## 2. OBJECTIVES

The overall objectives of this research were:

To explore and develop a pretreatment scheme for enzymatic hydrolysis of agricultural residues using nitric oxide and oxygen in the form of air.

To perform a preliminary process design based on the data collected.

To assess the results obtained using the pretreatment scheme developed by comparing the overall process economics with those of other processes.

### 3. PREVIOUS WORK

Lignocellulosic materials have been used as a source of sugar and ethanol in several nations in recent times. Russia and the Scandinavian countries, especially in times of war, have successfully extracted sugars from wood on an industrial scale. The practice is not widespread, however, owing to the relatively poor economics of the processes. A great deal of research has been done on the subject. However, most of this research centers on acid hydrolysis with little mention of enzymatic conversion. This chapter will discuss the results obtained by others on obtaining sugars from lignocellulose. Primary emphasis will be on the enzymatic method using Trichoderma viride cellulase coupled with various pretreatments. The effect of pretreatments on digestion of material by rumen organisms is also included.

The chapter will conclude with a discussion of previous work done at the University of California Forest Products Laboratory on the reaction between lignocellulose and nitrogen oxides.

#### 3.1 Pretreatments for Enzymatic Hydrolysis by T. Viride

Several methods of pretreatment have been investigated in recent years. These include using dilute sulfuric acid, chlorite-acetic acid, ethanol, ethylene glycol, cadoxen, and ball milling processes.

The majority of studies in this regard have been concerned with delignification. It is well established that as the lignin content of a material decreases the rate of enzymatic attack on the carbohydrates increases. Another approach which has not received a commensurate amount of attention involves altering the physical structure

of lignocellulose. Of the pretreatment methods mentioned above, chlorite-acetic acid, ethanol, and ethylene glycol are considered as delignifiers. The cadoxen, concentrated sulfuric acid, and ball milling processes are aimed at altering physical structure. The dilute acid process opens up the substrate to enzymes mainly by removing the hemicelluloses.

### 3.1.1 Dilute Sulfuric Acid

A pretreatment scheme which has received considerable study in this laboratory is the dilute acid process (1). It consists of reacting a material with a 1 w% sulfuric acid solution at 100°C for 5.5 hours at a liquor/solid ratio of 12 to 1. Studies have been conducted on the following materials: barley, corn stover, cotton gin trash, rice hulls, rice straw, sorghum straw, wheat straw, newsprint, and ground wood (1).

Figure 3.1 is a flowsheet of sugar yields obtained during the dilute acid pretreatment and subsequent enzymatic hydrolysis of wheat straw. As can be seen, the overall yield of sugars increases from 27% for untreated material to 53% for the acid treated straw.

Several economic studies of this process have been done (2,3) and conclude that the cost of glucose is between 7 and 10| per pound.

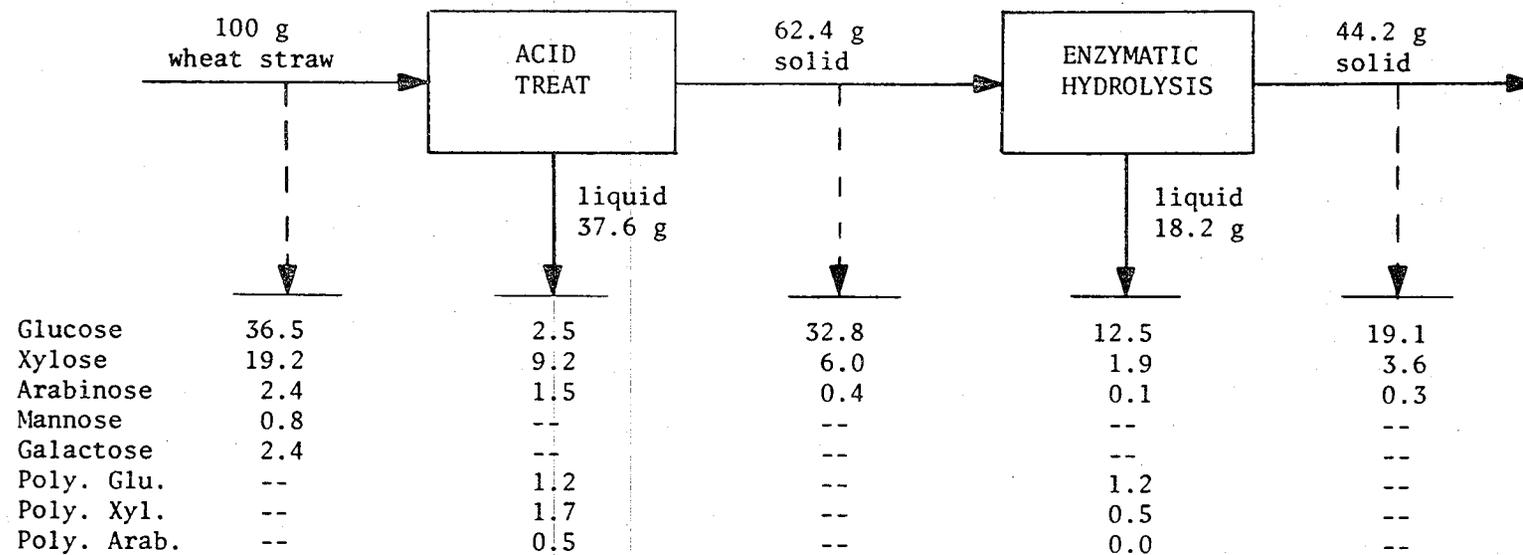


Fig. 3.1 Results obtained by enzymatic hydrolysis of dilute acid pretreated wheat straw. Pretreatment involves contacting straw with 0.09 M  $H_2SO_4$  for 5.5 hours at 100°C.

### 3.1.2 Chlorite-Acetic Acid

Benzene/ethanol extracted wheat straw was delignified using the common analytical holocellulose assay procedure (4). Studies on the effect of varying degrees of delignification by this method on the enzymatic conversion have been done by several investigators (5,6). The results show clearly that as the lignin content is lowered conversion increases. In the case of 2 mm Wiley milled wheat straw, a combined glucose and cellobiose yield of 38.2% was obtained upon enzymatic hydrolysis of treated wheat straw of 2% lignin content. Again, this is to be compared with a 24% yield of glucose conversion for untreated wheat straw.

When the chlorite-treated straw was ball milled to 150 mesh (0.105 mm) a yield of glucose and cellobiose of 66.7% resulted. The above two experimental results lead to the conclusion that the crystallinity of the cellulose is a major obstacle to hydrolysis.

### 3.1.3 Ethanol

The idea of using ethanol as a solvent for pretreatment seems especially attractive because of its availability in the process. Following Kleinert (7), Sciamanna (5) pretreated both wheat straw and hypochlorite bleached groundwood with a 50 w% ethanol-water mixture of pH 6 at 180 and 230°C for approximately 1 hour.

For wheat straw the obtained overall yields of glucose and cellobiose were 39.6% and 37.1% at 180°C and 230°C. The lignin values of the treated straw were 16.1% and 3.7% at 180 and 230°C, respectively.

#### 3.1.4 Ethylene Glycol

Pretreatments using ethylene glycol have been investigated (5). In this study 2 mm Wiley milled newsprint was hydrolyzed in 1 w% sulfuric acid for 5 hours. This hydrolyzed material was then reacted at 180°C for 30 minutes in ethylene glycol which contained 0.008 M sulfuric acid. The overall yield of glucose and cellobiose was 48 grams per 100 grams of the original newsprint. This corresponds to a yield of 73% of the available glucose. However, the majority of this glucose was in the ethylene glycol -- only 16.2 grams were found in the enzymatic hydrolysate. This is to be compared with a yield of 21.1 grams of glucose and cellobiose obtained upon enzymatic hydrolysis of the original, untreated newsprint.

#### 3.1.5 Cadoxen

Researchers at Purdue University have obtained glucose yields of 90% during the enzymatic hydrolysis of amorphously precipitated avicel (8). In this process avicel was dissolved in cadoxen solvent, precipitated by adding methanol and/or water, and separated from the liquid by filtration. Cadoxen is a solution containing 5 to 7% cadmium oxide in 28% aqueous ethylenediamine. When enzymatically hydrolyzed the precipitated avicel gave a 90% yield of glucose. The same process applied to agricultural residues gave a glucose yield of 80% after only 3 hours of enzymatic hydrolysis. This study, although of dubious economic feasibility owing to the high cost of cadmium and ethylenediamine, clearly shows that it is the crystalline structure of cellulose which prevents enzymatic hydrolysis.

### 3.2 Effect of Pretreatments on Rumen Digestion

Much study has been devoted to finding effective pretreatments of straws in order to increase their digestibility by ruminants. In this regard, a pretreatment with sodium hydroxide was patented by Beckman (9) and used extensively in Europe during both world wars.

Such an extensive amount of work has been done in this area that the different pretreatments will not be discussed. However, studies involving sodium hydroxide, ammonia, chlorine dioxide, ammonium bisulfite, sulfur dioxide, steaming, pulp mill wastes, grinding and ball milling, irradiation, photodegradation, high temperature, low temperature, and high pressures have all been studied and are summarized in a paper by Millett, Baker, and Satter (10).

### 3.3 Chemical Methods of Obtaining Glucose

The majority of studies on chemical saccharification involve the dilute and concentrated acid processes. However, recent research on a wet oxidation process shows promising results (11).

The dilute acid process involves reacting 0.2 w% acid solution at high temperature (170°C upward) with cellulose. Yields in a batch hydrolysis (Simonsen Process) are only 25-26% of the available sugar. However, higher yields can be obtained using pulse and continuous percolation (12). This method does give high pentose recovery.

Reacting lignocellulose with concentrated acids at room temperature results in an almost quantitative yield of monosaccharides. In one such process, first the hemicelluloses are removed with a dilute acid prehydrolysis. The residual solid is then reacted in a

a countercurrent fashion with 41 w% hydrochloric acid (12). Acid is removed by vacuum evaporation. This process exploits the observation that concentrated acids efficiently swell the cellulose structure.

Wet oxidation involves the treatment of an aqueous slurry of solid organic matter with air or oxygen at elevated temperatures and pressures (13,14). The solid flows countercurrent to the liquor -- the acidic solution used in the two hydrolysis stages being generated from wet oxidation of lignin in stage three. In the first hydrolysis step the solid undergoes mild hydrolysis at pH 2.2, 160°C, and 225 psig for 60 min. The liquor from this stage is the product of the process and contains the hemicellulosic sugars generated in the first stage, glucose (from cellulose) obtained in the second stage, and organic acids (primarily acetic acid) formed mainly during the third stage. The solids from the first stage are contacted with the organic acid solution generated in stage 3 and air is blown through this mixture at 170°C. In the second hydrolysis this mixture is heated to 200°C and reacted for 35 minutes, the main product being glucose. By this processing scheme glucose yields of 47% have been obtained from wood. The sugar concentration of the product steam is 46 grams/liter (15).

#### 3.4 Previous Work on Nitrogen Oxide Reactions

The use of nitrogen oxides in a pulping scheme has been investigated by Brink and coworkers (16,17) at the Forest Products Laboratory at the University of California over a period of years. In these studies wood chips of 10 to 20% moisture content were reacted with nitric oxide and oxygen at essentially atmospheric pressure. The

chips were then delignified in alkaline solution at elevated temperatures (160 - 180 °C). This processing scheme gave: 1) a rapid rate of delignification, 2) a higher overall yield at comparable lignin content than conventional pulping processes, and 3) high strength pulps. These results indicate that the use of nitrogen oxides in a pretreatment step for enzymatic hydrolysis may be effective.

In studies conducted by Lin (18) it was shown that a sequential mode of gas addition gave better results than simultaneous addition. Nitric oxide was first added to the reaction vessel, a few minutes were allowed to pass before addition of oxygen. By adding the gases in this manner the reactions occur uniformly throughout the chip. Nitric oxide and oxygen react to give nitrogen dioxide, which rapidly reacts with water to give nitric acid. If the addition of nitric oxide and oxygen is simultaneous, the nitrogen dioxide formed reacts with water near the surface of the chip. However, for the sequential mode of addition, the nitric oxide reacts with the oxygen in the chip, thereby giving uniform coverage.

Lin also concluded the optimal conditions of the gas phase reaction to be: 5 grams of NO per 100 grams of solid material, 24 hour reaction time, 20°C, and 20% moisture content of the solid.

Chapter 3. References

1. C.R. Wilke, "Pilot Plant Studies of the Bioconversion of Cellulose and the Production of Ethanol," LBL-6859, January 1977.
2. C.R. Wilke, R.D. Yang, A.F. Sciamanna and R.P. Freitas, "Raw Materials Evaluation and Process Development Studies for Conversion of Biomass to Sugars and Ethanol," LBL-7847, June 1978.
3. R.R. Lindsey, Master Thesis in Progress, U. of California, Berkeley.
4. "Holocellulose in Wood," Tappi Standard T9 m-54.
5. C.R. Wilke, "Pilot Plant Studies of the Bioconversion of Cellulose and Production of Ethanol," LBL-6860, June 30, 1977.
6. P. Carroad, Ph.D. Thesis, U. of California, Berkeley, Department of Chemical Engineering, LBL-4490, April 1976.
7. T.N. Kleinert, Tappi, 57, No. 8, 99 (1974).
8. Laboratory of Renewable Resources Engineering, Purdue University, U.S. Dept. of Energy, Solar Energy Division - Fuels from Biomass Program, Report of Project Progress, February 1978.
9. E. Beckman, "Food for Animals," Brit. Pat. 151,229, Aug. 15, 1919.
10. M.A. Millet, A.J. Baker and L.D. Satter, "Pretreatments to Enhance Chemical, Enzymatic, and Microbiological Attack of Cellulosic Materials," Forest Products Laboratory, Madison, Wisconsin, June 1974.
11. L.L. Schaleger and D.L. Brink, Tappi, 61, No. 4, 65 (1978).
12. N.I. Nikitin, The Chemistry of Cellulose and Wood, pp. 561-563, Israeli Program for Scientific Translations Ltd., Jerusalem, 1966.
13. J.G. Bicho and D.L. Brink, "Wet Oxidation of Solid Waste," Sanitary Eng. Res. Lab. Report 71-1, p. 1-86 (1971).

14. J.G. Bicho, Ph.D. Thesis, U. of California, Agricultural Chem.,  
December 1970.
15. L.L. Schaleger and D.L. Brink, Personal communication, U. of  
California For. Prod. Lab., Richmond.
16. D.L. Brink, M.M. Merriman, B.M. Collett, and S.Y. Lin, "Reaction  
of Lignocellulose with Oxides of Nitrogen," Abstract of Papers  
of the 167th Am. Chem. Soc. Meeting, Los Angeles, CA,  
March 31 - April 5, 1974.
17. D.L. Brink, U.S. Patent 4,076,579, February 1978.
18. S.Y. Lin, Ph.D. Thesis, U. of California, Dept. of Forestry  
and Conservation, Berkeley, 1976.

#### 4. THE CHEMISTRY OF LIGNOCELLULOSIC MATERIALS

##### 4.1 Chemical Composition

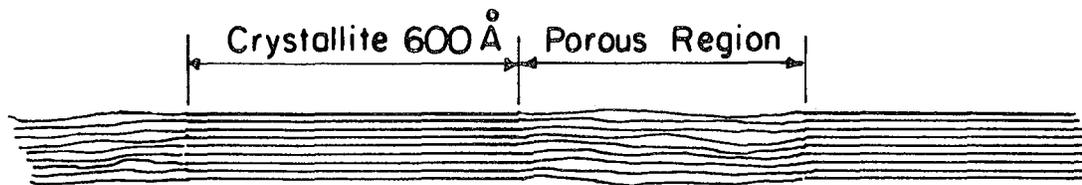
The chemical compounds occurring in lignocellulosic materials belong to one of the following general classes of compounds: 1) carbohydrates, 2) lignins, 3) extractives, and 4) inorganics. The relative abundance of these four classes is a function of species, climate, age, and location within the plant.

##### 4.1.1 Carbohydrates

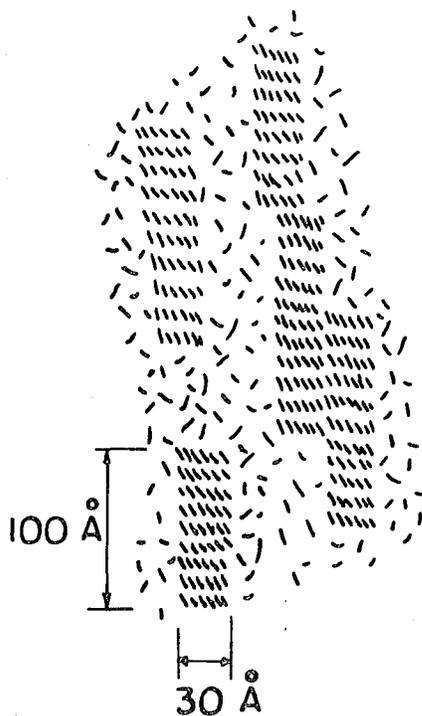
The carbohydrates consist mainly of cellulose and the hemicelluloses. Cellulose is the main constituent of the cell wall of plants. It is a linear polymer of  $\beta$ 1-4 linked anhydrous glucopyranose monomers. The number average degree of polymerization (DP) of cellulose ranges between 600 and 15,000 (1). The DP of wheat straw is approximately 3000 (2). Cellulose is not soluble in water or alkali.

The hydroxyl groups of adjacent cellulose chains interact with each other giving the superstructure of cellulose considerable lateral order. X-ray scattering experiments show that closely bound parallel cellulose chains form a distinct region of order. These regions are called microfibrils or micelles. This parallel arrangement of chains contains both crystalline and amorphous regions. Figure 4.1 (3) illustrates this proposed micellar theory of cellulose structure.

It is the crystalline region of the cellulose microfibril which makes it difficult to attack chemically. This can be better understood with the aid of Figure 4.2 (4). Due to the close proximity



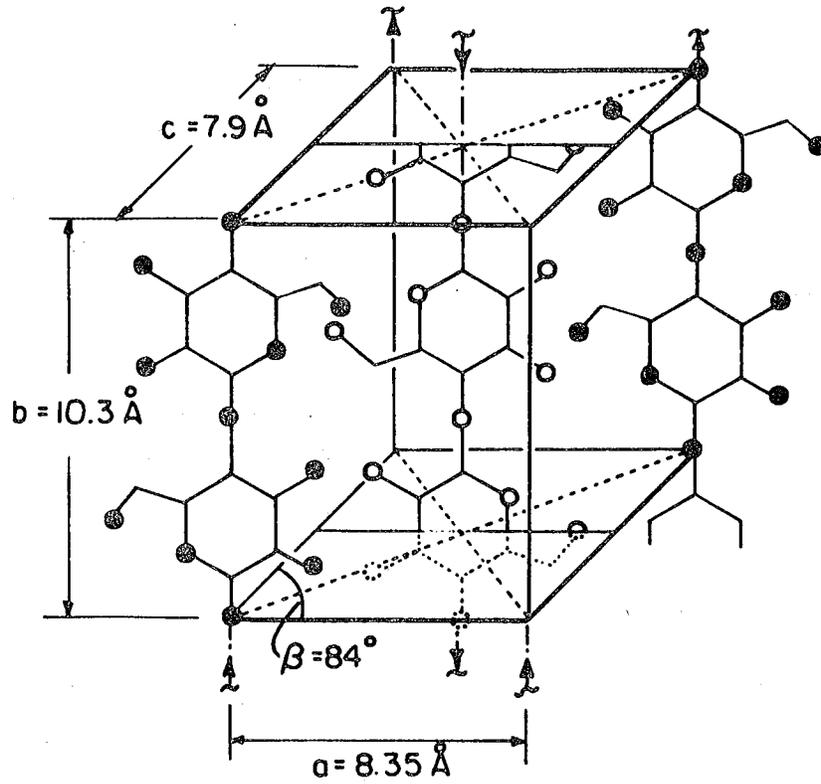
(a)



(b)

XBL 788-5680

Fig. 4.1 Proposed micellar theory of cellulose structure. (3)  
(a) longitudinal view, (b) cross sectional view.



XBL 788-5691

Fig. 4.2 The monoclinic elementary cell of cellulose. <sup>(4)</sup>

of neighboring glucose molecules ( $< 10 \text{ \AA}$ ) very low rates of diffusion occur in the microfibril. Incoming chemical reagent diffuses to the reaction sites slowly. Even more important, the bulkier degraded carbohydrates cannot diffuse out of the crystalline region. With regard to this study, it is obvious that the enzymes, being of high molecular weight, cannot penetrate the cellulose structure. Therefore, the heterogeneous kinetics are complex and exhibit little resemblance to the kinetics of soluble carbohydrates.

The hemicelluloses are amorphous polymers of sugar monomers which are for the most part water-insoluble, alkali-soluble. They form an integral part of the cell wall. There are many different types of hemicelluloses, as illustrated by Table 4.1 (5). Excellent papers on the hemicelluloses have been written by Timell (6,7).

The types and abundance of hemicelluloses found in a plant is a function of species. The hardwoods and grasses are to be contrasted against the softwoods. The former contain large amounts of 4-O-methylglucuronoxylan, whereas the latter usually contain mainly moderate amounts of glucomannan with smaller amounts of xylans. Softwood hemicelluloses become alkali soluble only after delignification.

#### 4.1.2 Lignins

Lignin is an amorphous, aliphatic-aromatic polymer of oxygen substituted phenylpropane units. It is a random, naturally occurring polymer of plants. In mature wood tissue the amount of lignin varies between 18 and 38 weight per cent, depending on the species. Lignin is generally present in smaller amounts in the grasses. It acts as a

Table 4.1 Summarizing data for polysaccharides (5).

Polysaccharide	Occurrence	Per cent of extractive -free wood	Composition	Parts	Linkages
Cellulose	All woods	42±2	β-D-Glup	All	1 → 4
<u>O</u> -Acetyl-4- <u>O</u> -methylglucurono-xylan	Hardwoods	20-35	β-D-Xylp 4- <u>O</u> -Me-α-D-GlupA <u>O</u> -Acetyl	10 1	1 → 4 1 → 2
Glucomannan	Hardwoods	3-5	β-D-Manp β-D-Glup	1-2 1	1 → 4 1 → 4
Arabino-4- <u>O</u> -methylglucurono-xylan	Softwoods	10-15	β-D-Xylp 4- <u>O</u> -Me-α-D-GlupA -Araf	10 2 1,3	1 → 4 1 → 2 1 → 3
Galactoglucomannan (water-soluble)	Softwoods	5-10	β-D-Manp β-D-Glup α-D-Galp <u>O</u> -Acetyl	3 1 1 0.24	1 → 4 1 → 4 1 → 6
Galactoglucomannan (alkali-soluble)	Softwoods	10-15	β-D-Manp β-D-Glup α-D-Galp <u>O</u> -Acetyl	3 1 0.1 0.24	1 → 4 1 → 4 1 → 6
Arabinogalactan	Larchwood	10-20	β-D-Galp L-Araf β-L-Arap β-D-GlupA	6 2/3 1/3 Few	1 → 3, 1 → 6 1 → 6 1 → 3 1 → 6
Pectin	All woods	1	α-D-GalpA D-Galp L-Araf	Most Few Few	1 → 4
Starch	All woods	Variable, but 5%			
Amylose			α-D-Glup	All	1 → 4
Amylopectin			α-D-Glup	All	1 → 4, 1 → 6

binding agent between adjacent fibers and sheaths the carbohydrates. Many studies have shown that as the lignin content is lowered, the rate of hydrolysis of the polysaccharide increases.

Lignins, as they occur in nature, are almost totally insoluble in known solvents, not hydrolyzable to monomeric units, and devoid of the highly regular structure characteristic of other natural polymers.

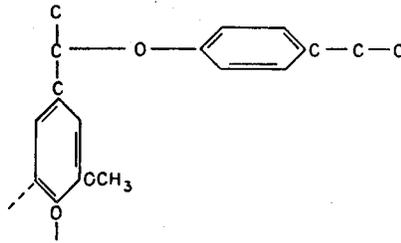
Plant lignins can be divided into three broad classes. These are called softwood, hardwood, and grass lignins. Softwood lignins represent polymers containing high percentages (usually over 95%) of guaiacylpropane units. Hardwood and grass lignins contain, as a first approximation, the same amounts of both guaiacylpropane and syringylpropane units. Grasses also contain a significant amount of parahydroxyphenyl units.

The randomness of lignin can be attributed to the various ways in which the phenylpropane units are linked. These linkages all belong to one of two general classes: ether and carbon-carbon linkages. The latter are highly resistant to chemical degradation. Typical lignin linkages are shown in Figure 4.3 (8).

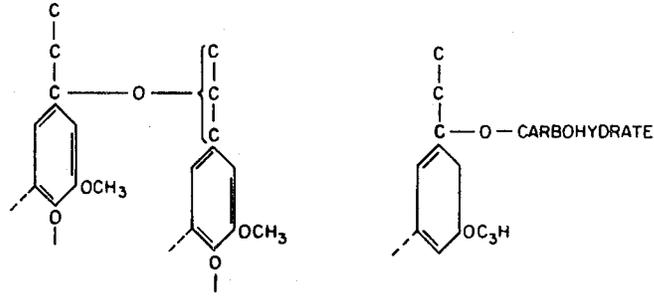
#### 4.1.3 Extractives

The extractives consist of a wide variety of compounds. They can be crudely divided into four groups: terpenes, resins, phenols, and carbohydrates. This project is not concerned with extractives to a great extent so the above groups will not be discussed. A knowledge of the occurrences of certain extractives in certain species

ETHER LINKAGES

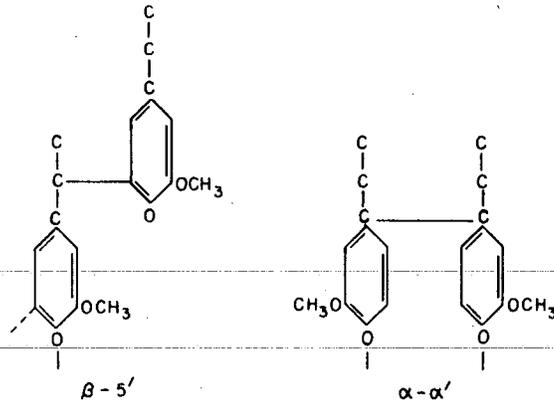


B - O - 4 ETHER



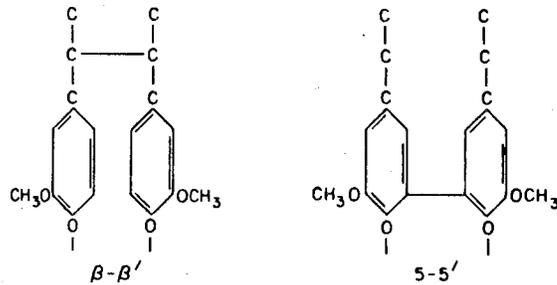
$\alpha$  - O ALKYL ETHERS

CARBON-TO-CARBON LINKAGES



$\beta$  - 5'

$\alpha$  -  $\alpha'$



$\beta$  -  $\beta'$

5 - 5'

XBL 789-5766

Fig. 4.3 Postulated intermonomeric linkages in lignin.(8)

should be known, however, as they can participate in cross-linking reactions with the lignin. Taxofolin and pinosylvin, found in douglas fir and the pines, for example, condense with the aromatic moiety of lignin during acidic treatments.

#### 4.1.4 Inorganic Compounds

The inorganic compounds usually constitute less than 1% of wood weight. However, they can range up to 18% in the case of rice straw. They are primarily alkali and earth alkali carbonates. Large amounts of silicates are present in cereal straws (4).

### 4.2 Carbohydrate Reactions

#### 4.2.1 NO<sub>x</sub> Reactions

When exposed to an environment containing nitrogen oxides, cellulose is oxidized. Studies conducted employing NO<sub>2</sub> and N<sub>2</sub>O<sub>4</sub> have shown that the oxidation is more or less specific to the primary hydroxyl group of carbon 6 (9). However, studies with NO<sub>2</sub> have also shown that once the primary hydroxyl is oxidized the secondary hydroxyls at carbons 2 and 3 may be oxidized also. The degree of oxidation is determined by the time, temperature, and amount of NO<sub>2</sub> added.

The oxidation preferentially occurs in the amorphous regions of the cellulose microfibril according to Parkinson (10). The rate of reaction is enhanced by the presence of nitric oxide.

#### 4.2.2 Alkaline Reactions

In an alkalkine solution monosaccharides are isomerized and degraded by  $\beta$ -hydroxyl elimination and subsequent reactions (11). The

chemical reactions of polysaccharides to be considered are of the following types (4):

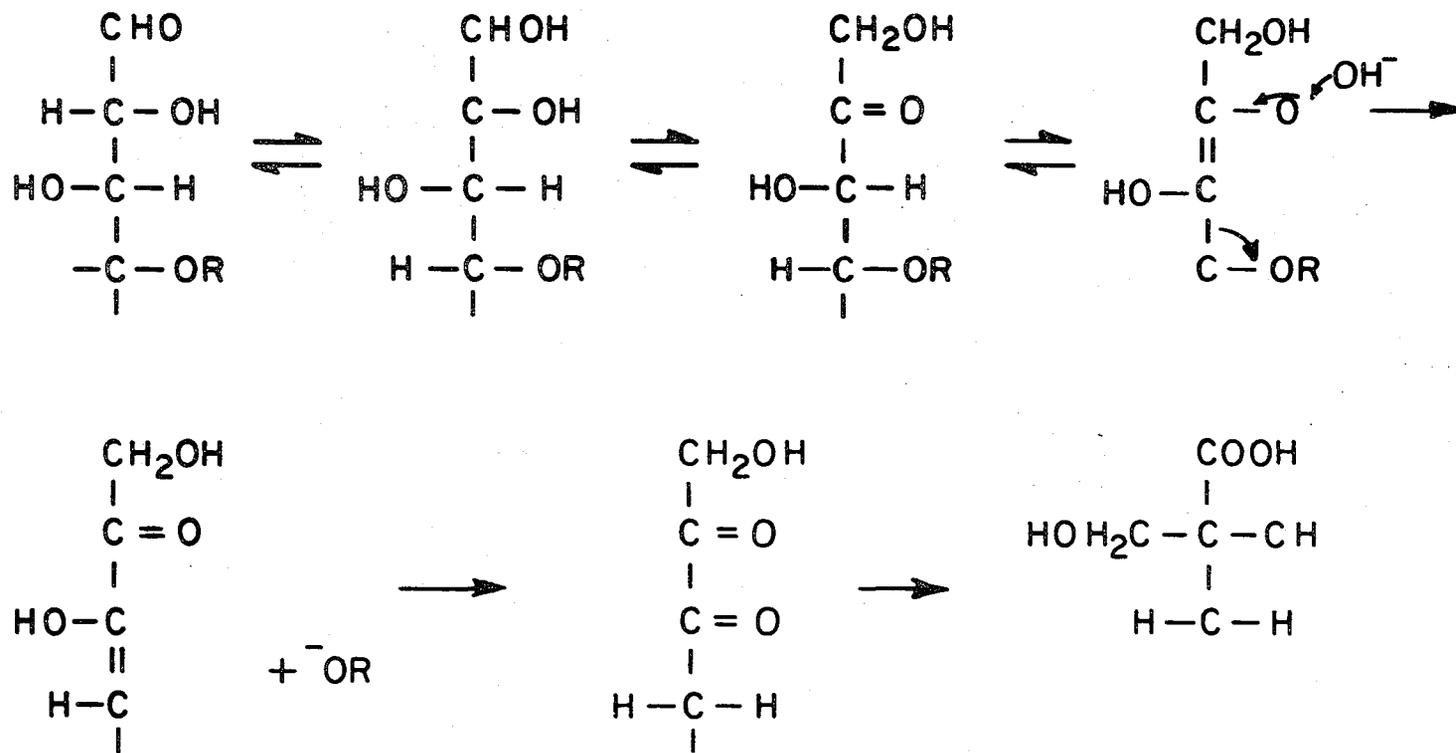
- 1) Alkaline dissolution of polymeric carbohydrates
- 2) Alkaline hydrolysis of acetyl groups from the hemicelluloses
- 3) Alkaline peeling
- 4) Stopping reactions
- 5) Alkaline hydrolysis of  $\beta$ -glycosidic bonds

The reaction of major importance is the peeling reaction. It occurs only at the reducing end group of the polysaccharide. Figure 4.4 (4) illustrates the peeling reaction. From this figure it can be seen that all peeled sugar monomers have been destroyed. The major product of the peeling reaction is isosaccharinic acid.

The peeling reaction eliminates approximately 65 monomeric units from the reducing end before a "stopping" reaction occurs (12). The stopping reaction renders the polysaccharide stable toward alkaline peeling. The most predominant stopping reaction mechanism is the one by which metasaccharinic acid is formed.

New reducing end groups can be formed by the base catalyzed hydrolysis of glycosidic bonds. However, this reaction is much slower than the peeling reaction and does not appreciably effect the kinetics. Under oxygen pressure alkaline hydrolysis does become significant at temperatures in excess of 130°C by a different mechanism.

Xylan is less reactive in alkali than cellulose because the carbon 2 and 3 substituents retard the peeling reaction. For this reason, large amounts of undegraded xylan are found in alkaline solutions. Other carbohydrates of sufficiently low molecular weight are also



XBL 788-5689

Fig. 4.4 Mechanism of primary peeling.

dissolved as they become accessible to solution. This results from two factors: alkaline swelling and previous chain shortening.

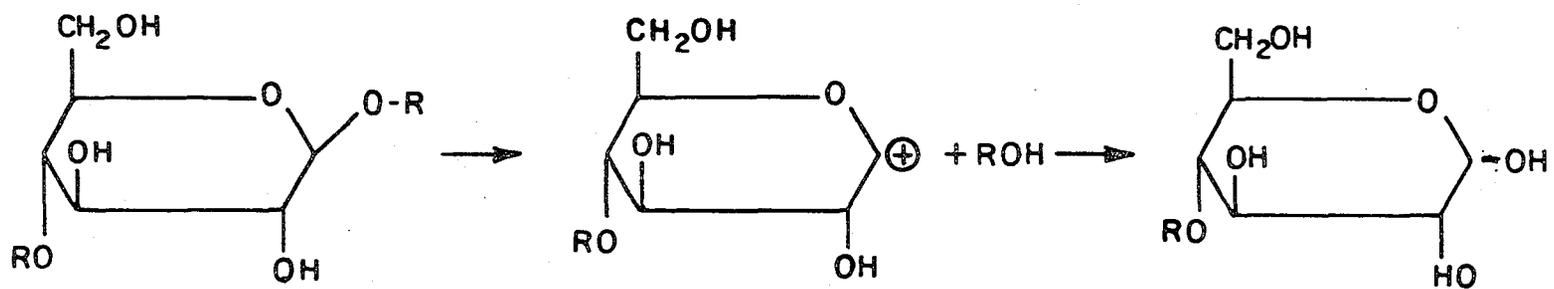
#### 4.2.3 Acidic Reactions

In acidic solution the carbohydrate reaction occurring most frequently is acid hydrolysis. Hydrolysis of polysaccharides involves the scission of the acetal bonds between two sugar residues and the addition of water at the place of the scission. A proposed mechanism for this is shown in Figure 4.5 (7).

Homogeneous acid hydrolysis is approximately first order with respect to hydrogen ion concentration and the amount of polysaccharide present in solution, that is, rate of sugar formation =  $k[H^+]P$ . Here P is the total amount of polysaccharide, in grams, found in solution at time t. The hydrolysis of the readily accessible hemicelluloses follows these kinetics at 100°C (9). However, crystalline cellulose hydrolysis kinetics depart radically from this rate law.

In Figure 4.6 the intermediate carbonium ion can also be attacked by a free sugar molecule or polysaccharide fragment to yield a larger polysaccharide. This process is called reversion and is common in concentrated acid solutions.

The rate of acid hydrolysis is effected little by electrophilic groups introduced to the polysaccharide. Rånby (13) studied the effect of oxidation on the rate of hydrolysis and found very little change. He concluded that one of the linkages is made more stable by oxidation, while the other adjacent 1-4 linkage is made more susceptible to hydrolysis.



XBL 788-5688

Fig. 4.5 Proposed acid hydrolysis mechanism.

Concomitant with sugar formation is their decomposition. Free sugars in acidic solution are degraded to a mixture of products. Hexoses decompose to hydroxymethylfurfural, formic acid, levulinic acid, and humic substances. Pentoses yield furfural, formic acid, and humic substances. The rate of decomposition is approximately first order in sugar concentration at a constant pH.

The ratio of the rates of formation and decomposition determine the maximum sugar yield obtainable. For any consecutive pair of first order reactions  $A \rightarrow B \rightarrow C$ , the maximum yield of the intermediate is given by:

$$(C_B)_{\max} = C_{A_0} \left( \frac{k_1}{k_2} \right)^{\frac{k_2}{k_2 - k_1}}$$

#### 4.2.4 Enzymatic Hydrolysis

Fractionation studies of the Trichoderma viride cellulase system have shown three distinct general types of enzymes to exist:

1) 1,4- $\beta$ -glucan glucanhydrolase (also called  $C_x$ ); 2) 1,4- $\beta$ -glucan cellobiohydrolase (also called  $C_1$ ); and 3)  $\beta$ -glucosidase.

$C_x$  enzymes are endoglucanases which randomly attack the amorphous regions of the cellulose, increasing the number of end groups. Acting alone they are incapable of hydrolyzing the crystalline region.

It is generally accepted that the  $C_1$  component acts as exoglucanase. Starting at a non-reducing end group, the enzyme works its way down the cellulose chain, sequentially hydrolyzing the cellulose to cellobiose molecules. Cellobiose and higher oligosaccharides inhibit the  $C_1$  and  $C_x$  components. For this reason,  $\beta$ -glucosidase,

which hydrolyzes cellobiose to glucose, plays an integral role in enhancing hydrolysis kinetics.

An important feature of the cellulase system is the capability of  $C_1$  and  $C_x$  enzymes to act synergistically. In purified form neither of these can hydrolyze the crystalline regions. However, when acting together they can extensively hydrolyze crystalline cellulose. It is believed that the cellulase action involves sequential attack, where the randomly-acting endoglucanases ( $C_x$ ) initiate the attack and the new chain ends that are produced are then hydrolyzed instantly by the  $C_1$  component, in order to prevent reformation of the glucosidic linkage.

It has been shown repeatedly that the kinetics of hydrolysis do not follow Michaelis-Menton form but are considerably influenced by the heterogeneous aspects of the reaction. Whittaker (14) has estimated the T. viride enzyme molecule to be cylindrically shaped and to have a length of 200 Å and a diameter of 33 Å. This large molecule cannot possibly diffuse into the cellulose crystallite, where the passages are approximately 10 Å. Several empirical rate equations have been developed (15,16).

Enzymatic hydrolysis is a very localized phenomenon. This is confirmed by considering the experiments of Reese, Segal, and Tripp (17). They hydrolyzed cotton and mercerized cotton with acid and enzymes. For a 5% weight loss of the starting material the number average DP was found to drop from approximately 5000 to 150 during acid hydrolysis. For enzymatic hydrolysis a 30% weight loss of starting material resulted in a DP of 3500. It is also worth noting that in the case of acid hydrolysis the DP of 150 corresponds to a length of 700 Å, which is

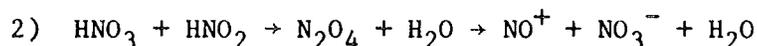
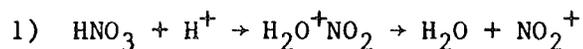
the approximate length of a crystallite.

#### 4.3 Reactions of Nitrogen Oxides with Lignin

Phenolic or benzene rings rapidly undergo reaction with electrophiles, even at low temperature. An electrophile is defined as any atom or molecule which seeks, and reacts with, electron-rich centers or functional groups. Chlorination and nitration are two of the most commonly studied electrophilic reactions of lignin. The discussion which follows is for the most part that of Dence (18).

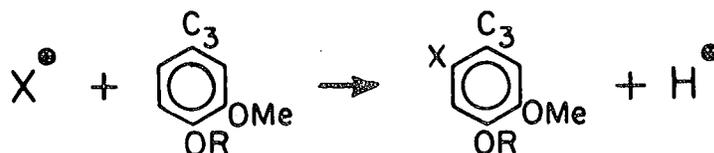
The major kinds of reactions which occur during the nitration of lignin are summarized in Figure 4.6 (18). Under severe reaction conditions lignin is degraded to water soluble fragments such as CO<sub>2</sub>, oxalic acid, and acetic acid.

Nitration rates are independent of the substrate concentration. The rate determining step is the formation of the electrophile. In the nitric oxide-oxygen system electrophiles are formed by many different mechanisms, as discussed by Lin (19), and will not be discussed here. However, in the case of nitric acid, electrophiles are produced mainly by the mechanisms:

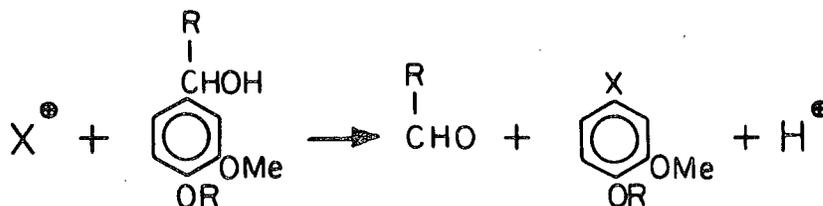


Due to the rapid rate of protonation of nitric acid and H<sub>2</sub>ONO<sub>2</sub><sup>+</sup> the rate of formation of the electrophiles is slow. In an environment of nitrogen oxides the rate of electrophile production is rapid. For this reason, nitrogen oxides more selectively attack lignin than

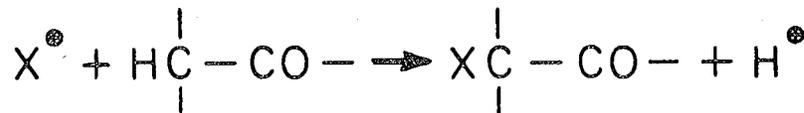
AROMATIC SUBSTITUTION:



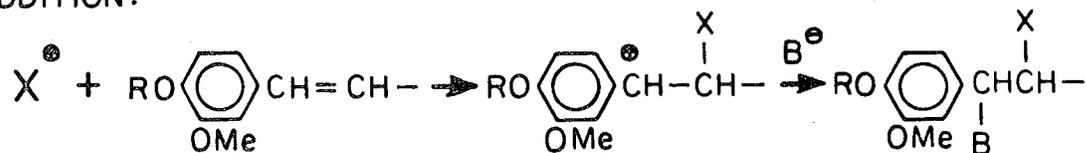
ELECTROPHILIC DISPLACEMENT:



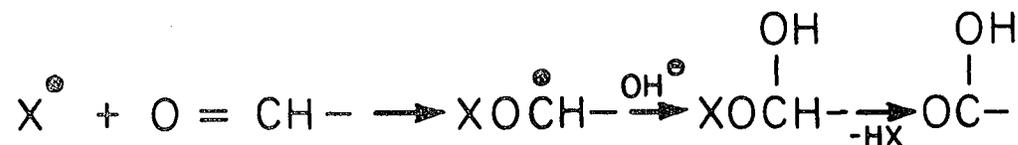
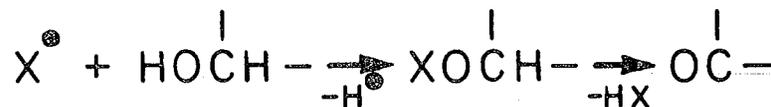
ALIPHATIC SUBSTITUTION:



ADDITION:



OXIDATION:



XBL 788-5690

Fig. 4.6 Summary of electrophilic reactions occurring during the nitration of lignin. X<sup>⊕</sup> denotes electrophile. R denotes alkyl substituent. (13)

nitric acid.

Nitrolignin is most commonly extracted from the parent material with glacial acetic acid or dilute alkali. For economic reasons the use of dilute alkali is preferred on an industrial scale, and, in combination with the use of elevated temperatures, has been found to be especially selective (20).

#### Chapter 4. References

1. D.A.J. Goring and T.E. Timell, Tappi, 45, 454 (1962).
2. T.E. Timell et al., Tappi, 40, 749 (1957).
3. A.J. Panshin and C. DeZeeuw, Textbook of Wood Technology, Vol. 1, 3rd Ed., p. 76, McGraw-Hill, New York, 1970.
4. S.A. Rydholm, Pulping Processes, p. 114, 596, 599, and 682, Interscience, New York, 1965.
5. B.F. Hrutfiord, Personal communication, College of For. Res., U. of Washington, Seattle, October 1974.

---

6. T.E. Timell, Adv. in Carb. Chem., 19, 247-302 (1964).

---

7. T.E. Timell, Adv. in Carb. Chem., 20, 410-483 (1965).
8. K.V. Sarkanen, in The Chemistry of Wood, B.L. Browning Ed., p. 239, Interscience, New York, 1963.
9. N.I. Nikitin, The Chemistry of Cellulose and Wood, p. 161, 555, and 556, Israeli Program for Scientific Translations Ltd., Jerusalem, 1966.
10. I.R. Parkinson, Tappi, 41, 661 (1958).
11. Meller, Holzforschung, 14, No. 3, 78-89 (1960).
12. D.W. Haas, B.F. Hrutfiord and K.V. Sarkanen, J. App. Poly. Sci.,

- 11, 587 (1967).
13. B.G. Ranby, J. Poly Sci., 53, 131 (1961).
  14. D.R. Whitaker, Arch. Biochem. Biophys., 53, 439 (1954).
  15. Y. Yamanaka, Ph.D. Thesis, U. of California, Dept. of Chem. Eng., Berkeley, December 1975.
  16. S. Wei, Master Thesis in Progress, U. of California, Dept. of Chem. Eng., Berkeley.
  17. E.T. Reese, L. Segal and V.W. Tripp, Textile Research J., 24, 345 (1954).
  18. C.W. Dence, in Lignins, Ed. by K.V. Sarkanen and C.H. Ludwig, p. 373, Interscience, New York, 1971.
  19. S.Y. Lin, Ph.D. thesis, U. of California, Dept. of Forestry and Conservation, Berkeley, 1976.
  20. D.L. Brink and S.Y. Lin (in press).

## 5. EXPERIMENTAL PROCEDURES

### 5.1 Overall Process Flow Scheme

The overall process flow scheme is illustrated by Figure 1.1, for enzymatic hydrolysis of lignocellulose employing a  $\text{NO}_x$  pretreatment step. The process consists of three distinct steps. The first and second steps constitute chemical pretreatments for the enzymatic hydrolysis of step 3.

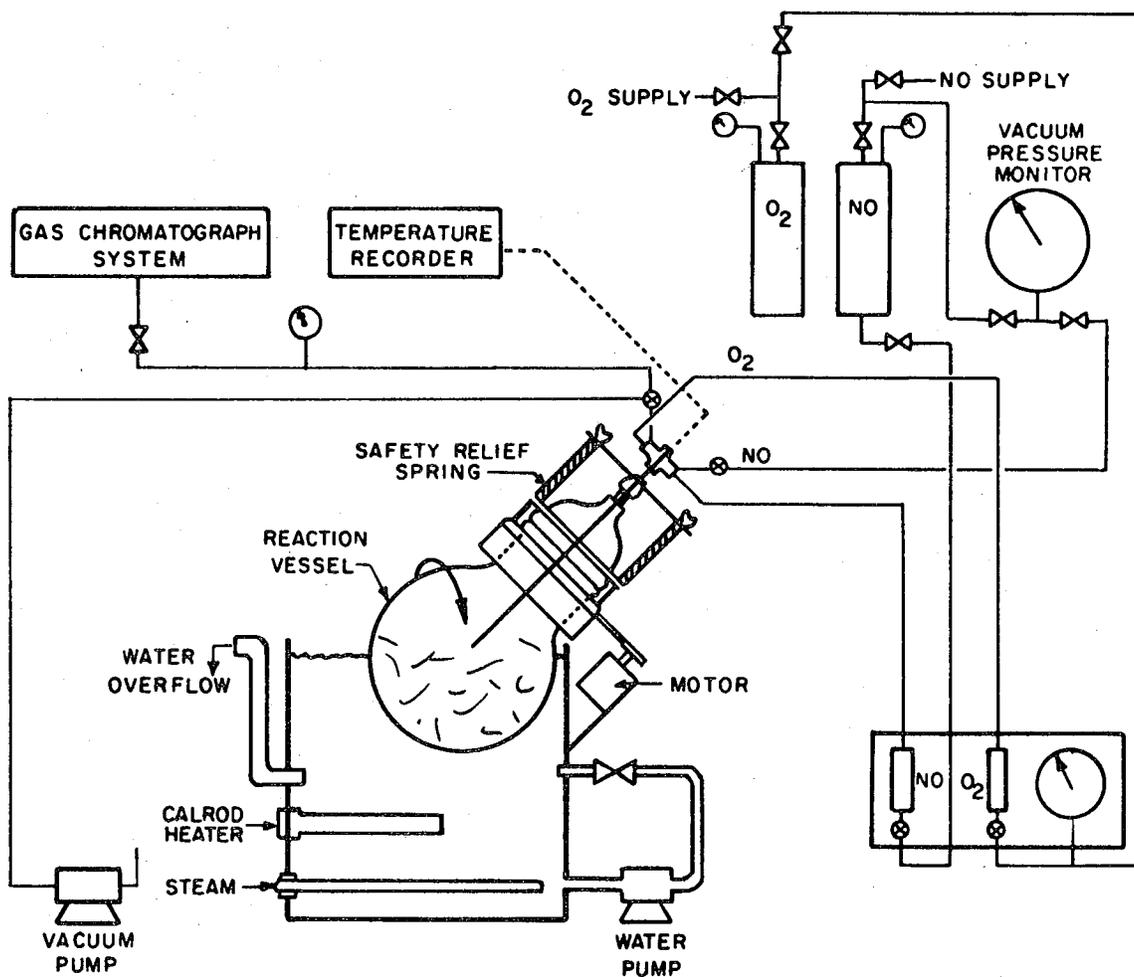
In the first pretreatment step wheat straw of approximately 10% moisture is reacted with nitric oxide and air at essentially atmospheric pressure. The second pretreatment step is an aqueous extraction of the solid material from the  $\text{NO}_x$  stage. The liquor recovered from this stage is rich in xylose. The residual solid after extraction is enzymatically hydrolyzed by T. viride cellulase of 3.6 Filter Paper Activity (FPA) at 45°C. These steps are described in detail below.

#### 5.1.1 Reaction of Wheat Straw with Nitric Oxide and Air

A 22 liter round bottom glass flask reactor system located at the University of California Forest Products Laboratory in Richmond was used for these gas-solid reactions. Figure 5.1 is a schematic diagram of this system. It was designed to be airtight when rotating.

The flask is partly submerged in a controlled temperature water bath. It is inclined at an angle of 45° from the vertical and rotates at 20 rpm. The head to the flask is connected to a stationary, non-rotating glass manifold. There are five headers on the manifold:

- 1) a thermocouple well
- 2) an air/oxygen inlet



XBL 789-5767

Fig. 5.1 Schematic diagram of gas-phase reaction system.(1)

- 3) a nitric oxide inlet
- 4) an outlet for reading reactor pressure and gas sampling
- 5) a spare

The gas holding tanks were used to deliver gas to the reactor.

Since the holding tank volumes are constant, the calculated amount of gas to be added to the reactor can be accomplished accurately by controlling the holding tank pressure differential.

A procedure similar to that described by Lin (1) was used for the gas phase reaction:

- 1) Between 50 and 400 grams of wheat straw were added to the reactor. The reactor was then sealed, evacuated, and checked for leaks.
- 2) Three successive purges with helium were performed, the final pressure being adjusted to approximately 720 mm Hg vacuum.
- 3) 5 grams of nitric oxide per 100 g of dry wheat straw were quantitatively added to the reactor.
- 4) Approximately 5 minutes were allowed to pass before starting air addition. The rate of addition was kept low in order to maintain a low temperature inside the reactor. Typically, this lasted for 10 to 20 minutes. In most cases 25.8 grams of air per 100 grams of dry straw were added. In one experiment oxygen was added rather than air. Final reactor pressure was 1 atm. or less, depending upon the amount of wheat charged to the flask.
- 5) The reaction was allowed to proceed for the specified reaction time. Periodically, gas samples were withdrawn and analyzed

on a gas chromatograph. This procedure is explained in 5.2.5.

- 6) At the end of the reaction period the gases were evacuated from the flask. This was followed by three successive purges with air. The gas treated straw was then removed from the flask, weighed, and extracted as described in the section which follows.

### 5.1.2 Aqueous Extraction Stage

After reacting with nitric oxide and air ( $\text{NO}_x$ ) the wheat straw was extracted in aqueous solution. This was done in one of two extraction systems: 1) a 3 liter round bottom flask equipped with a stirring rod and an overhead water condenser; and 2) a 2 liter Ehrlemeyer flask equipped with overhead water condenser which was stirred manually by swirling.

The extraction procedure consisted of the following steps:

- 1) A weighed amount of straw was placed in the extraction system.
- 2) A known volume of aqueous solution was added to the flask.

The amount of solution added was such that the ratio of solution weight to that of the weight of straw was between 7 and 15. In the majority of the experiments the aqueous solutions was simply water. However, some experiments using NaOH solutions were done.

- 3) The contents of the extraction system were brought to  $100^\circ\text{C}$  as rapidly as possible. This took about 15 minutes. The extraction was then carried out for the specified time, using the time at which the contents reached  $100^\circ\text{C}$  as the zero time.

In some experiments liquor samples for sugar determination were taken during the course of the extraction.

- 4) The extraction flask was then removed from the heating element, a cap was put on it, and it was cooled in an ice bath.
- 5) The contents of the flask were filtered on a Buchner funnel through Watman No. 1 filter paper. The filtrate was saved for determination of sugar content. The retained solid was washed with hot water until the underflow contained less than 1 g/l total reducing sugar by the DNS assay. Approximately 4 ml of wash water per ml of liquor was typically required.
- 6) The solid was removed from the funnel, air-dried and weighed. A moisture determination of this solid was then made in order to calculate the yield for the extraction step. The chemical composition of the solid was determined in many of the experiments by the procedure of 5.2.4. The residual solid was then enzymatically hydrolyzed as described below.

### 5.1.3 Enzymatic Hydrolysis

The extracted wheat straw was next enzymatically hydrolyzed by T. viride cellulase system of FPA 3.6 at 45°C and pH 5.0. The hydrolysis apparatus was a tall 600 ml glass beaker with a large rubber stopper sealing the mouth. Two holes were in the stopper. Through the hole located in the center of the stopper a shaft for the mixing impeller passed. This impeller rotated at approximately 150 rpm during the hydrolysis. The second hole was a sampling port capped with a small

rubber stopper.

In all experiments air-dried extracted wheat straw corresponding to about 12.5 grams dry was added to the beaker. This was followed by the addition of about 237.5 grams of cellulase solution which was buffered at pH 5.0 by 0.05 M acetate buffer. A 5 w% suspension resulted from this ratio of straw and cellulase solution.

The beaker was placed in a controlled temperature water bath of 45°C and the impeller motor was started. The hydrolysis was allowed to proceed for about 40 hours. Hydrolysate liquor samples taken during the reaction were analyzed for total reducing sugar by the DNS method.

At the end of the hydrolysis the contents of the beaker were filtered through a thin glass filter pad on a Buchner funnel. The filtrate was saved for chromatographic determination of sugar content. The retained solid was washed with hot water until the underflow contained less than 1 g/l total reducing sugar by the DNS assay. The solid was air-dried and weighed. A moisture determination of the solid was then made in order to calculate the solid yield for the hydrolysis step. The chemical composition of the solid was determined in many of the experiments.

## 5.2 Assay Procedures

### 5.2.1 DNS Reducing Sugar Assay

In some experiments a quick method for the determination of total reducing sugars is helpful. Such a method is particularly useful during the enzymatic hydrolysis step where the majority of the sugar produced is glucose. To this end, the total reducing sugar concentration

on a glucose basis was determined by the 3, 5 dinitrosalicylic acid (DNS) method (2). The assay is performed as follows:

- 1) Centrifuge the samples at 10,000 rpm for 10 minutes.
- 2) Dilute the supernatant to contain between 0.35-2.5 g/l total reducing sugar on a glucose basis.
- 3) To one milliliter of the diluted sample add 3.0 ml of DNS reagent.
- 4) Heat the sample for 5 minutes in a boiling water bath.
- 5) Remove the sample from the water bath and immediately place it in an ice bath to stop the reaction.
- 6) Add 20 ml of distilled water to the sample.
- 7) Read the absorbance at 600 nanometers with a 0.03 mm slit.
- 8) Convert the absorbance reading to concentration with a standard curve prepared for each batch of DNS reagent.

Other functional groups capable of being reduced interfere with the DNS assay. For this reason, the test was of no value on the extraction liquors which contain great quantities of oxidized organic materials from the  $\text{NO}_x$  reaction step.

#### 5.2.2 Filter Paper Activity

The activity of the cellulase enzyme system was determined by the Filter Paper Activity Assay (3). This assay measures the amount of reducing sugar generated by an enzyme solution acting on a 50 milligram sample of Whatman No. 1 filter paper after a 1 hour incubation at 50°C. The procedure was as follows:

- 1) To 50 milligrams of Whatman No. 1 filter paper (1.0 cm x 6.0 cm strip) were added 1.0 ml of enzyme solution and 1.0 ml of 0.05 M acetate buffer of pH 5.0.
- 2) The tube was incubated for 1 hour at 50°C in a heated water bath.
- 3) Reducing sugar concentration in the filtrate was measured by the DNS reducing sugar assay.
- 4) Enzyme activity was determined such that Filter Paper Activity units were equivalent to the milligrams of reducing sugar per ml of filtrate generated by the undiluted enzyme.

#### 5.2.3 Sugar Determination by Gas-Liquid Chromatography

Liquors were quantitatively analyzed for sugars by gas-liquid chromatography. Trimethylsilylether (TMS) derivatives of the sugars were prepared as follows:

- 1) 1 ml sample of the liquor in a screw cap vial was freeze-dried.
- 2) 1 ml of equilibrating solvent was added to the vial. The solvent consisted of 0.65 g/l myoinositol and 4.0 g/l  $\alpha$ -hydroxypyridine in dimethylsulfoxide. The sample was allowed to equilibrate in this solvent for at least 4 hours at 40°C.
- 3) Approximately 0.5 ml of silylating agent was added to the vial. This consisted of 2 volumes of hexamethyldisilazane and 1 volume of trimethylchlorosilane. The contents were then allowed to react for one hour at 22°C. The two phases in the vial were periodically mixed during the hour.

- 4) The lower phase was withdrawn from the vial and discarded. One ml of water was then added to the vial, which was then thoroughly mixed. The lower phase was again removed and discarded.
- 5) A generous portion of anhydrous sodium sulfate was added to the solution remaining in the vial. The sample was now ready for injection into the chromatograph.

A column which was found to give good separation of the sugars typically found in these analyses was a 1.5 ft. long, 3/32 in. i.d. column packed with a 3% loading of SE-30 on Chromosorb-G.

The column temperature was manually controlled to afford maximum separation of sugar peaks. The ratio of the area of each emerging peak to the area of the internal standard inositol peak was then determined. This ratio was multiplied by the corresponding sensitivity factor for each peak to give the sugar concentration in the liquor.

---

#### 5.2.4 Solid Assay

The determination of the chemical composition of solids was done by the procedures of Freitas, Wilke, Long, and Sciamanna (4). This method of analysis is an adaptation of the autoclave method of Moore and Johnson (5). A brief description of the procedure is given below and depicted in Figure 5.2.

The solid materials are first milled to 40-60 mesh. One portion of the solids is ignited at 600°C for 6 hours. The residual solids after ignition are defined as ash.

Another portion of the solid is extracted with a 2:1 v/v benzene/

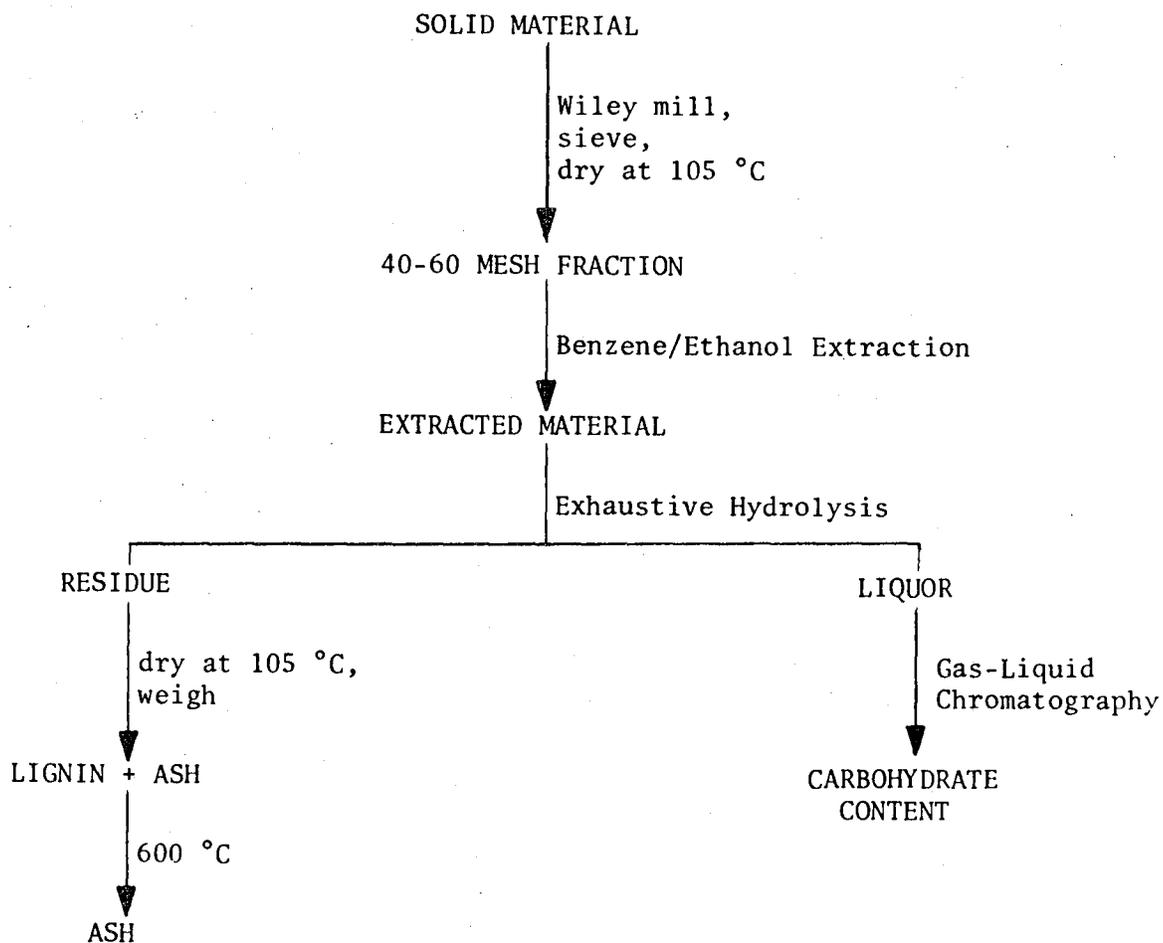


Fig. 5.2 Solid analysis procedure.

Sieve particles were arranged in series. The first column was operated at 195°C and the second column was at ambient temperature. Helium flow rate was 50 ml/min. This chromatograph was equipped with a constant volume sampling loop of 0.267 ml and two thermal conductivity detectors.

The polarity switch was used to change the polarity of the output signal from the gas chromatograph. This was necessary because O<sub>2</sub>, N<sub>2</sub>, NO, and CO were detected when eluting from the second column onto the reference side of the detector, whereas N<sub>2</sub>O and CO<sub>2</sub> eluted from the first column onto the sample side of the detector.

The NO<sub>2</sub> absorption flask was a 50 ml round bottom flask to which 10 ml of a solution containing glacial acetic acid and N-1-naphthyl-ethylenediamine dihydrochloride was added (7).

The acetone-dry ice bath functioned to prevent NO<sub>2</sub> and water vapor from entering the column.

The procedure was as follows:

- 1) The sample line and NO<sub>2</sub> absorption flask as shown in Figure 5.3 were evacuated and purged with helium three times. Vacuum reading and ambient temperature were then recorded.
- 2) A gas sample was let into the sampling system from the reaction vessel bypassing the absorption flask. Gas pressure reading and sampling port temperature were recorded. The sample injection valve was opened and the recorder was activated simultaneously.
- 3) After closing the sample injection valve, the stopcock

leading to the NO<sub>2</sub> absorption flask was opened, allowing a gas sample to enter the flask. A gas pressure reading was again recorded and the stopcock leading to the absorption flask closed.

- 4) The absorption flask was removed from the system with the stopcock closed. The flask was swirled occasionally for 15 minutes before absorption readings at 550 nm with a Leitz photometer.

The peak areas of each emerging gas were printed out on the digital integrator. These areas were then converted to concentration by the method developed by Lin (1). NO<sub>2</sub> absorbance was also converted to concentration by the method of Lin.

#### Chapter 5. References

1. S.Y. Lin, Ph.D., U. of California, Dept. of Forestry and Conservation, Berkeley, 1978.
2. J.B. Summer and G.E. Somers, Laboratory Experiments in Biological Chemistry, Academic Press, New York, 1944.
3. M. Mandels and J. Weber, "The Production of Cellulases," in Cellulases and Their Applications, Ed. by R.F. Gould, p. 391-414, Washington, D.C., American Chemical Society, 1969.
4. R.P. Freitas, B. Long, A. Sciamanna, and C.R. Wilke, "Procedures for Analysis of Solids and Liquors from Cellulosic Sources," LBL-5967, December 1977.
5. W.E. Moore and D.B. Johnson, "Procedures for the Chemical Analysis of Wood and Wood Products (as used at the U.S. Forest Products

Laboratory)," Forest Products Laboratory Forest Service, U.S.

Dept. of Agriculture.

6. R. Mauch, M.S. Thesis, U. of California, Dept. of Forestry and Conservation, Berkeley, 1966.
7. B.E. Saltzman et al., Health Lab. Sci., 6, No. 2, 106 (1969).

## 6. RESULTS AND DISCUSSION

The experimental apparatus, techniques, and assay procedures have been described in general in the previous chapter. In this chapter each experiment will be described, analyzed, and discussed.

In all of the experiments in this study 5 grams of nitric oxide and 6 grams of oxygen in the form of air were reacted with the straw in the first step, the gas phase reaction. The reason for restricting the study to this quantity of reactants has been discussed in Section 3.4. The effects of changing the gas phase reaction time and temperature were investigated, however.

The majority of the study was concerned with changing conditions of the second reaction step, the aqueous extraction stage. Temperature, extraction time, ratio of solids to liquor, pH, and effect of recycling the extraction liquor were all investigated.

The enzymatic hydrolysis was carried out at 5 w% consistency, pH 5.0, and temperature of 45°C with T. viride enzyme of filter paper activity (FPA) 3.6 in all experiments. This has previously been described in 5.1.

All of the sugar weights in this chapter, both in solution and solid, are listed in terms of hydrated monomeric units. For this reason, the shown composition of a solid may total more than 100% due to the addition of the water of hydration to the polymer weight.

### 6.1 Enzymatic Hydrolysis of Untreated Wheat Straw

In Experiment 1 the effect of the enzyme system on wheat straw which had not been pretreated was investigated. The purpose of doing

this experiment was to give a base case with which subsequent results could be compared. This experiment and the results are shown in Figure 6.1.

Low yields were obtained in this experiment. The yield of total available sugars was 17.0%. 20% of the available glucose was obtained and the xylose yield was only 8.9%. These results clearly showed that the enzyme system possessed a low activity toward the substrate. Using X-ray powder diagrams Hermans and Weidinger (1) estimated that purified native cellulose was between 69 and 71% crystalline. Assuming this value to be applicable to wheat straw it can be seen that not all of the amorphous regions of the cellulose had been hydrolyzed. This indicates that the lignin-cellulose matrix may be of sufficient order, even in parts of the amorphous region, to prevent the enzyme from diffusing to the reaction site.

The activity toward the xylan was especially low. Owing to the fact that the hemicelluloses are amorphous, the low activity can be interpreted to mean that the activity of the enzyme toward readily accessible xylan is low or the xylan is inaccessible. For this reason, a high yield of xylose is not expected during enzymatic hydrolysis.

## 6.2 General Studies of the Extraction Stage

It was necessary to obtain basic data and trends concerning the extraction stage. This section examines the effect of pH during extraction and also whether the extraction stage is actually needed.

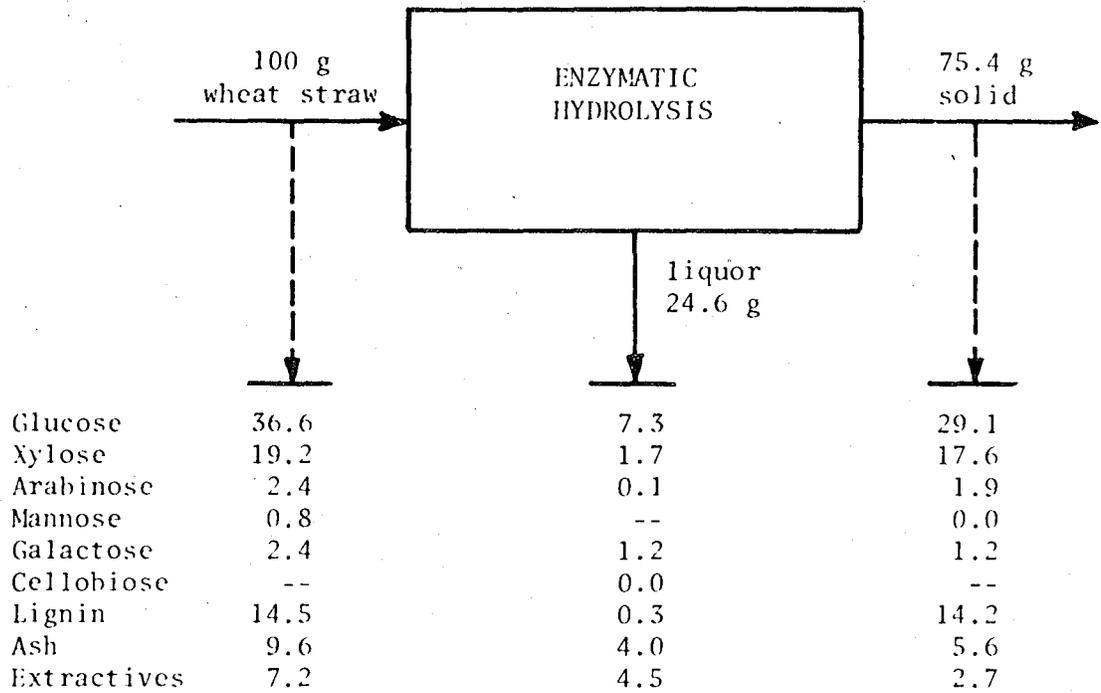


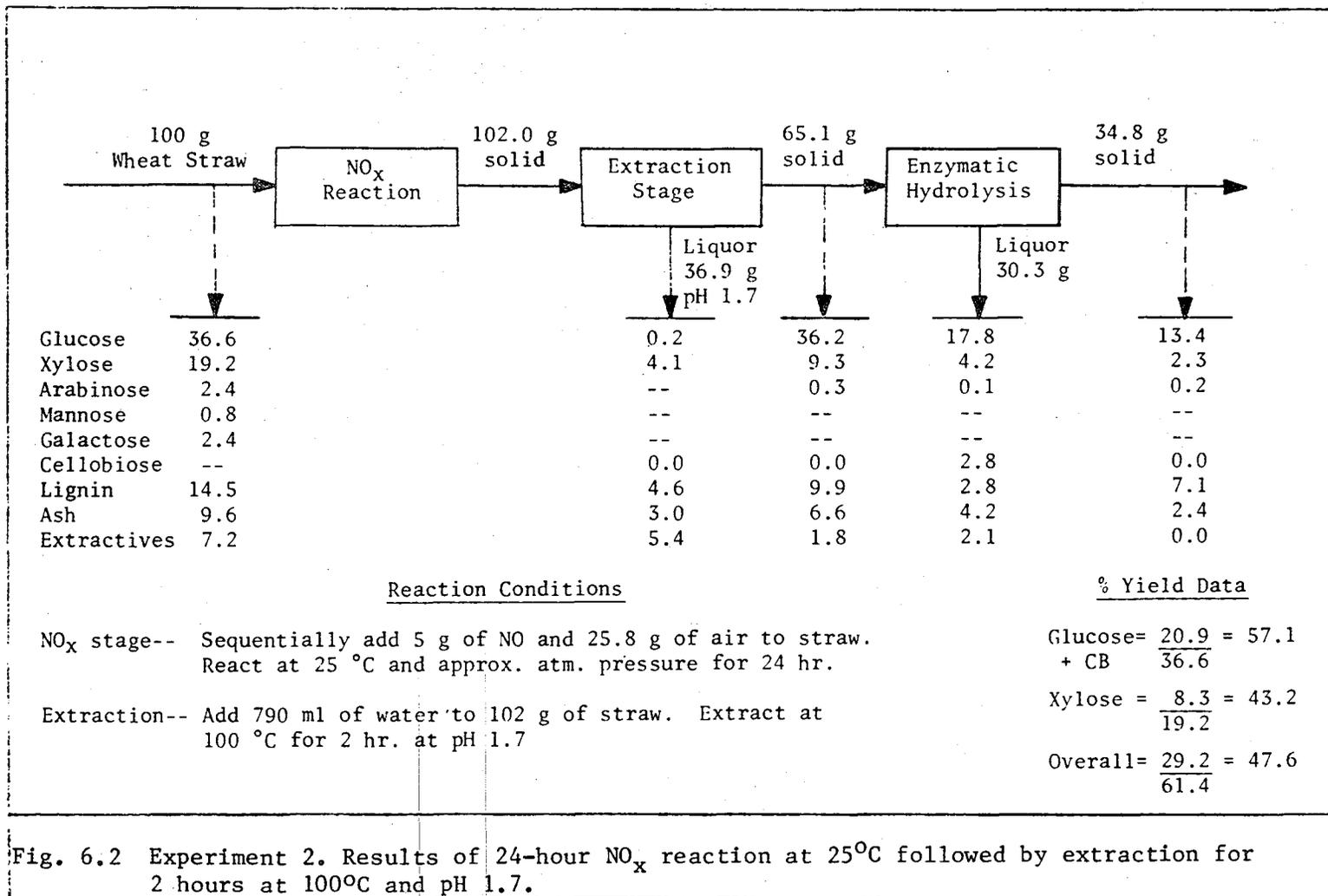
Fig. 6.1 Experiment 1. Enzymatic hydrolysis of untreated wheat straw.

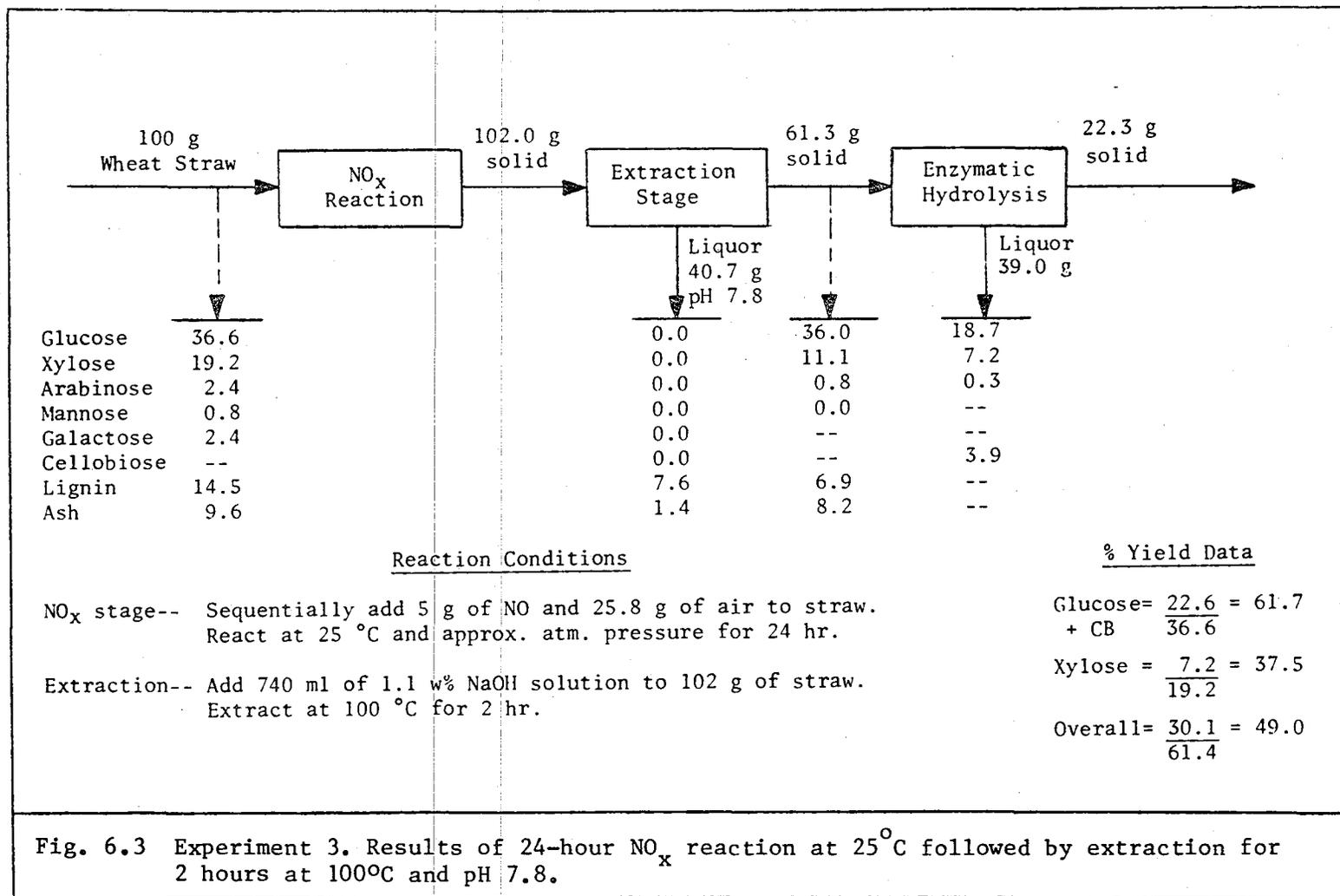
### 6.2.1 Effect of pH

In Experiments 2-6 the effect of varying the pH in the extraction stage was investigated. Experiments 2, 3, and 4, which are described in Figures 6.2, 6.3, and 6.4, give results obtained by enzymatic hydrolysis of wheat straw which was reacted with  $\text{NO}_x$  for 24 hours followed by extraction at pH 1.7, 7.8, and 12.1, respectively. By comparing these figures it can be seen that increasing the alkalinity of the extraction liquor results in solubilizing more of the straw. This effect appears to be particularly strong at alkaline pH values.

The lignin, ash, extractives, and hemicelluloses are removed in appreciable quantities at all 3 pH values. The fraction of each of these components dissolved increases with increasing pH. This is illustrated by Figure 6.5. Very little of the glucan in the wheat straw is removed from the solid during the extraction for all pH values. Essentially all of the glucan remains intact after two hours at pH 1.7 and 7.8. Even at pH 12.1 86% of the glucan units are remaining in the straw after a one hour extraction period.

The minimum in the xylan curve of Figure 6.5 at approximately pH 7 can be explained by considering the two major modes of dissolution. At acidic pH values the xylan is hydrolyzed, resulting in a shortening of the xylan chain, thereby increasing its solubility and also in the formation of free xylose. Under neutral conditions the rate of acid hydrolysis is very slow and the solubility of the undegraded xylan is not as great as in an alkaline solution. However, the xylan shows marked solubility under alkaline conditions, which is typical of the hemicelluloses in wheat straw (2).





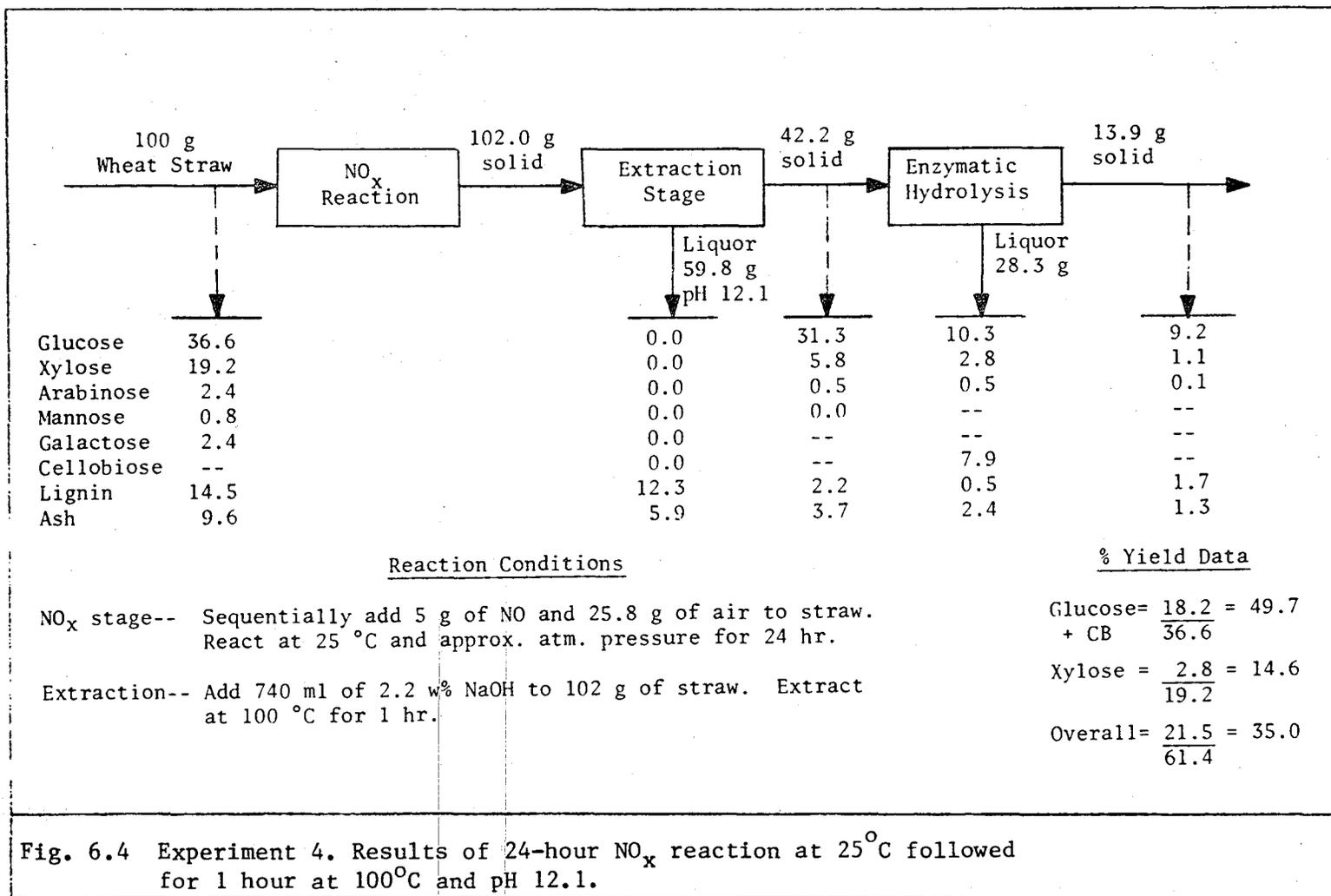
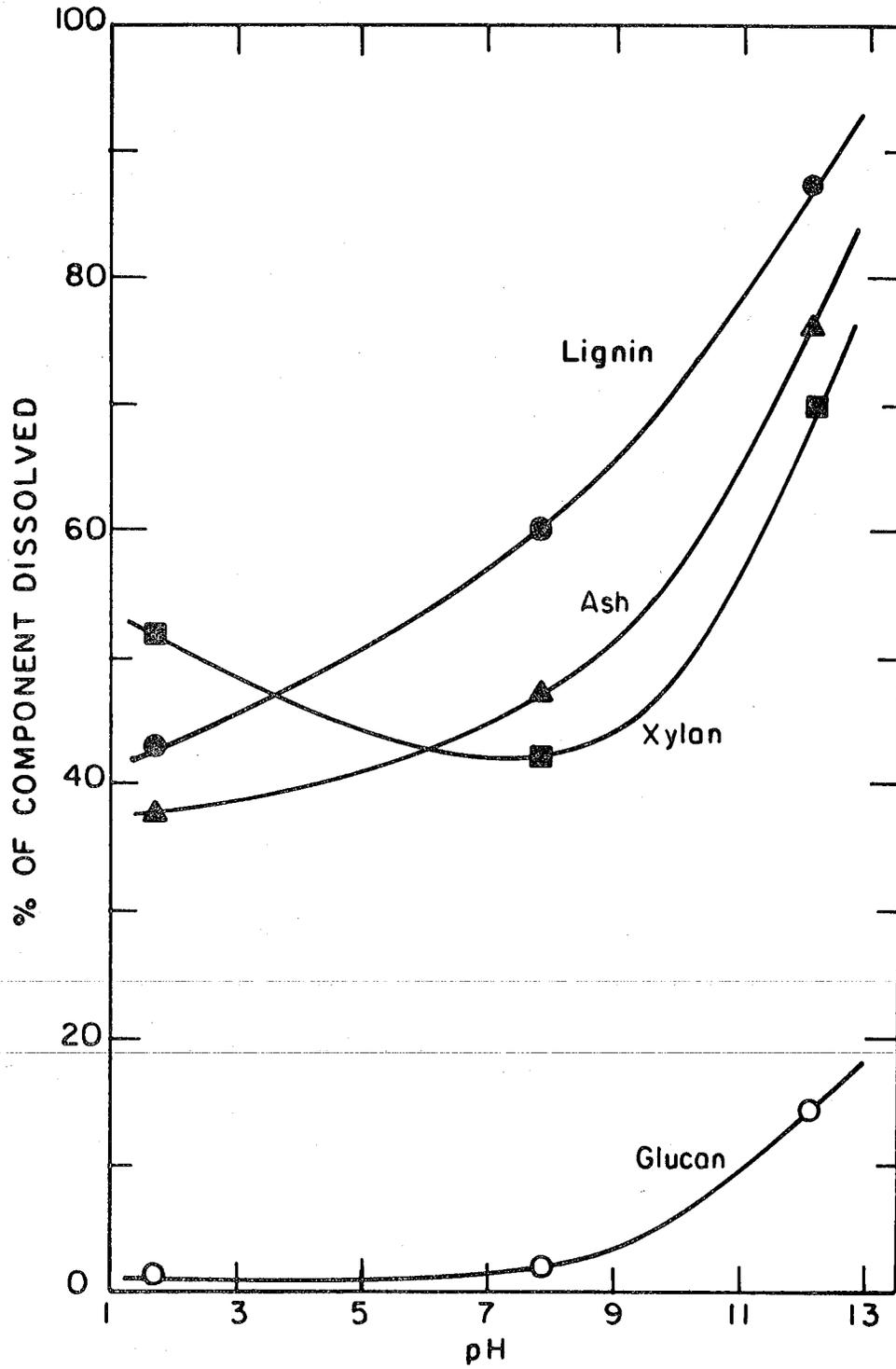


Fig. 6.4 Experiment 4. Results of 24-hour NO<sub>x</sub> reaction at 25°C followed for 1 hour at 100°C and pH 12.1.



XBL 788-5682

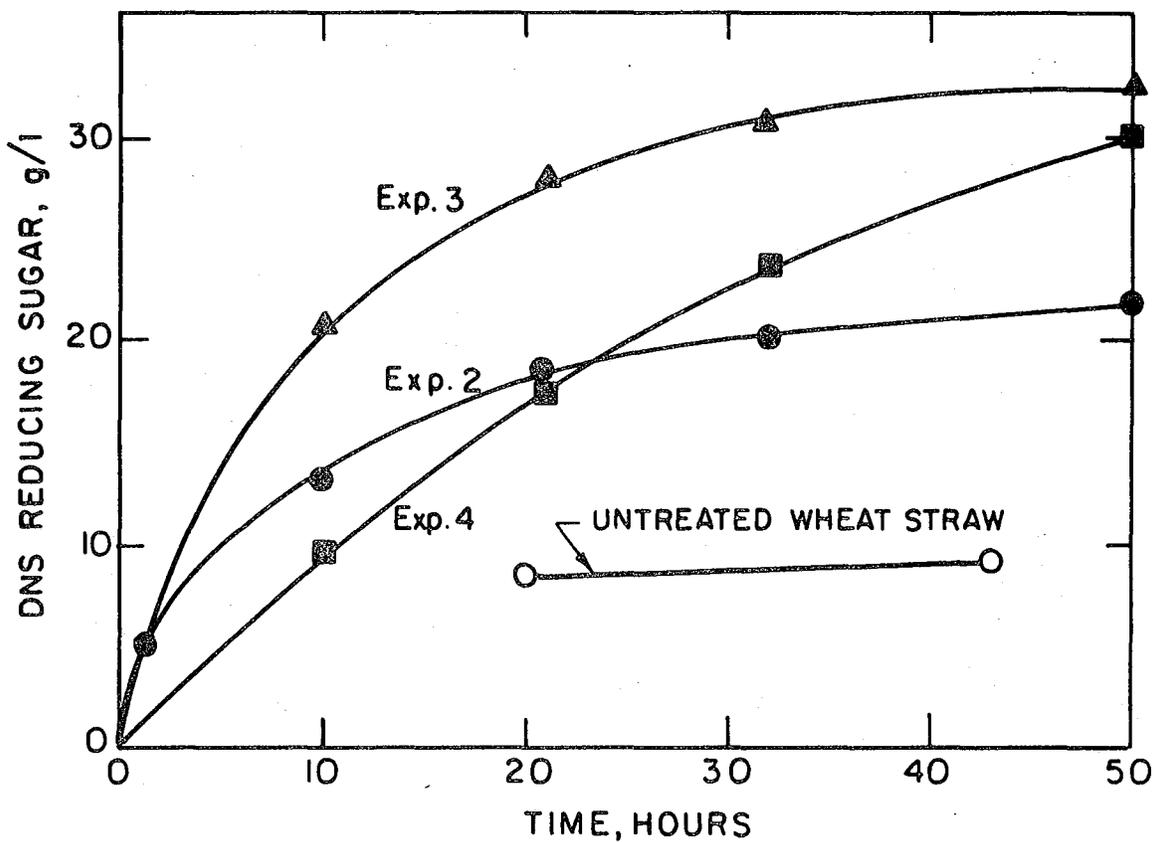
Fig. 6.5 Percentage of components dissolved in extraction liquor at various pH values.

The fact that free xylose in solution does not appear for Experiments 3 and 4, in which the extraction liquor pH values were 7.8 and 12.1, respectively, is expected. Since the extraction liquor in each case was alkaline, the only reactions occurring to a large extent were the peeling and stopping reactions discussed in 4.2.2. The xylan monomer is converted to a saccharinic acid upon peeling and other degradation products in alkaline solution. Also, the amount of peeling of the xylan occurring would not be expected to be large. This is because the temperature is below that of which peeling is extensive.

The responses of the extracted solids of Experiments 2, 3, and 4 during enzymatic hydrolysis were vastly different (Figure 6.6). Based on the total sugars feed to hydrolysis the conversions for this step were 55%, 63%, and 58.5%, respectively. Overall glucose yields, based on the entering straw, were 57.1, 61.7, and 49.0, respectively.

The low yield during enzymatic hydrolysis of the solid extracted at pH 12.1 (Experiment 4) is difficult to explain in light of the low ash and lignin content. However, an examination of the total reducing sugars by the DNS assay indicates that the hydrolysis of the solid from Experiment 4 may still be occurring at a substantial rate. This is shown in Figure 6.6. It is also worth noting that the treated solid of Experiment 3 is rapidly hydrolyzed in comparison to the other two.

Since the highest overall yield of sugar was obtained at the intermediate pH of 7.8, a more thorough investigation of possible pretreatments was done at relatively neutral conditions. In



XBL788-5684

Fig. 6.6 Hydrolysis curves for Experiments 1 to 4. Exp. 1 is enzymatic hydrolysis of untreated wheat straw. Exp. 2, 3, and 4 the  $\text{NO}_x$  24-hour reacted straw was extracted at  $100^\circ\text{C}$  for 2 hours at pH of 1.7, 7.8, and 12.1 respectively.

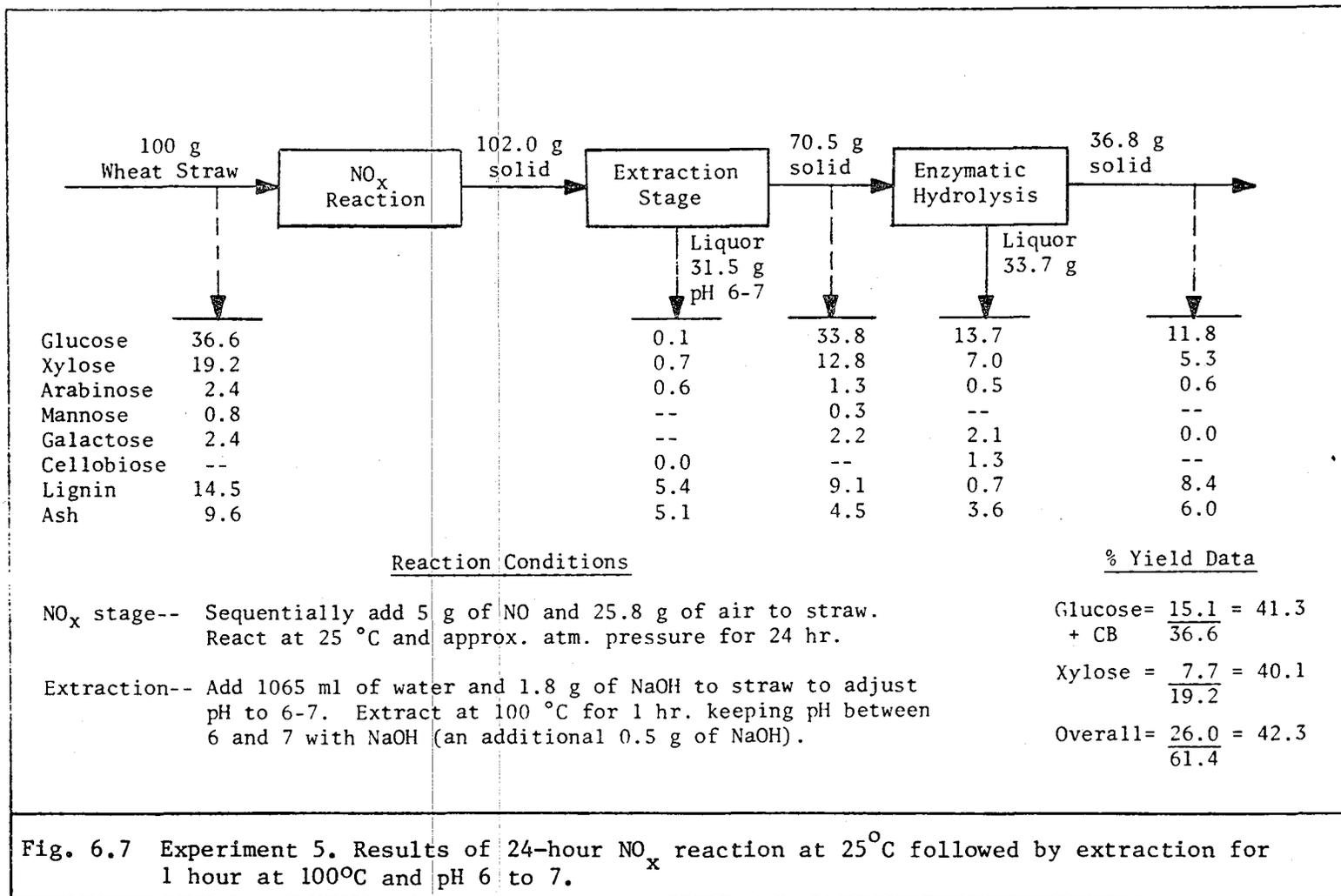
Experiment 5 the pH of the extraction liquor was maintained between 6 and 7 at 100°C (Figure 6.7) The overall glucose yield of 41.3% is well below the yields obtained in Experiments 2-4. This possibly can be attributed to the fact that the extraction time was one hour in Experiment 5, compared to 2 hours in the previous experiments.

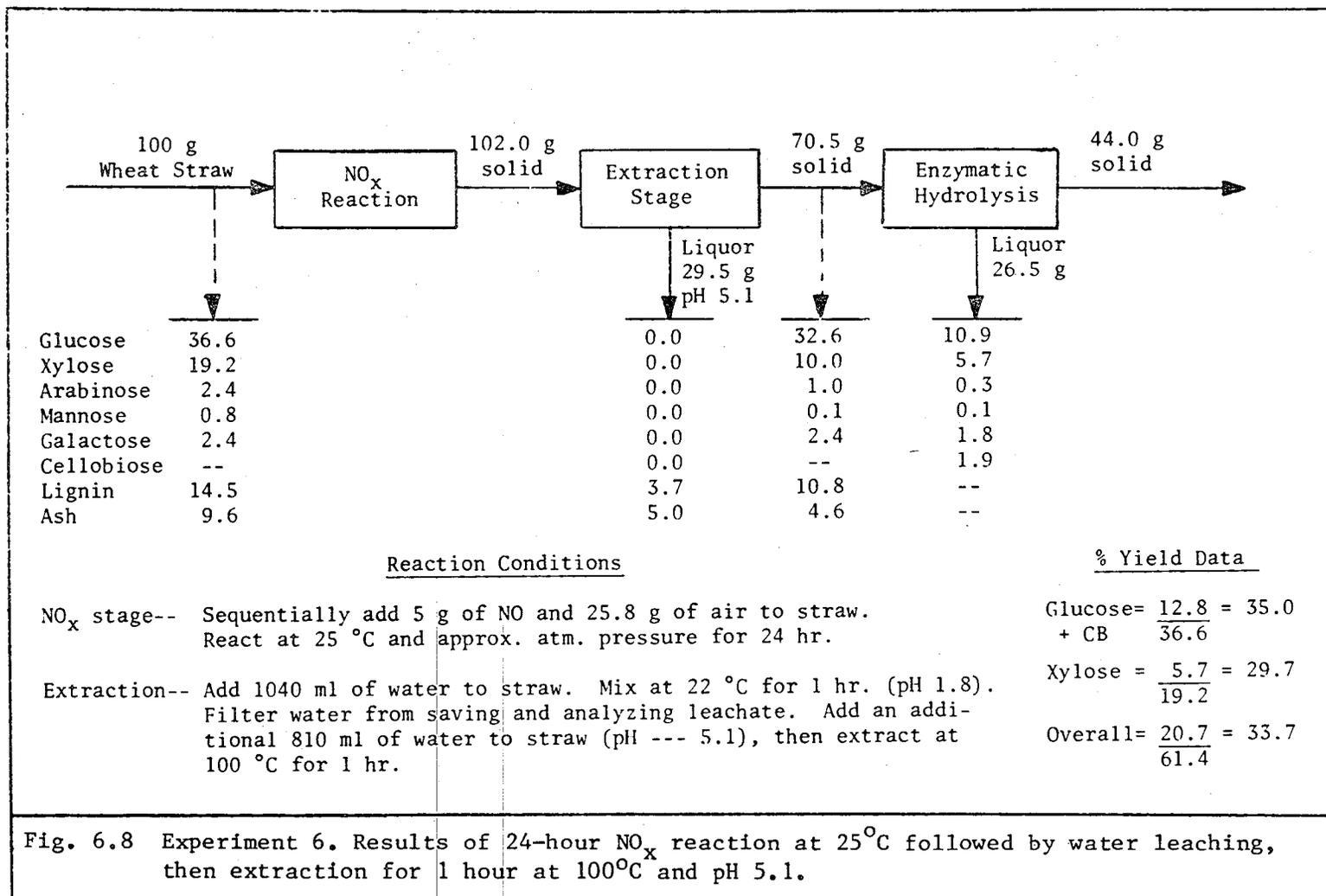
The effect of a leaching step, with respect to overall sugar yields, was examined. In Experiment 6 a water and straw mixture was agitated one hour at 22°C. The resulting liquor of pH 1.7 was then filtered from the straw and an equal volume of fresh water added to the straw. This caused the pH to rise to 5.3. The mixture was then extracted at 100°C for one hour. The results are given in Figure 6.8. The overall glucose yield was 35.0% and that of xylose was 29.7%.

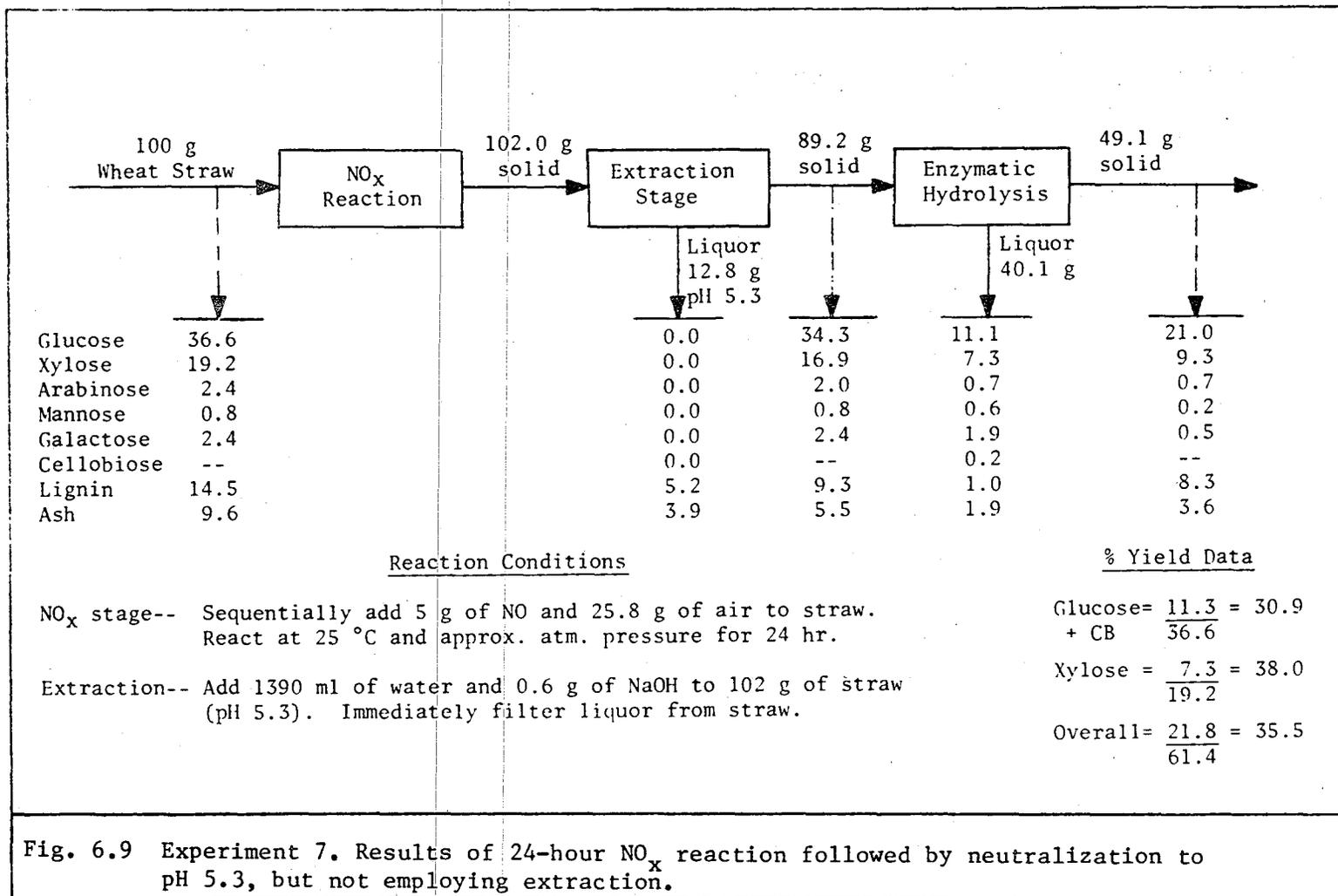
#### 6.2.2 Neutralization Versus Extraction

If high glucose yields could be obtained without employing an extraction stage, pretreatment costs would be greatly decreased. In order to determine this, an experiment was conducted in which the  $\text{NO}_x^-$  reacted straw was simply neutralized at room temperature prior to enzymatic hydrolysis. Although the neutralization was done in an aqueous suspension, it was envisioned that the neutralizing agent could possibly be a gas, such as ammonia. The pretreatment would be entirely gaseous. The results, shown in Figure 6.9, clearly demonstrate that an extraction stage is needed. Glucose yield was only 30.9%.

On the basis of Experiments 2-7 it was decided to focus this study on the extraction using water only, with no caustic additions.







Three reasons for this are: 1) the use of caustic does not increase the yield significantly enough to offset its cost; 2) by increasing the extraction time more xylose can be recovered by acid hydrolysis; and 3) xylose and glucose are separated at an early stage in the process.

### 6.3 The Use of Oxygen Versus Air in the Gas Phase Reaction

An experiment using pure oxygen rather than oxygen in the form of air was done to see if significantly different results were obtained. A priori, there is no reason the results would be expected to differ greatly.

The gas phase reaction procedure in this experiment, labeled No. 8, was the same as in the previous experiments only oxygen instead of air was used and the extraction was for 5 rather than 2 hours. The results are shown in Figure 6.10. For comparison a two hour extraction as done in Experiment 2 was also done here. Inspection of the two experiments shows the results obtained to be in close agreement. Because air and oxygen appear to give nearly the same results it was decided to restrict this study to the use of air. The cost of using air is much less than using oxygen.

### 6.4 Studies of Xylose During the Extraction Stage

Since xylose is the major product of the extraction stage, a knowledge of its formation and decomposition is necessary.

#### 6.4.1 Rate of Formation

The rate of formation of xylose in acidic solution has been

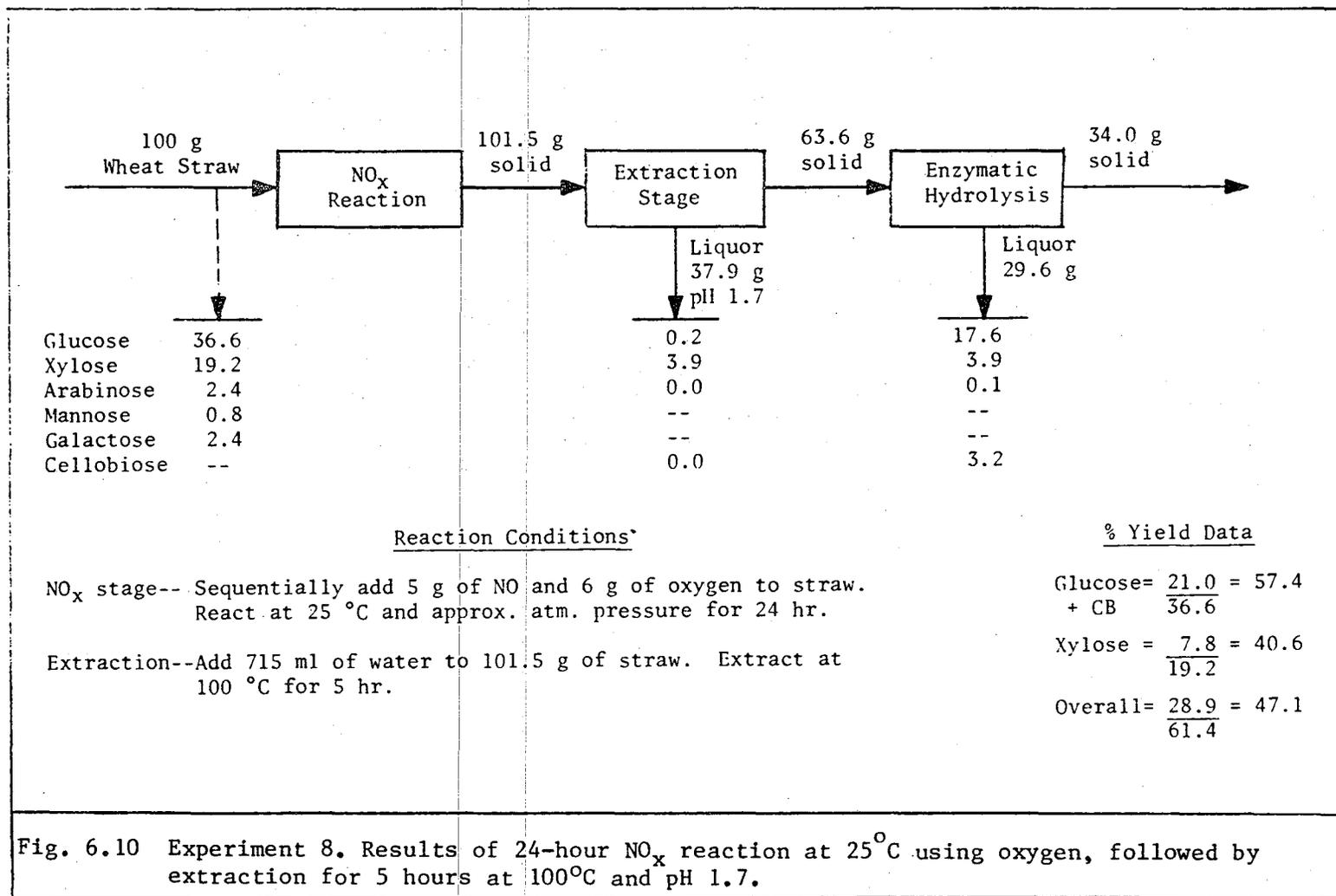


Fig. 6.10 Experiment 8. Results of 24-hour NO<sub>x</sub> reaction at 25°C using oxygen, followed by extraction for 5 hours at 100°C and pH 1.7.

found to depend on both the hydrogen ion concentration and the initial xylan concentration of the solution by Saeman (3). To a good approximation the rate of hydrolysis of a soluble polysaccharide at a constant pH can be represented by the equation:

$$\frac{dX}{dt} = k_1 (a-X) \quad (6.1)$$

which upon integration gives:

$$\ln \frac{(a-X)}{(a)} = -k_1 t \quad (6.2)$$

where:

a = the initial equivalent amount of sugar present in the polysaccharide

t = the time of hydrolysis

X = the amount of sugar formed after time t

$k_1$  = the rate constant of hydrolysis of the polysaccharide

In order to investigate the rate of formation of xylose during the extraction stage a series of experiments were conducted over a range of  $\text{NO}_x$  contact times and solid-liquid ratios in the extraction step. In all cases the extraction step was done at 100°C.

The straw of Experiment 9 had been reacted with  $\text{NO}_x$  for 24 hours. For the extraction, a weight ratio of 7.4 grams of water to 1 gram of straw were mixed resulting in a pH of 1.7.

In Experiment 10 the straw used had also been reacted with  $\text{NO}_x$  for 24 hours. However, the weight ratio of water and straw was 15.3 to 1, which gave a pH of 1.9.

Wheat straw which had been treated for two hours was used in

Experiment 11. The weight ratio of water to straw was 7.9 to 1 resulting in a pH of 1.55.

The xylose concentrations of the liquors determined at various reaction times are listed in Table 6.1. These results were plotted as  $\ln(a-X)$  versus  $t$  to give nearly straight lines of slope  $-k_1$ . This is shown in Figure 6.11. Some of these lines have been shifted vertically so that they fit on the same graph.

The values of  $k_1$  determined for Experiments 9, 10, and 11 are 0.099, 0.057, and 0.124  $\text{hr.}^{-1}$  respectively. Much of the disparity between these values can be accounted for by considering the effect of pH. Several investigators have determined that the rate of hydrolysis is nearly proportional to acid concentration for dilute acids. With the addition of a term for acid concentration Equations (6.1) and (6.2) become:

$$\frac{dX}{dt} = k_1' (a-X)[H^+] \quad (6.3)$$

and:

$$\ln \frac{(a-X)}{(a)} = -k_1' [H^+]t = -k_1 t \quad (6.4)$$

where:

$$[H^+] = \text{hydrogen ion concentration}$$

The values of  $k_1'$  calculated from Equation (6.4) are 4.96, 4.53, and 4.40 (liter/gmole)( $\text{hr.}^{-1}$ ) respectively. The near constancy of  $k_1'$  is in agreement with the assumption that the hydrolysis rate is proportional to the hydrogen ion concentration.

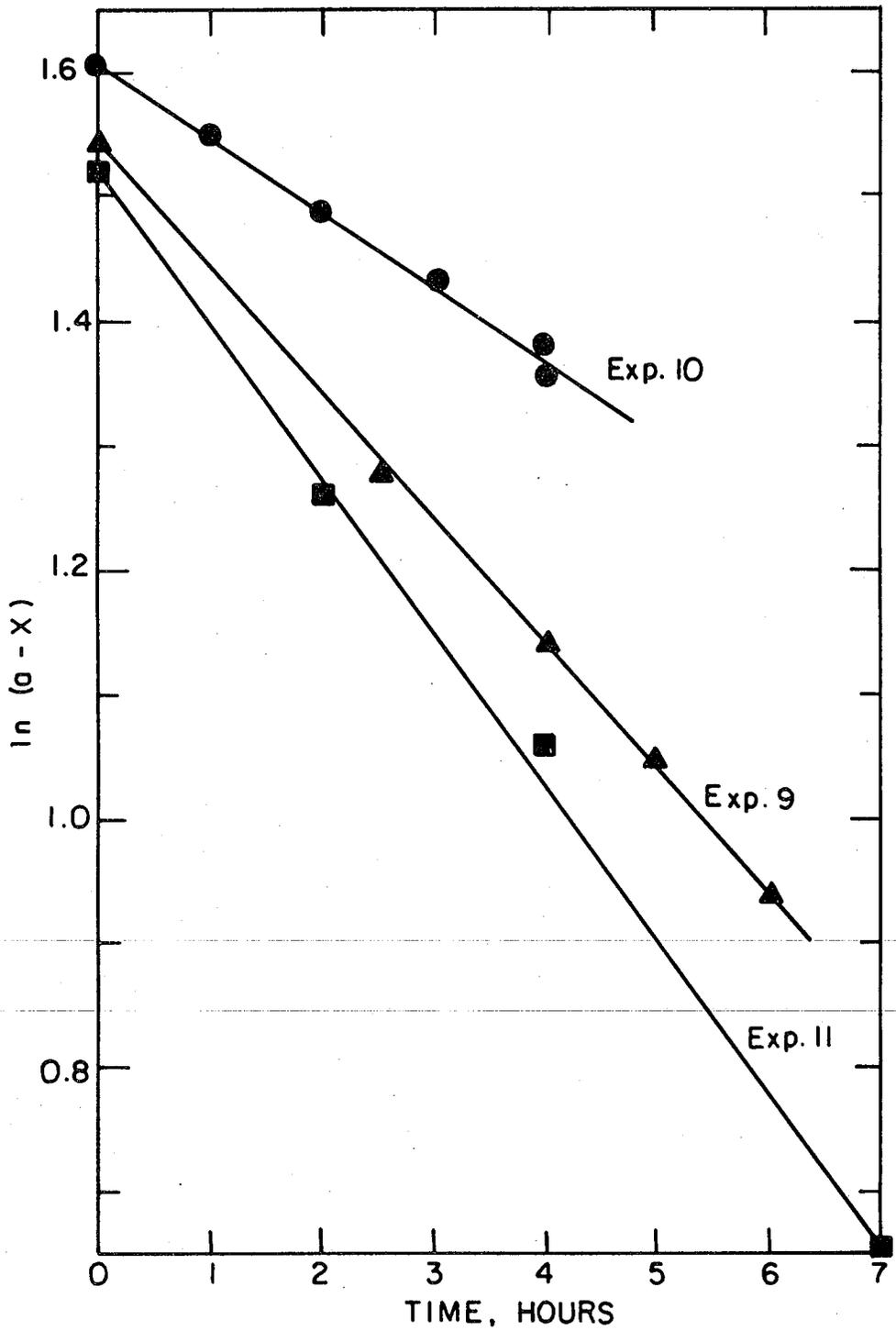
#### 6.4.2 Xylose Stability Experiment

The rate of decomposition of xylose in aqueous acidified solution

TABLE 6.1

## DATA FOR XYLOSE FORMATION KINETICS EXPERIMENTS

Exp. No.	$\bar{a}$ initial xylose as xylan, g	liquor volume, ml	pH	time, hours	xylose conc., g/l	xylose in solution, g	$\frac{X}{a-X}$ total xylose - initial xylose, g	$\ln(a-X)$
9	4.68	184	1.7	0	0.0	0.0	0.0	1.540
				2.5	6.0	1.10	1.10	1.275
				4	8.5	1.56	1.56	1.138
				5	10.0	1.84	1.84	1.044
				6	11.6	2.13	2.13	0.936
10	13.54	1082	1.9	0	0.0	0.0	0.0	2.606
				1	0.72	0.80	0.80	2.545
				2	1.42	1.54	1.54	2.485
				3	2.03	2.20	2.20	2.428
				4	2.55	2.76	2.76	2.377
					2.78	3.00	3.00	2.354
11	7.57	310	1.55	0	0.0	0.0	0.0	2.020
				2	5.58	1.73	1.73	1.765
				4	9.19	2.85	2.85	1.552
				7	14.18	4.40	4.40	1.155
15	4.00	163	1.6	0	13.02	2.12	0.0	1.386
			1.5	1	28.02	4.57	2.45	0.438
			1.5	3	33.49	5.41	3.34	-0.416
			1.5	5	36.26	5.91	3.79	-1.561



XBL 7 88-5683

Fig. 6.11 Xylose formation kinetic experiments in extraction liquor at 100°C.

to furfural, formic acid, and humic substances has been found to follow first-order kinetics by several investigators. Also, the effect of catalyst acid concentration on the rate of decomposition of xylose is nearly directly proportional to hydrogen ion concentration above 0.1 N (3). Therefore, the rate of decomposition can be written as:

$$\frac{-dC_x}{dt} = k_2' C_x [H^+] \quad (6.5)$$

where

$C_x$  = xylose concentration

$k_2'$  = rate constant for decomposition of xylose

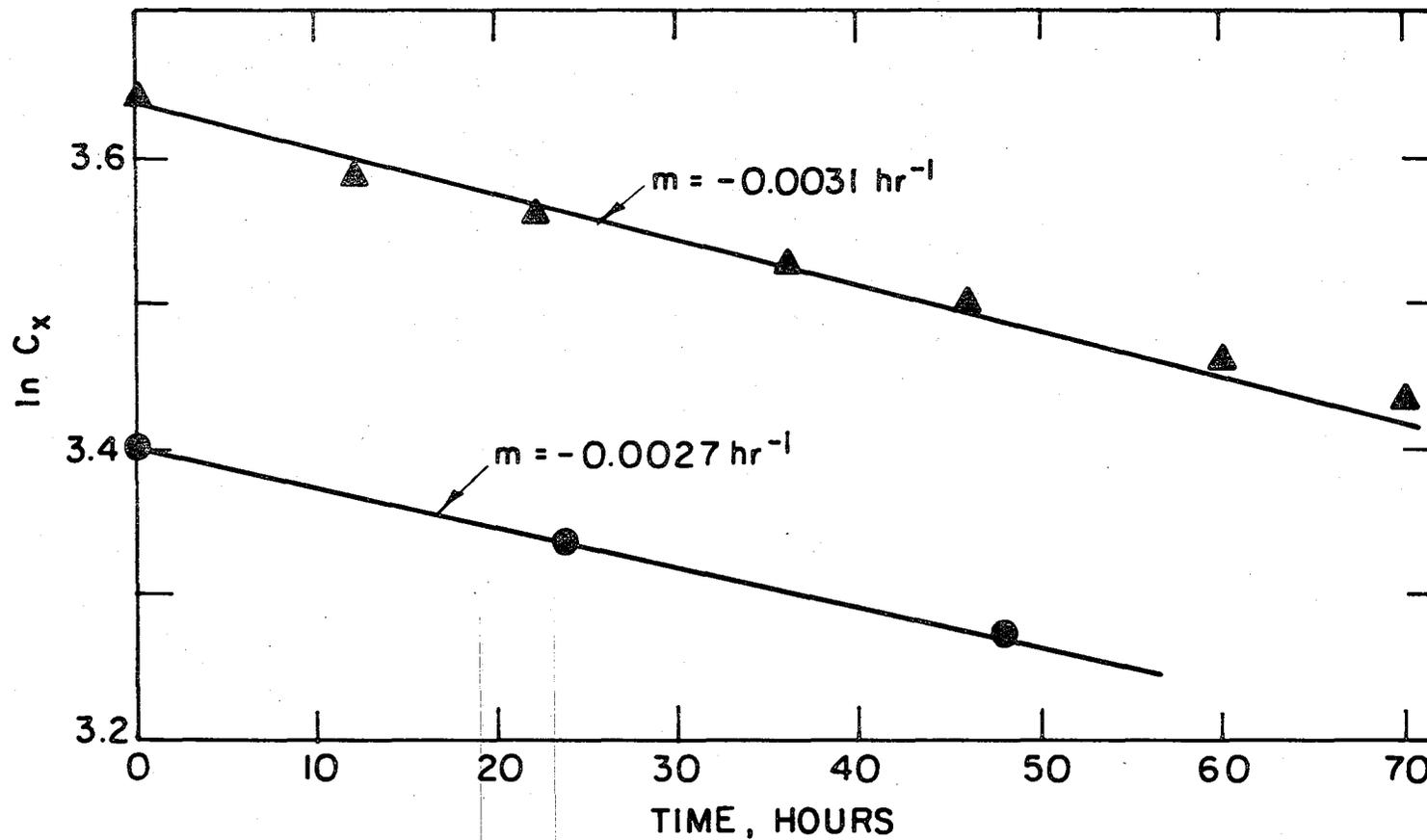
In order to investigate the stability of xylose, extraction liquor from Experiment 15 was put in a tightly capped vial and submerged in a 100°C boiling water bath. Liquor samples were taken periodically, neutralized to pH 7.0, and analyzed for xylose concentration by gas-liquid chromatography. The results are listed in Table 6.2.

Assuming first-order kinetics, the results were plotted as  $\ln C_x$  versus time. Figure 6.12 shows that the results do follow the expected first-order kinetics. From this figure, the rate constant  $k_2$  was determined to be 0.0031 hr.<sup>-1</sup>

The above result compares favorably with the data of Freitas (4), which are also plotted in Figure 6.12. From these data a  $k_2$  value of 0.0028 hr.<sup>-1</sup> is obtained. In the work of Freitas, 99% pure Grade II Sigma Company xylose was boiled in a sulfuric acid solution of pH 0.75 at 100°C. Empirical equations developed by Smuk (5) and Root, Saeman, and Harris (6) predict  $k_2$  values of 0.0022 and 0.0019 hr.<sup>-1</sup>, respectively, at pH 0.75 and 100°C.

Table 6.2 Xylose Stability Experiment Results

time, hours	pH	xylose conc., g/l	ln. xylose conc.	fraction of xylose remaining
0	1.5	38.1	3.640	1.000
12	1.5	36.05	3.585	0.946
22	1.5	35.2	3.561	0.924
36	1.55	33.9	3.523	0.890
46	1.55	33.0	3.496	0.866
60	1.6	31.8	3.459	0.835
70	1.6	30.85	3.429	0.810



-71-

XBL 788-5681

Fig. 6.12 Stability of xylose in acidic extraction liquor.  
 ▲ this work, ● data of Freitas (4).

### 6.4.3 Conclusions of the Xylose Experiments

From the rate constants of xylose formation ( $k_1$ ) and decomposition ( $k_2$ ) the maximum obtainable yield of xylose can be calculated. Being consecutive first-order reactions this maximum is given by:

$$X_{\max} = a \left( \frac{k_1}{k_2} \right)^{\frac{k_2}{k_2 - k_1}} \quad (6.6)$$

and occurs at time:

$$t = \frac{\ln(k_2/k_1)}{k_2 - k_1} \quad (6.7)$$

Applying the  $k_1$  value from Experiment 9 and the  $k_2$  value derived above gives  $X_{\max} = 0.895a$  at a reactor space time of 36 hours.

These constants can also be used to calculate the xylose yield for any reaction time by using:

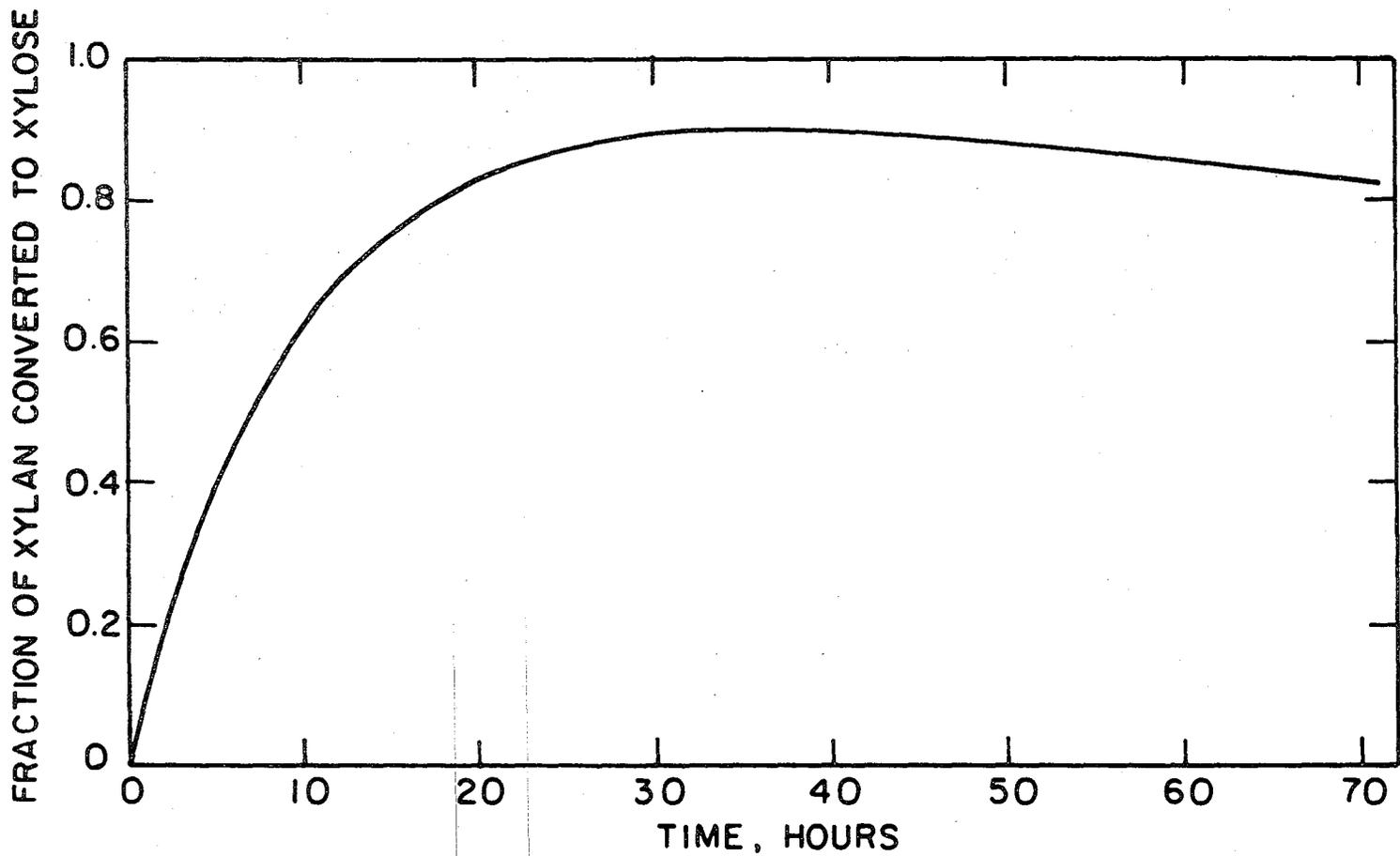
$$X = \frac{ak_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad (6.8)$$

Equation (6.8) is plotted in Figure 6.13 assuming  $k_1 = 0.1 \text{ hr}^{-1}$  and  $k_2 = 0.0031 \text{ hr}^{-1}$

### 6.5 The Effect of Varying Reaction Time of $\text{NO}_x$ Reaction Time at 25°C

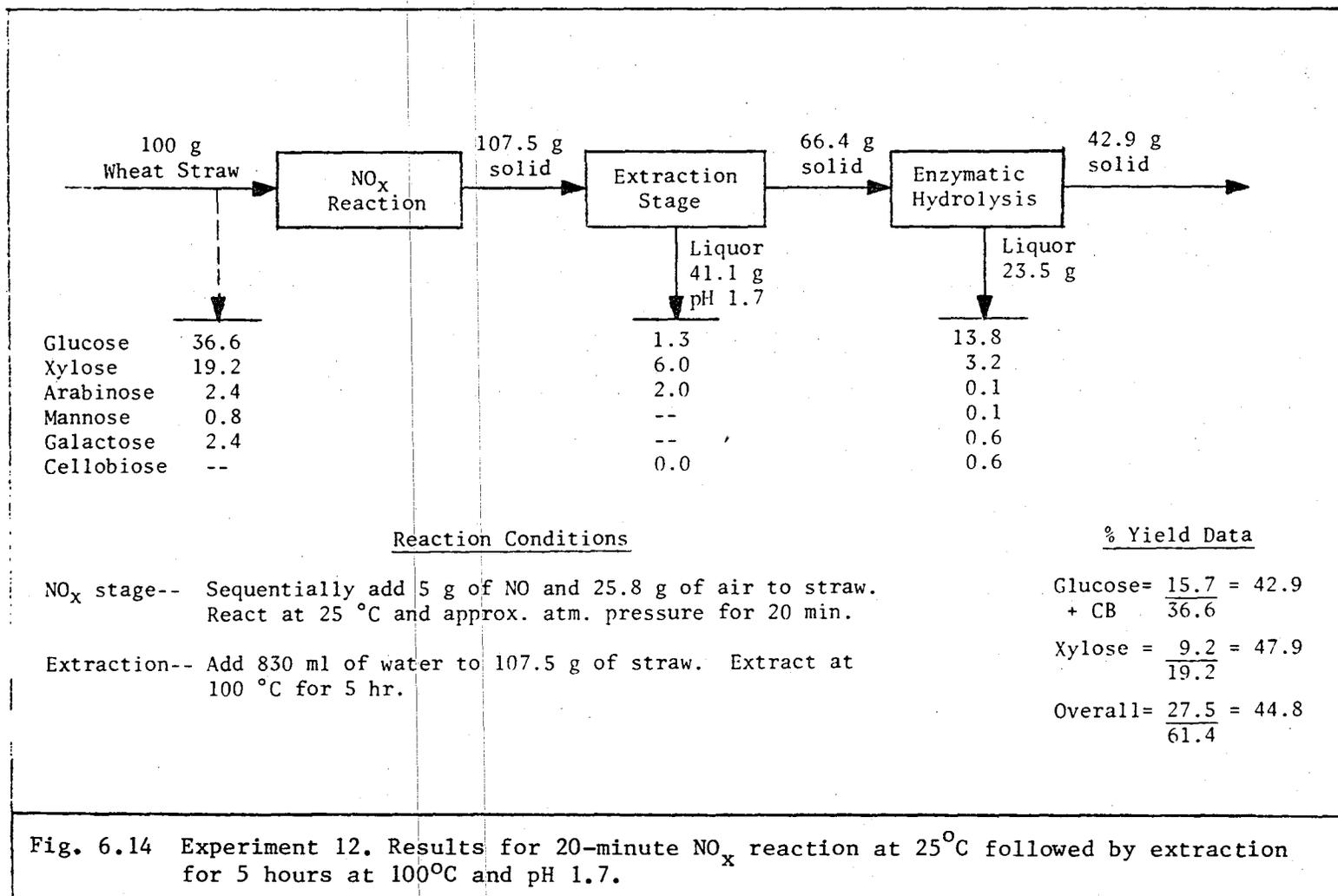
In order to minimize the reactor space time an investigation of the effect of gas phase reaction time at 25°C on the sugar yields was done. Experiments 12, 13, and 14 shown in Figures 6.14, 6.15, and 6.16 are for reaction times of 20 minutes, 1 hour, and 2 hours.

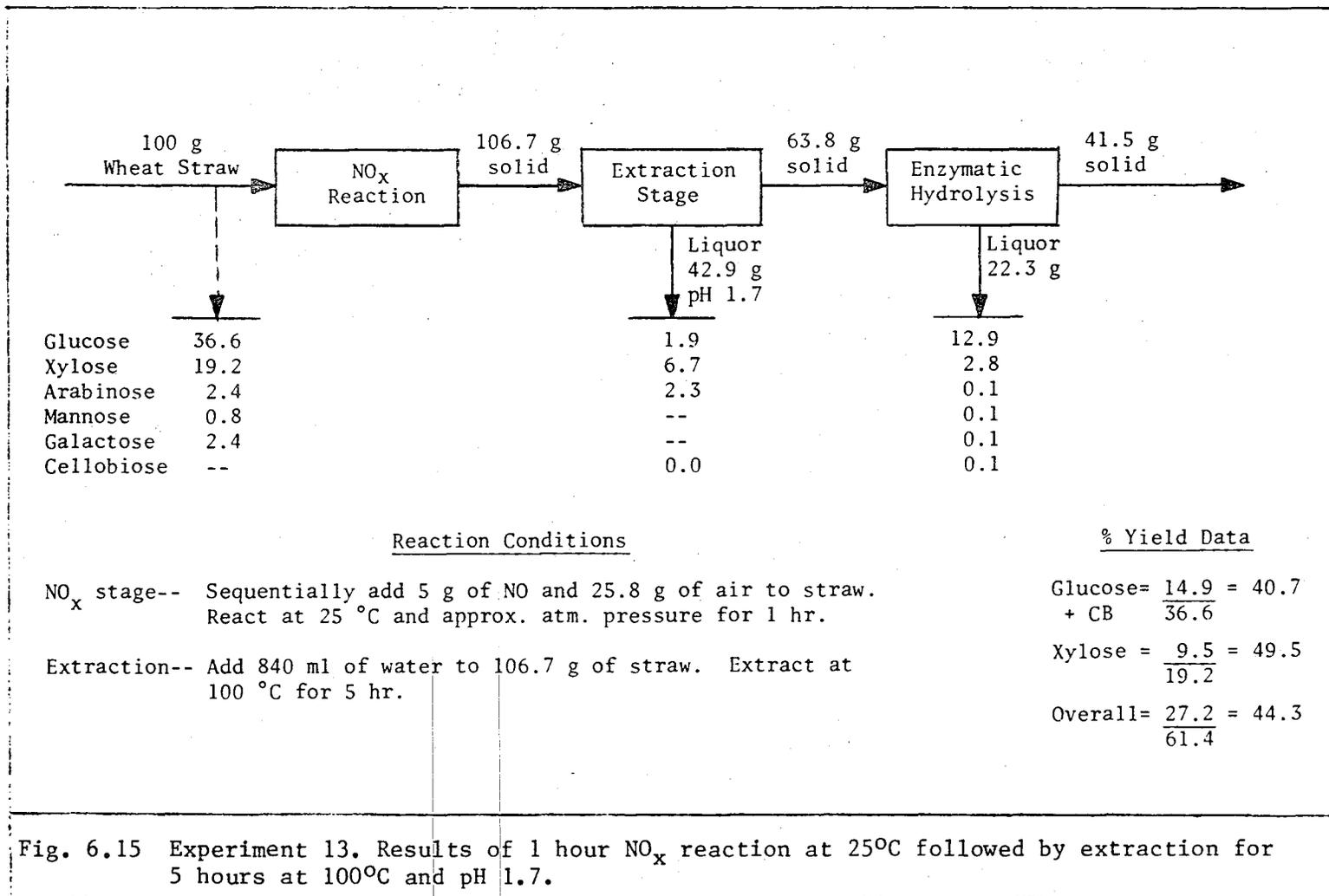
The two hour reaction appears to give significantly better results than the experiments of shorter duration. Comparing the results of

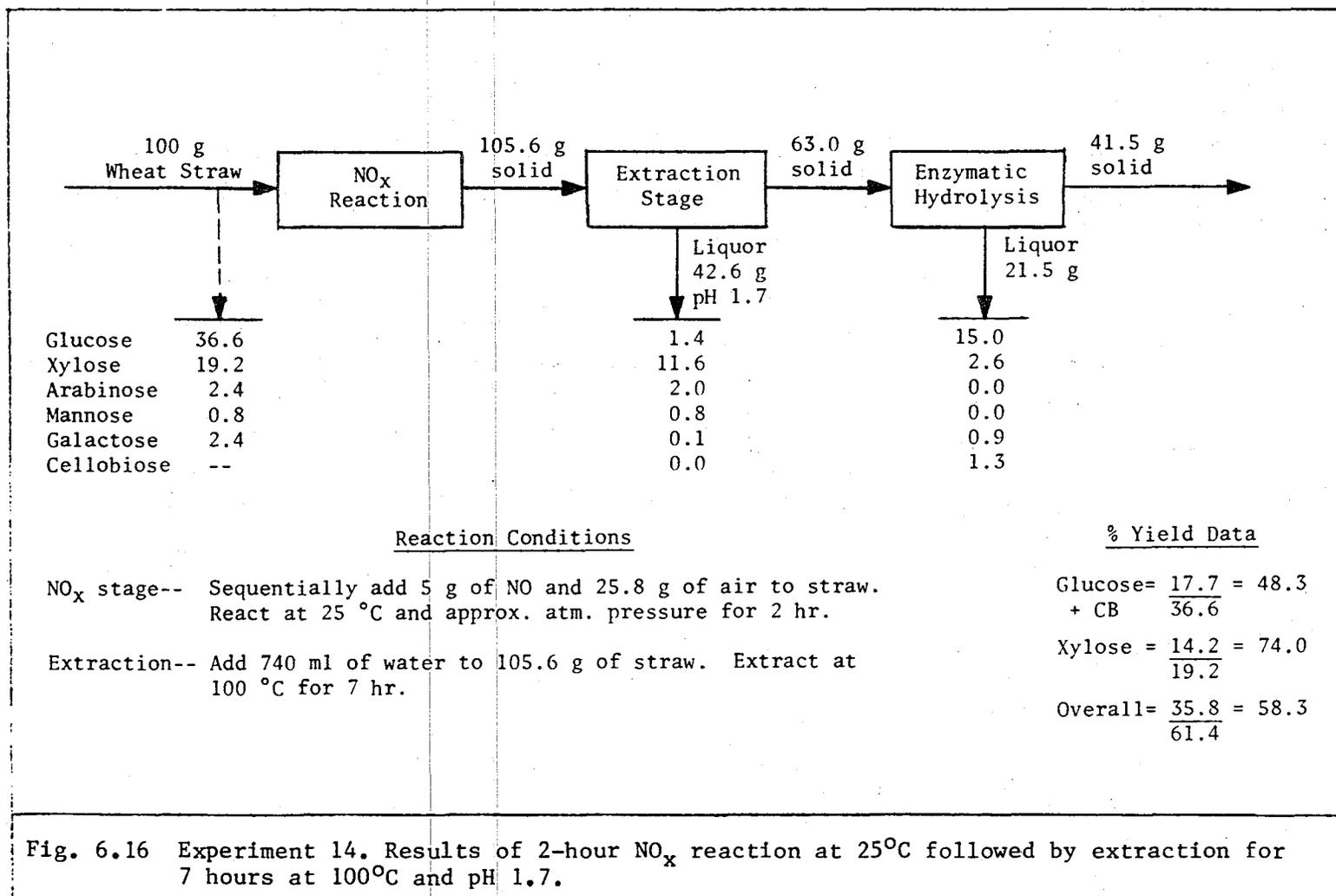


XBL 788-5685

Fig. 6.13 Predicted xylose yields during extraction assuming  $k_1 = 0.1 \text{ hr}^{-1}$  and  $k_2 = 0.0031 \text{ hr}^{-1}$ .







these experiments it can be seen that substantially more xylose is formed during the extraction stage for the two hour NO<sub>x</sub> reacted straw. This may be to some degree attributable to the 7 hour extraction time used in Experiment 14 versus 5 hours used in Experiments 12 and 13.

In the above experiments, especially No. 14, for the two hour NO<sub>x</sub> contact, the relatively high total sugar yields are due to the large amount of xylose recovered. However, glucose yields are actually lower than in Experiment 2, where a 24 hour gas phase treatment was used. Two factors which may be responsible for the latter are: 1) the enzymatic hydrolysis of glucan is dependent upon the gas phase reaction time beyond 2 hours; and 2) the amount of acidic hydrolysis occurring during the extraction may, after a certain point, retard enzymatic hydrolysis. The results of Experiments 15 and 16 on extraction liquor recycle discussed in the next section indicate that both of these factors are important.

#### 6.6 The Effect of Recycling the Extraction Liquor

By recycling the extraction liquor considerable process economic gains can be made -- provided that the recycling does not adversely affect the overall sugar yields. A priori, the primary advantages of reusing the extraction liquor are: 1) higher concentrations of xylose in the liquor improve the economics of xylose conversion to other products, such as furfural, 2) more dissolved solids in a given volume of liquor reduces the problem of disposal of undesirable compounds, and 3) the expected lowering of pH expected by recycling increases

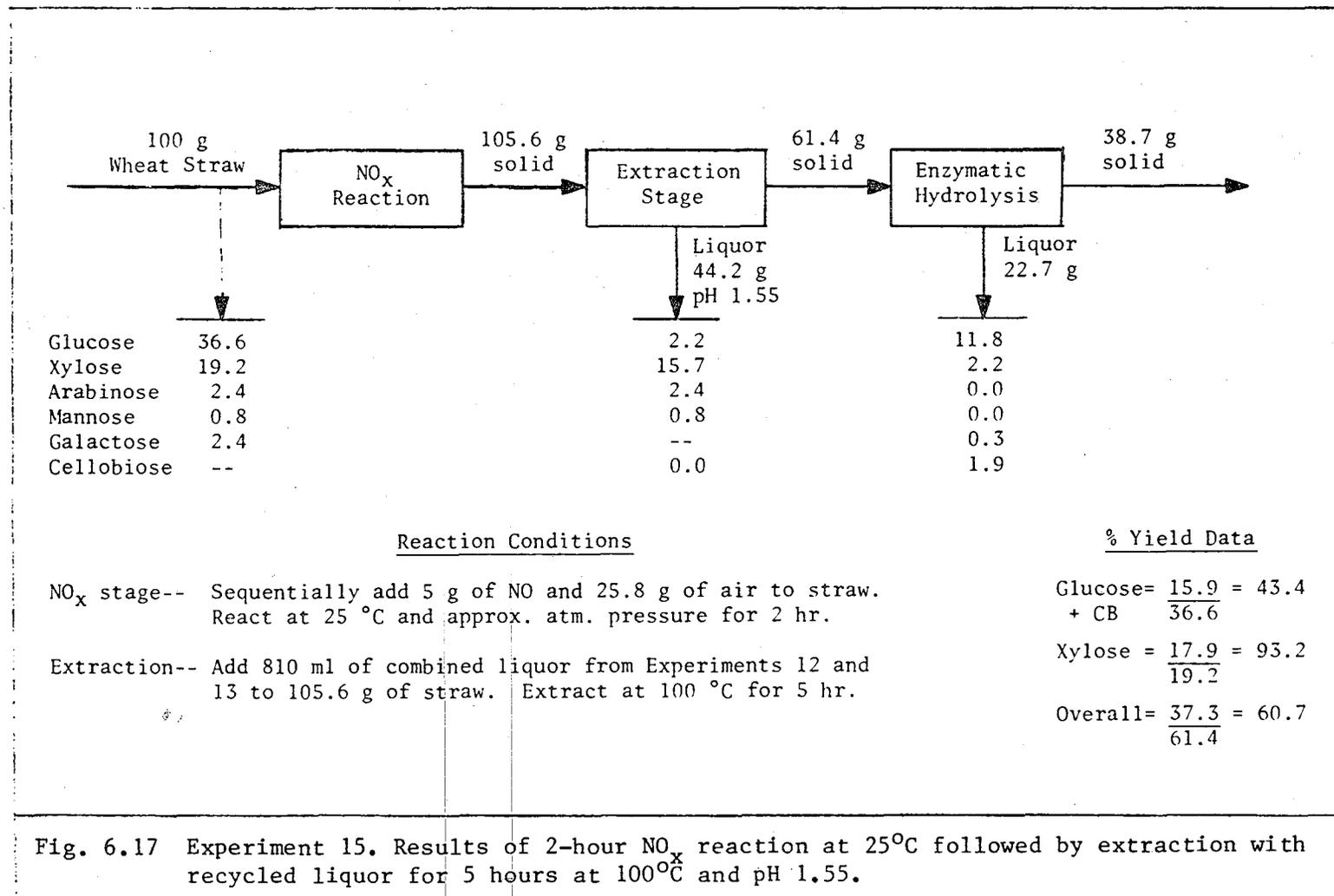
the rate of hydrolysis of the xylan.

Two batch experiments were conducted examining the effect of recycle. These are labeled Experiments 15 and 16 and are shown in Figures 6.17 and 6.18.

Experiment 15 involved using the combined liquors of Experiments 12 and 13 as a solvent. This solvent was mixed with the straw that had been reacted for two hours with  $\text{NO}_x$ . The overall sugar yield is quite high at 61%. This experiment is to be compared with Experiment 14, Figure 6.16 where a yield of 57% was obtained with no recycle. Whether or not the 4% increase in sugar yield is real is not clear, however, because a large portion of the increase could have come from polymeric xylan previously dissolved in the recycle liquor during Experiments 12 and 13.

The rate of xylose formation was monitored in this experiment. The results are listed in Table 6.1. As in Section 6.4.1 these results were plotted as  $\ln(a-X)$  versus  $t$ . In calculating  $\ln(a-X)$  credit was not taken for xylose present in the liquor before the extraction started. In other words, the previously formed xylose was subtracted out of solution. In this way, hopefully only xylose derived from the straw of Experiment 15 was counted.

From Figure 6.19 the rate constant for the rate of formation of xylose,  $k_1$ , was determined to be  $0.645 \text{ hr.}^{-1}$ . This corresponds to a  $k_1'$  value (defined in Equation (6.4)) of  $20.4 \text{ (liter/gmole)(hr}^{-1}\text{)}$ , about 4 times greater than that calculated in the previous xylose experiments of Section 6.4.1. The reason for the higher value of  $k_1'$  is unknown, although 3 possible explanations can be made: 1) previously



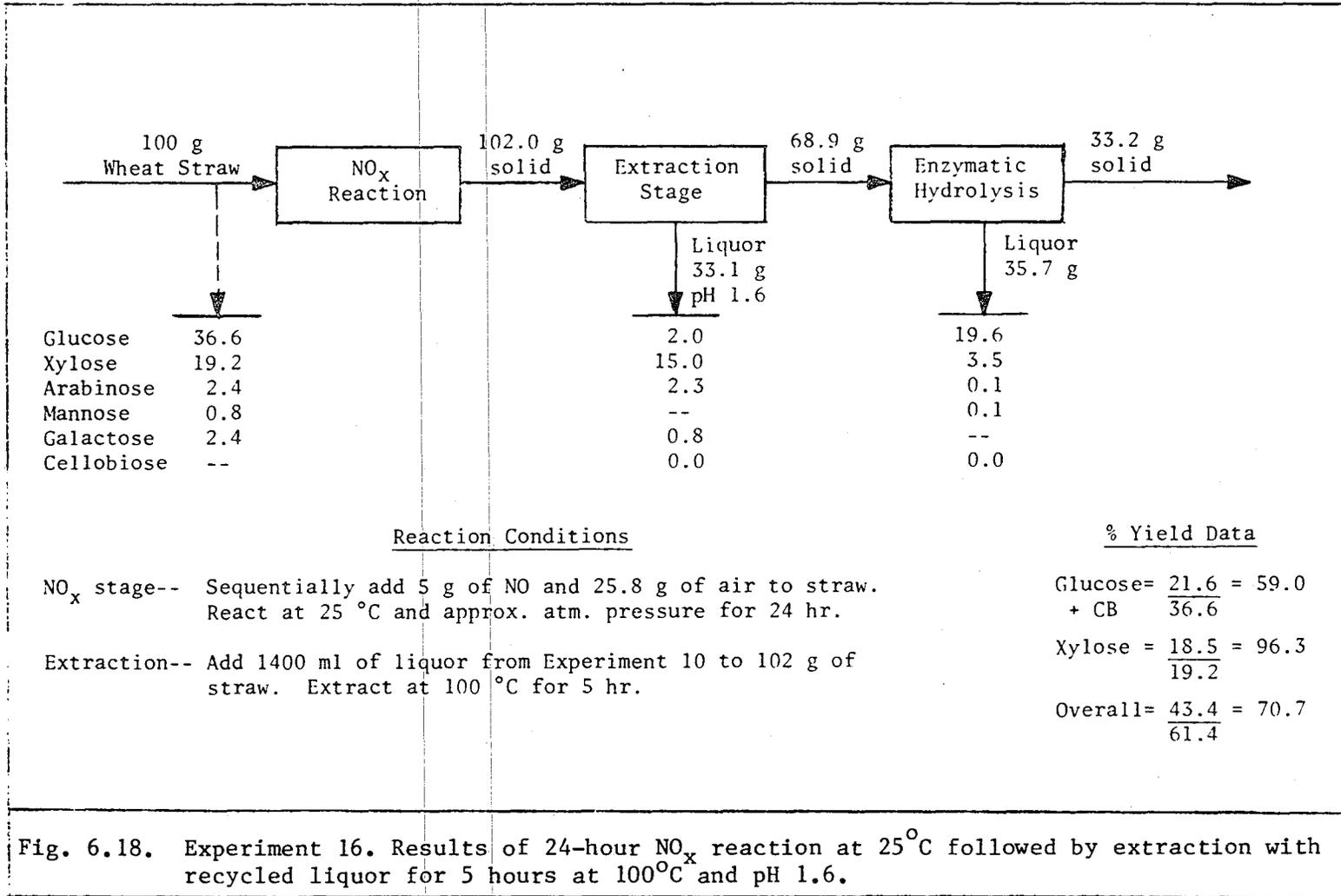
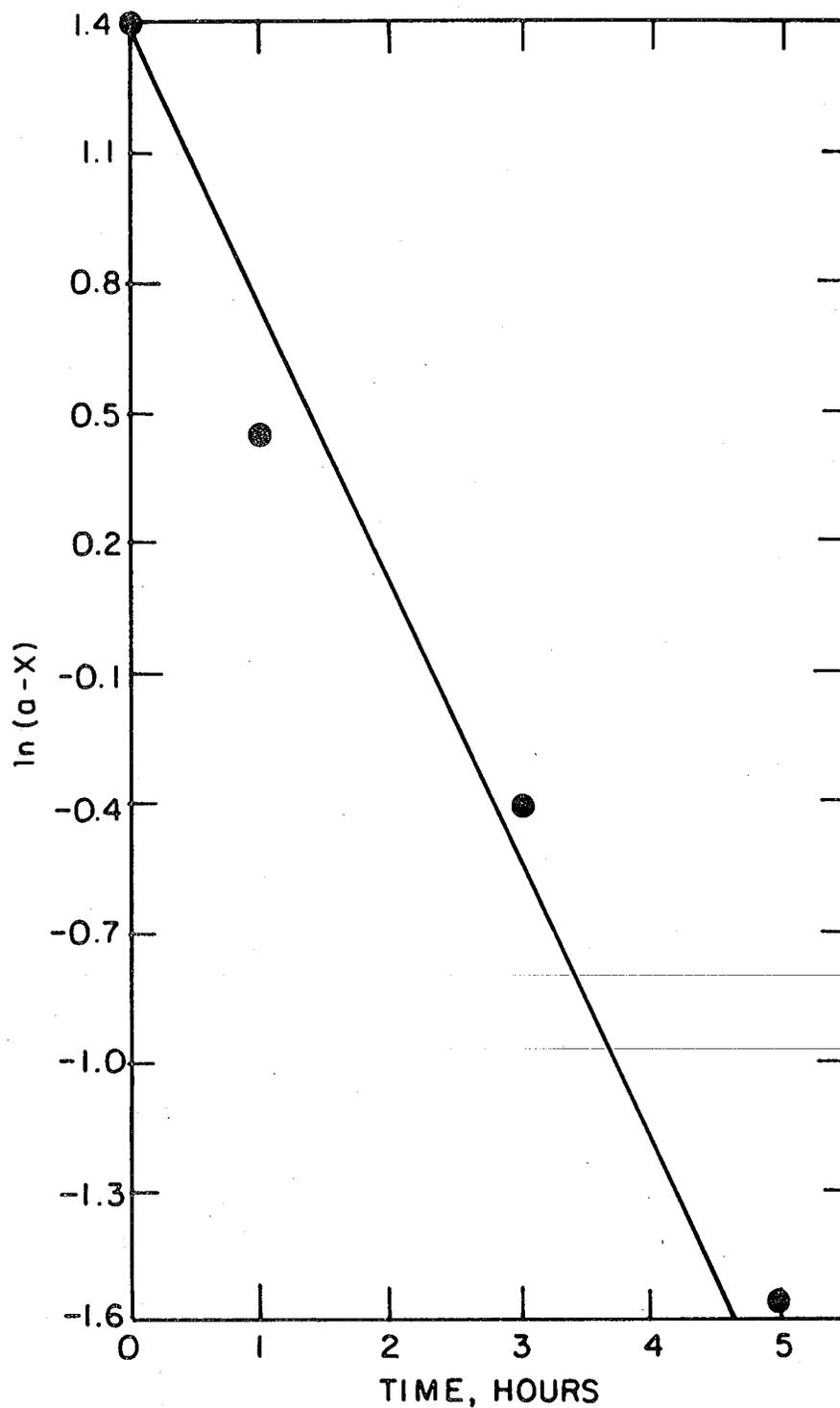


Fig. 6.18. Experiment 16. Results of 24-hour NO<sub>x</sub> reaction at 25°C followed by extraction with recycled liquor for 5 hours at 100°C and pH 1.6.



XBL 788-5686

Fig. 6.19 Xylose formation kinetics with recycled extraction liquor (Exp. 15).

dissolved xylan is being continuously hydrolyzed, 2) the effective acidity differs from that measured by pH, and 3) a different mechanism is involved in xylose formation in this experiment. The first two seem most likely.

The glucose yield of 43.7% for this experiment is decreased relative to the 48.6% yield obtained under the similar conditions of Experiment 14 where no recycle was used.

In Experiment 16 the liquor from Experiment 10 was added to a fresh batch of 24 hour reacted straw. This mixture was then boiled for 5 hours at 100°C. The overall yield of sugars is nearly 71%. The combined xylose from extraction and enzymatic hydrolysis corresponds to a yield of 96.4%. The glucose yield is 59%.

#### 6.7 NO<sub>x</sub> Reactions at 80°C

Previous work done by Brink and coworkers (8,9,10) at the University of California Forest Products Laboratory indicate that the mechanisms of the NO<sub>x</sub> gas phase reaction with cellulase are significantly different at 80°C than at 25°C. Satisfactory pulping was obtained only at the lower temperature. For this reason, an investigation of the effect of 80°C gas phase reactions on the overall sugar yield in the subsequent extraction step and enzymatic hydrolysis was conducted.

Because of the higher temperature it was anticipated that the reactions would occur rapidly. Therefore, NO<sub>x</sub> reactions lasting 1 and 2 hours were conducted. The effect of these two reaction times on the overall sugar yield were nearly identical. Only the results of

the 1 hour reaction, labeled Experiment 17, are shown in Figure 6.20. As can be seen, the sugar yield is poor. The reason for xylose not appearing in the extraction liquor is that the relatively high pH value of 3 is not conducive to acid hydrolysis.

A  $\text{NO}_x$  reaction at  $80^\circ\text{C}$  lasting 24 hours was then performed on wheat straw. The results obtained are shown in Figure 6.21, and labeled as Experiment 18. Although better than the 1 and 2 hour reactions these results showed that any further experiments at  $80^\circ\text{C}$  would be futile.

#### 6.8 Composition of Off-Gas from $\text{NO}_x$ Stage

In several experiments the composition of the gases found in the head space of the  $\text{NO}_x$  gas phase reactor was determined. This was done by the procedures outlined in 5.2.5. The results for  $\text{NO}_x$  reactions at  $25^\circ\text{C}$  are shown in Table 6.3.

The results are somewhat surprising in the fact that no  $\text{N}_2\text{O}$ ,  $\text{NO}$ ,  $\text{CO}_2$ , or  $\text{CO}$  were found to be present. This is in contradiction with the results of Lin (8), who found sizable quantities of all of these gases. However, Lin's experiments were on wood and used pure oxygen instead of air.

The concentration of  $\text{NO}_2$  was found to range between 1 and 2% of the total gases. Clearly, this  $\text{NO}_2$  must be removed in an absorber. The composition is relatively constant after the initial addition of air.

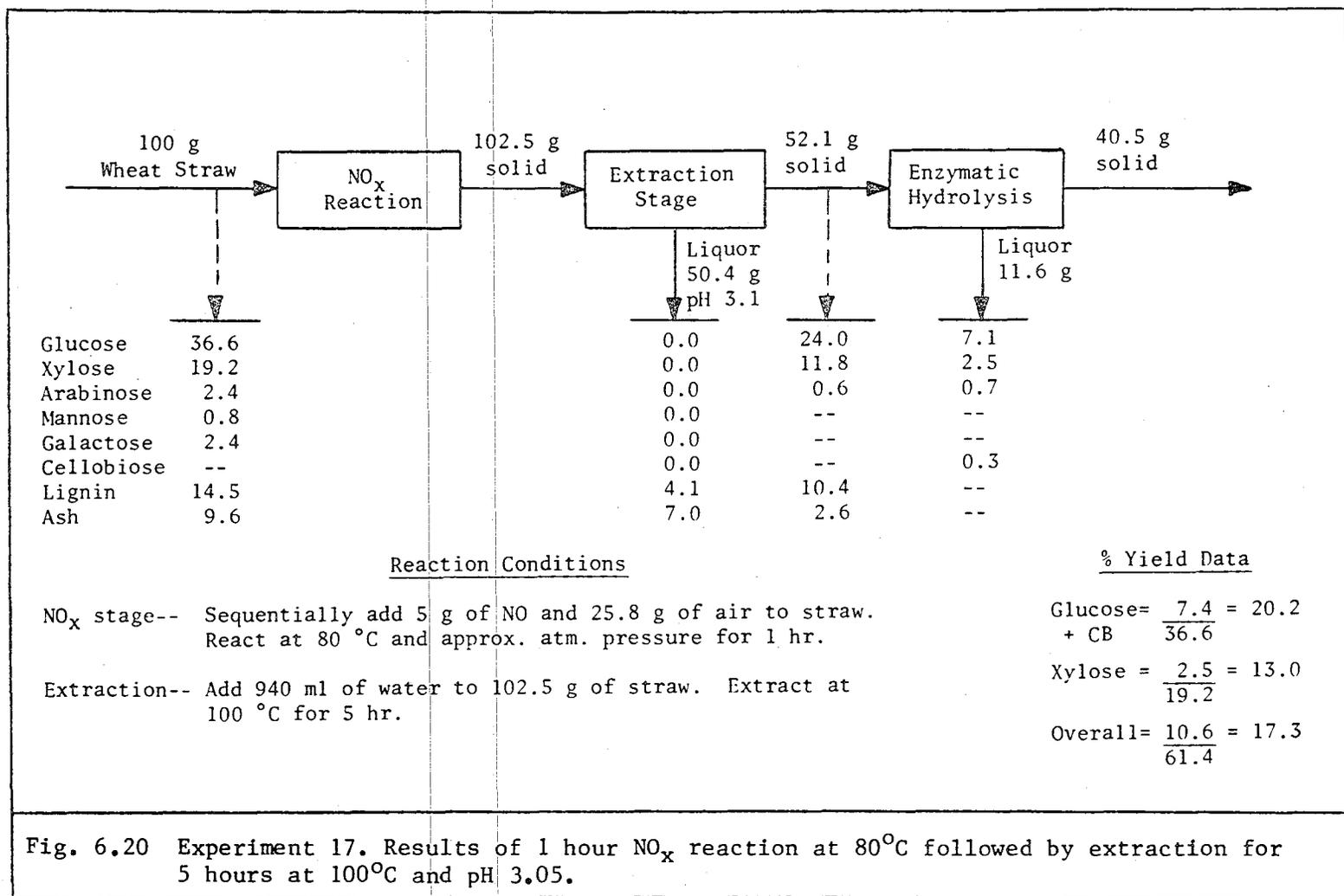


Fig. 6.20 Experiment 17. Results of 1 hour NO<sub>x</sub> reaction at 80°C followed by extraction for 5 hours at 100°C and pH 3.05.

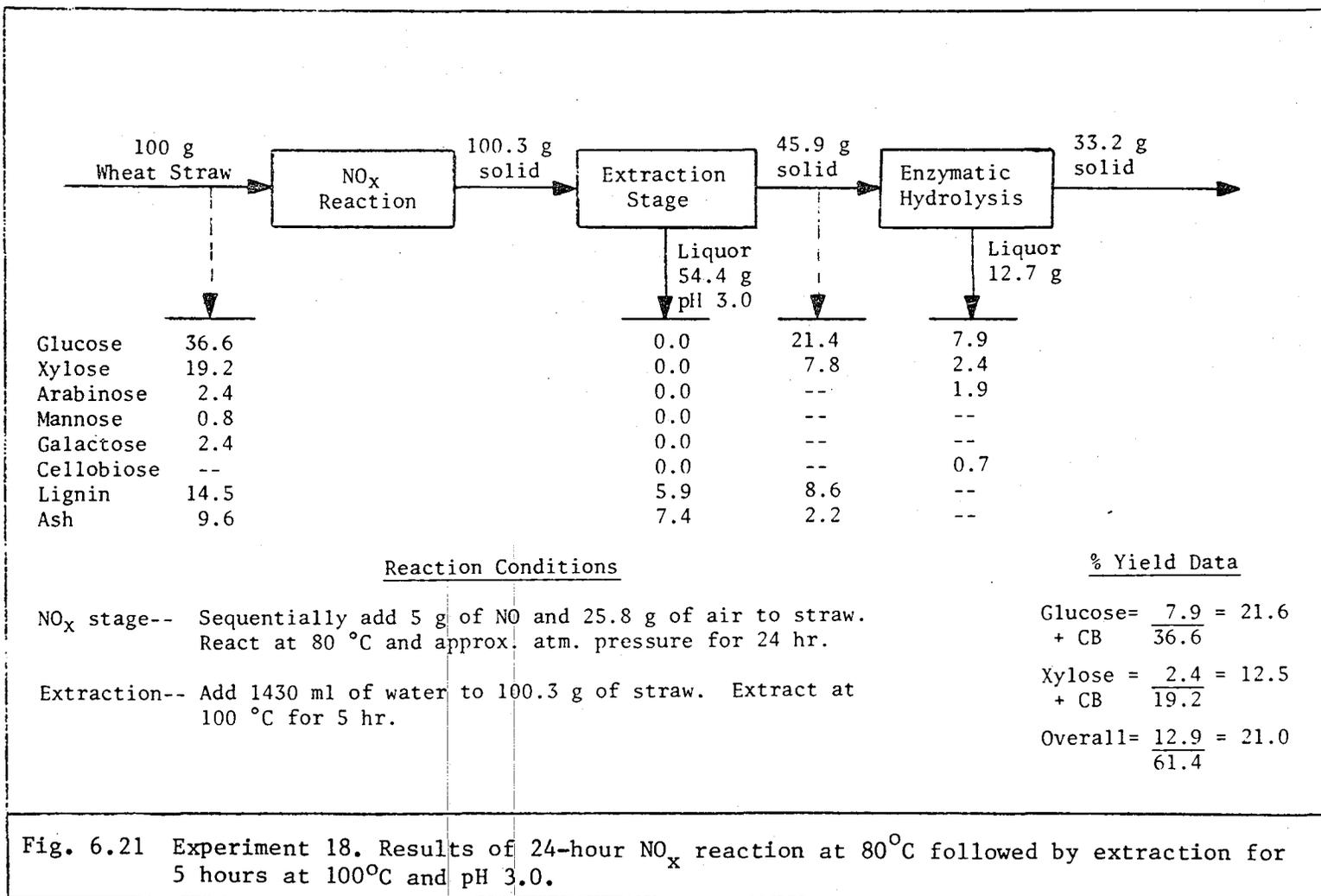


Table 6.3 Off-Gas Analysis Results for NO<sub>x</sub> Reaction

COMPOSITION (mole fraction)					
GAS	0.25 hr.	1 hr.	4 hr.	12 hr.	24 hr.
N <sub>2</sub>	0.874	0.880	0.885	0.930	0.943
O <sub>2</sub>	0.116	0.110	0.099	0.054	0.037
NO <sub>2</sub>	0.010	0.010	0.016	0.016	0.020

TOTAL MOLES OF OFF-GAS/100 GRAMS OF STRAW					
GAS	0.25 hr.	1 hr.	4 hr.	12 hr.	24 hr.
N <sub>2</sub>	0.707	0.714	0.718	0.720	0.715
O <sub>2</sub>	0.094	0.089	0.080	0.042	0.028
NO <sub>2</sub>	0.008	0.009	0.013	0.012	0.015
TOTAL	0.809	0.812	0.811	0.774	0.758

Chapter 6. References

1. P.H. Hermans and A. Weidinger, J. Poly. Sci., 4, 317 (1949).
2. T.E. Timell, Adv. in Carb. Chem., 19, 247-302 (1964).
3. J.F. Saeman, Ind. and Eng. Chem., 37, No. 1, 43 (1945).
4. R.P. Freitas, Personal communication, U. of California, Dept. of Chem. Eng., Berkeley.
5. J.M. Smuk, Ph.D. Thesis, U. of Wisconsin, Dept. of Chem. Eng., Madison, 1960.
6. D.F. Root, J.F. Saeman, and J.F. Harris, For. Prod. J. 9, 158 (1959).
7. N.I. Nikitin, The Chemistry of Cellulose and Wood, p. 557, Israeli Program for Scientific Translations Ltd., Jerusalem, 1966.
8. S. Lin, Ph.D. Thesis, U. of California, Dept. of Forestry and Conservation, Berkeley, 1976.
9. D.L. Brink, M.M. Merriman, B.M. Collett, and S.Y. Lin, "Reactions of Lignocellulose with Oxides of Nitrogen," Abstract of Papers of the 167th Am. Chem. Soc. Meeting, Los Angeles, CA, March 31-April 5, 1974.
10. D.L. Brink, U.S. Patent 4,076,579, February 1978.

## 7. PROCESS ECONOMICS AND EVALUATIONS

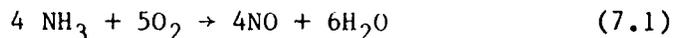
### 7.1 Process Description

Figure 7.1 is a schematic flow diagram for the plant. The operating conditions for this plant are designed to approximate Experiment 16 of this study, in which the  $\text{NO}_x$  reaction temperature and time were  $25^\circ\text{C}$  and 24 hours, respectively. The extraction stage is designed for a solid residence time of 5.5 hours at  $100^\circ\text{C}$  with an extraction liquor recycle. Mass flows of the principal streams are indicated on the diagram.

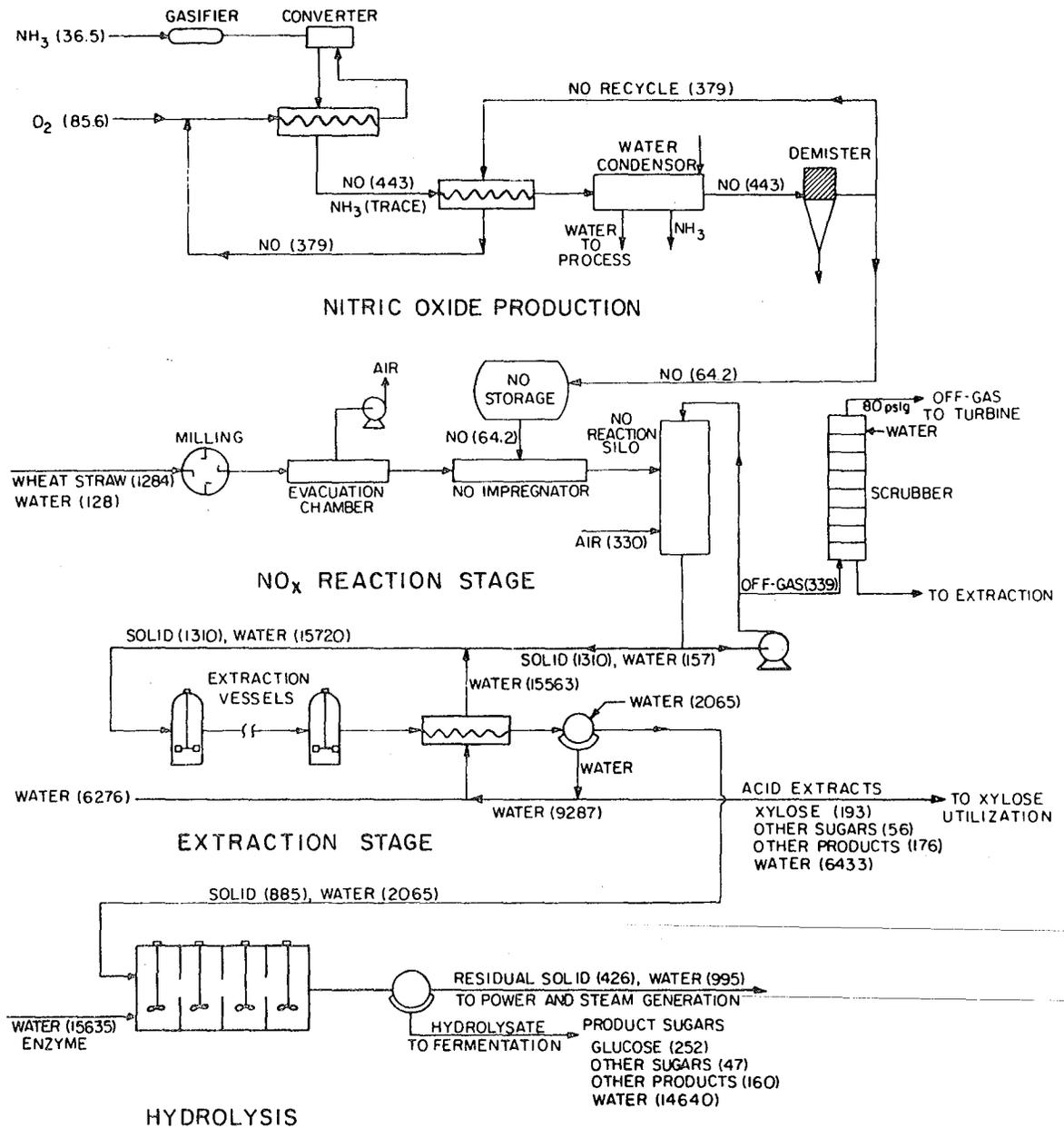
#### 7.1.1 Nitric Oxide Production

Nitric oxide, an intermediate in nitric acid manufacture, is not commercially available in bulk quantities. However, on-site production of  $\text{NO}$  by slightly modifying the nitric acid process should present no problems.

The DuPont Pressure Process for nitric acid production by catalytic oxidation of ammonia is described by Chilton (1). It involves reacting ammonia and air over a platinum alloy catalyst at  $900\text{--}1000^\circ\text{C}$  with a very short contact time to produce nitric oxide and water. The equation is:

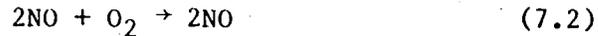


Product gases from the nitric oxide converter are then cooled to  $50^\circ\text{C}$ , condensing the water. Below  $100^\circ\text{C}$ ,  $\text{NO}_2$  is formed by the reaction between nitric oxide and excess oxygen:



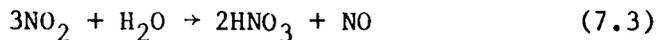
XBL 788-56 92

Fig. 7.1 Material balance flow diagram for processing scheme.



Equation (7.2) is a termolecular reaction and as the temperature is raised, it goes slower. The equilibrium heavily favors reactants at moderately high temperatures.

The final step in nitric acid manufacture is accomplished by absorbing nitrogen dioxide in water, usually in a plate tower:



The nitric oxide formed at each plate reacts with oxygen, forming nitrogen dioxide, which is absorbed on the next plate.

A critical factor in producing nitric acid is avoiding the lower explosive limit in the NO converter. This occurs at an ammonia concentration of 14 w% and is prevented by using an excess of air (1).

The process described above can be adapted to nitric oxide production by making the following changes (2): 1) using a nearly stoichiometric amount of oxygen instead of air, and 2) employing a nitric oxide recycle to the converter in order to avoid the lower explosive limit. This is depicted in Figure 7.1.

Nitrogen dioxide formation upon cooling of the reaction gases can be prevented by using a slight excess of ammonia. This ensures that nearly all of the oxygen is reacted. The ammonia in the product stream will for the most part be removed in the water condenser (2).

#### 7.1.2 $\text{NO}_x$ Reaction Stage

The primary plant feed consists of 1425 tons per day of wheat straw containing 10% moisture. This corresponds to a solids feed of

885 tons per day to enzymatic hydrolysis, which is used as the design basis following Wilke, Yang, and von Stockar (3). Table 7.1 gives the base case design specifications.

The straw is hammermilled to approximately -10 mesh. The size reduction is not critical so long as the straw will form suspensions which can be pumped, agitated, and filtered.

The straw is then fed to an air evacuation chamber. Here the air is removed from the solid by use of a vacuum pump. Retention time is approximately 5 minutes and exit pressure is 40 mm Hg.

Nitric oxide (64.2 tons/day, corresponding to 5 w% usage on a dry straw basis) is then added to the degassed straw in the nitric oxide impregnator. The vessel is essentially a gas-tight stainless steel tank providing a straw residence time of 5 minutes. Conditions within the vessel are 25°C and 1 atmosphere pressure of NO.

The straw and nitric oxide are fed to the NO<sub>x</sub> reaction silo. This is a large stainless steel silo which provides a straw residence time of 24 hours. 330 tons per day of air is added to the silo and approximately 339 tons per day of off-gas containing 1% mole fraction of NO<sub>2</sub> passes to the absorber. 1310 tons per day of NO<sub>x</sub> reacted solid containing 12% moisture leaves the reaction silo.

### 7.1.3 Extraction

The gas-treated solid is next passed to the aqueous extraction section, which is nearly identical to that described by Wilke, Yang, Sciamanna, and Freitas (4). Five Extraction stages in series are employed with each stage consisting of an agitated stainless steel

Table 7.1 NO<sub>x</sub> pretreatment and enzymatic hydrolysis of wheat straw base case design specification.

---

FEED (2mm WHEAT STRAW)	1425 T/D @ 10% Moisture Content
CARBOHYDRATE CONTENT	61.4%
NO <sub>x</sub> REACTION STAGE	5 w% NO, 25.8 w% air, 25°C, 24 hr.
EXTRACTION STAGE	7.7 w% Suspension, 100°C, 5.5 hr.
EXTRACTION LIQUOR (75% XYLOSE)	425 T/D
EXTRACTED SOLIDS	885 T/D
ENZYMATIC HYDROLYSIS	3.6 FPA, 5 w% Suspension, 45°C, 40 hr.
CELLULOSE CONVERSION	54% to Glucose
HYDROLYSATE (84% GLUCOSE)	299 T/D
SUGAR SOLUTION	2.0%

---

vessel providing a residence time of 1.1 hours. The extraction stages are maintained at 100°C by internal coils. In the first stage, a solid suspension of 7.7% is employed based on the entering streams. The resulting pH is 1.6. Effluent from the last stage is filtered on a vacuum belt filter and 53% of the filtrate is recycled to the first stage. The product stream contains 3% xylose. The possibility of using a higher recycle ratio appears quite favorable as it has been shown in this study that recycling the liquor increases the rate of xylose formation. However, experimental data are not sufficient to predict the optimum recycle ratio.

#### 7.1.4 Enzymatic Hydrolysis

The extracted solid is contacted countercurrently in two mixer-filter stages with the product sugar stream from the hydrolyzer for enzyme recovery as discussed by Wilke, et al. (4). Solid and recovered enzyme are then fed to enzymatic hydrolysis. The hydrolysis vessels consist of 5 agitated cylindrical digesters of the type used for solid waste treatment in sanitary engineering. Hydrolysis is conducted for 40 hours at 45°C at a solid suspension of 5 w% based on inputs to the hydrolyzer. Enzyme strength is maintained at 3.6 Filter Paper Activity Units by addition of make-up enzyme. A sugar concentration of 2.0% is obtained.

#### 7.2 Cost Estimation Procedures

The economics for the process described above were determined by the cost estimation procedures recommended by Peters and Timmerhaus (5). Values were obtained for the fixed capital investment and cost

per pound of sugar.

For the  $\text{NO}_x$  reaction stage, fixed capital cost was estimated as a multiple of purchased cost of the principal items of equipment. In the present case a multiplier of 3.1 was used. The fixed capital investment was taken as 85% of the total capital. Estimated costs of equipment correspond to the fourth quarter of 1977 when the Marshall and Stevens Index was 518. The total manufacturing cost is subdivided into capital related costs, utility costs, labor related costs, and raw material costs. Multipliers and utility rates are listed in Tables 7.2, 7.3, and 7.4. A base labor rate of \$5.60 per man-hour and a 8,500 hour working year was assumed. The plant operates 330 days per year. The calculation procedures are explained in the Appendix.

The fixed capital investment determination for milling, extraction, and hydrolysis has previously been determined by Wilke, et al. (4) and will not be repeated here. The milling fixed capital and the utilities for all processes were assumed to be linearly proportional to straw flowrate and were obtained by comparison to the value of Wilke et al. (4). In a similar fashion, the FCI for extraction and hydrolysis was obtained, but a 0.6 size exponent was used.

The fixed capital investment needed to produce nitric oxide was determined by comparison to nitric acid production. Values for the total capital investment as a function of plant size are given by Guthrie (6). The equipment used for nitric oxide production is the same as that used in nitric acid manufacture, except the air compressor and off-gas scrubber are not needed. By assuming a Lang factor of 3.65 ( $= 3.1/0.85$ ) the equipment costs for the nitric acid plant were

Table 7.2 Capital Related Cost Factors  
(Annual Cost = Factor X Fixed Capital)

ITEM	COST FACTOR
Depreciation	0.10
Interest	0.06
Maintenance	0.06
Insurance	0.01
Plant Supplies	0.01
Taxes	0
TOTAL	0.24

Table 7.3 Labor Related Cost Factors  
(Cost = Factor x Labor Cost)

ITEM	COST FACTOR
Direct Labor Cost	1.00
Supervision	0.15
Payroll Overhead	0.15
Laboratory	0.15
Plant Overhead	0.50
TOTAL	1.95

Table 7.4 Base Utility Rate

UNIT	UNIT COST	UNIT HR.
Power kw-hr.	3 **	9875
Steam 1000 lb.	32.5 *	206
Water 1000 gal.	12.8	196

\*Self generated from residual solids.

\*\*Bought from public utility.

determined, the costs of the compressor and scrubber subtracted out, and the resulting value multiplied by 3.1 to give the nitric oxide production fixed capital investment. The calculations are found in the Appendix.

### 7.3 Cost Estimation Results

The resulting fixed capital investment, total manufacturing costs, and costs per pound of glucose produced during hydrolysis and total sugar produced in the entire process are listed in Table 7.5 for each of the major processing sections: 1) nitric oxide production, 2) milling, 3) NO<sub>x</sub> reaction, 4) extraction, and 5) hydrolysis associated processing (includes enzyme production and recovery).

The glucose cost of 10.86 cents per pound is to be compared with the dilute acid pretreatment processing scheme cost of 10.0 cents per pound from corn stover. However, for dilute acid pretreatment followed by hydrolysis, glucose yields are typically about 20% higher for corn stover than those obtained with wheat straw (4). From this point of view, it can be calculated that the cost per pound of glucose using the dilute acid pretreatment is approximately 12 cents per pound in the case of wheat straw.

The annual capital related costs for nitric oxide production and the NO<sub>x</sub> reaction stage are low in comparison to hydrolysis costs. The reasons for this are: 1) the simplicity and exothermicity of nitric oxide production, and 2) the fact that the NO<sub>x</sub> reaction occurs at atmospheric pressure and ambient temperature.

The results of a sensitivity analysis for a \$30 per ton increase

Table 7.5 Hydrolysis Process Cost Analysis -- Base Case  
Raw Material (Wheat Straw) Cost Excluded.

	NITRIC OXIDE PRODUCTION	NO <sub>x</sub> REACTION	MILLING	EXTRACTION	HYDROLYSIS ASSOCIATED	TOTAL
Fixed Capital Cost x 1000 \$	2,018	6,085	3,116	4,976	20,882	37,077
Annual Capital Related Costs x 1000 \$	484	1,460	756	1,194	5,011	8,905
Annual Labor Related Costs x 1000 \$	96	96	96	191	478	957
Annual Utilities Costs x 1000 \$	-	225	102	429	1,528	2,284
Annual Material Costs x 1000 \$	2,711	-	-	-	3,211	5,922
Annual Manufacturing Cost x 1000 \$	3,291	1,781	954	1,814	10,228	18,068
Glucose Cost, /lb.	1.98	1.07	0.57	1.09	6.15	10.86
Total Sugars Cost, /lb.	0.91	0.50	0.26	0.50	2.83	5.00

in wheat straw and corn stover are shown in Table 7.6, where it can be seen that the higher glucose yield obtained upon NO<sub>x</sub> pretreatment offers a substantial savings over acid pretreatment. Similarly, for one cent per pound increases in the prices of ammonia and oxygen, the cost increases per pound of glucose are 0.15 and 0.34 cents per pound, respectively. These results are shown in Table 7.7.

Table 7.6 Effect of Raw Material Cost on Glucose Cost  
NO<sub>x</sub> Pretreatment vs. Dilute-Acid Pretreatment

GLUCOSE COST  /lb.	ASSUMING NO RAW MATERIAL COST	RAW MATERIAL AT \$30/ton	TOTAL
NO <sub>x</sub> Pretreated Wheat Straw	10.86	7.64	18.50
Acid Pretreated Corn Stover	9.97	9.66	19.63

Table 7.7 Incremental Effect of Variables on Glucose Cost  
for NO<sub>x</sub> Pretreatment Scheme

RAW MATERIAL	INCREASE	EFFECT ON GLUCOSE COST,  /lb.
Wheat Straw	\$30/ton	+7.64
Ammonia	1 /lb.	+0.15
Oxygen	1 /lb.	+0.34

Chapter 7. References

1. T.H. Chilton, The Manufacture of Nitric Acid by the Oxidation of Ammonia, Chem. Eng. Prog. Monograph Series, 56, No. 3 (1960).
  2. T. Vermeulen, Personal communication, U. of California, Dept. of Chem. Eng., Berkeley, July 1978.
  3. C.R. Wilke, R.D. Yang, and Urs von Stockar, "Preliminary Cost Analyses for Enzymatic Hydrolysis of Newprint," Biotechnol. Bioeng. Symp. No. 6, 155-175 (1976).
  4. C.R. Wilke, R.D. Yang, A.F. Sciamanna, and R.P. Freitas, "Raw Material Evaluation and Process Development Studies for Conversion of Biomass to Sugars and Ethanol," LBL-7847, June 1978.
  5. M.S. Peters and K.D. Timmerhaus, Plant Design and Economics for Chemical Engineers, 2nd Edition, McGraw-Hill, New York, 1968.
  6. K.M. Guthrie, Chem. Eng., 77, No. 13, 140 (1970).
- 
-

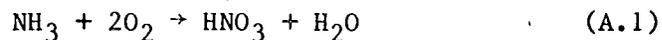
APPENDIX

COST CALCULATIONS FOR NITRIC OXIDE PRODUCTION AND NO<sub>x</sub> REACTION STAGE

Nitric Oxide Production

The fixed capital necessary to produce nitric oxide was determined by comparison to nitric acid production. Values for the total capital investment as a function of plant size are given by Guthrie (1). The equipment used for nitric oxide production is the same as that used for nitric acid manufacture, except an air compressor and gas scrubber are not needed. However, these are used for scrubbing the off-gases of the NO<sub>x</sub> reaction stage. Compressed oxygen is used instead of air.

The overall equation for nitric acid formation is:



From Equation (A.1) it can be calculated that 46.2 tons of nitric oxide correspond to 135 tons/day of nitric acid. From Guthrie (1) the total capital investment required for a nitric acid plant of this size is \$2,730,000. Assuming a Lange Factor of 3.65 (= 3.1/.85) gives the equipment cost to be \$748,000.

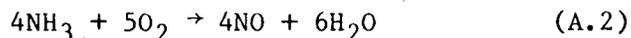
By referring to Bell (2), it is seen that in a plant of this size the compressor capacity is 22,000 cu. ft. per minute at 120 psig. This costs approximately \$175,000. The scrubber cost will not be subtracted out in this section because of the difficulty in assessing its cost. Therefore, it should be recognized that the fixed capital costs for nitric oxide production are somewhat high and the fixed capital investment for the NO<sub>x</sub> reaction stage somewhat low. However, they cancel

each other out in terms of overall cost.

A 10 minute storage capacity for nitric oxide in order to compensate for possible system upsets is needed. At 100 psia this requires a spherical storage tank of 16,000 gallon capacity, which costs approximately \$78,000 (stainless construction).

Subtracting the compressor cost and adding the nitric oxide storage tank cost to the FCI of the nitric acid plant gives an equipment cost of \$651,000 for nitric oxide production. This corresponds to an FCI of \$2,018,000.

The cost of raw materials for nitric oxide production is obtained by considering Equation (A.2). Given that ammonia costs 6.5¢ per pound and oxygen 2.0¢ per pound (3), the total cost of the chemicals necessary to make nitric oxide can be calculated:



$$\frac{\text{raw material cost}}{\text{lb. NO}} = \frac{4(6.5)(17 \text{ lb./mole}) + 5(2.0)(32 \text{ lb./mole})}{(4 \text{ moles NO})(30 \text{ lb./mole})}$$
$$= 6.35\text{¢/lb.} = \$127/\text{ton}$$

$$\frac{\text{raw material cost}}{\text{ton of straw}} = \$127.(0.05 \text{ ton NO/ton of straw}) = \frac{\$6.35}{\text{ton of straw}}$$

In addition, consumed catalyst amounts to \$0.05/ton of straw (4). Therefore, the total cost of raw materials for nitric oxide production is \$6.40/ton of straw, which corresponds to \$2,711,000 per year. No cost has been charged to the straw, as this was considered in a sensitivity analysis.

NO<sub>x</sub> Reaction Stage

As discussed in Chapter 7, the following major items of equipment are required for the NO<sub>x</sub> reaction stage: air evacuation chamber, nitric oxide impregnator, NO<sub>x</sub> reaction silo, air compressor, and gas scrubber. In order to estimate the FCI the equipment was sized as follows:

1) Evacuation Chamber -- Setting the residence time equal to 5 minutes, for a straw specific gravity of 0.1 the minimum required volume is:

$$\text{Volume} = \frac{(1284 \text{ T/D})(\text{day}/1440 \text{ min.})(5 \text{ min.})(2000 \text{ lb./T})}{(0.1)(62.4 \text{ lb./cu.ft.})}$$
$$= 1429 \text{ cu.ft.} = 10,700 \text{ gallons}$$

To allow for overhead space and system upsets a 15,000 gallon carbon steel vacuum vessel will be used. Being a vacuum vessel capable of withstanding a pressure of 40 mm Hg absolute, this costs approximately \$47,000 installed.

2) Vacuum Pump for Evacuation Chamber -- A pump is needed which will evacuate to 40 mm Hg pressure. The capacity of the pump is equal to the volume of the solid fed to the evacuation chamber per unit time, which yields 285 cu.ft. per minute. The horsepower for a pump of this size and pressure differential was determined by a method in Peters and Timmerhaus (5) to be 85 HP. This pump costs \$24,000.

3) Nitric Oxide Impregnation Chamber -- For a residence time of 5 minutes a 15,000 gallon stainless steel tank is required. This costs \$52,000.

4) Solid Feeders -- Five feeders, capable of handling 107,000

pounds per hour, are required to move the solids between the four major pieces of equipment of the  $\text{NO}_x$  reaction stage. These cost \$11,000 each (including motors), giving a total cost of \$55,000. Belt conveyors are required to handle the high solid feed rate.

5)  $\text{NO}_x$  Reaction Silo -- After air addition to the silo, 100 grams of straw require about 22.5 liters of overhead reactor space. In Section 6.7 it was shown that the gas composition remains relatively constant after air addition. Therefore, it can be concluded that only the straw residence time is critical so long as the gas residence time is at least 0.5 hour. The limiting factor for silo capacity is the volume occupied by the straw for a 24 hour reaction time. Assuming the silo to be 70% full, the volume can be calculated by:

$$\text{Volume} = \frac{(1284 \text{ T/D})(2000 \text{ lb./T})(1 \text{ day})}{(0.7)(0.1)(8.34 \text{ lb./gal})} = 4.40 \times 10^6 \text{ gallon}$$

Three silos of equal volume,  $1.47 \times 10^6$  gallons, will be used. These cost \$547,000 each (stainless steel).

6) Off-Gas Scrubber -- Past experience has shown scrubbing of nitrogen dioxide to be more economical at high pressure (6). This is one of the main reasons for development of the DuPont Pressure Process. Because the  $\text{NO}_x$  reaction occurs at atmospheric pressure it is necessary to compress the off-gases. Prior experience with the DuPont Process has determined 100 psi to be near the economic optimum (6). On the basis of gas flow rates it appears reasonable to use the scrubber from a similar sized nitric acid plant described by Chilton (6). This scrubber is 10 feet in diameter and 40 feet high with 35

absorption plates. No attempt will be made to price the tower here, as its cost was included under nitric oxide production. Although the approximate nature of the scrubber sizing should be realized, it is believed the procedure gives a fairly accurate cost estimation.

7) Compressor for Scrubber -- The compressor used in a similar sized nitric acid plant will be required. As discussed under nitric oxide production, a compressor of 22,000 cu.ft. per minute at 120 psig is used, which costs \$175,000.

A summary of the equipment for the  $\text{NO}_x$  reaction stage is listed in Table A.1. From the total equipment cost of \$1,994,000 a fixed capital investment of \$6,085,000 is obtained.

The utilities required for the  $\text{NO}_x$  reaction stage may be taken as that required to power the compressor. This has been reported as 160 kw.hr/ton of  $\text{HNO}_3$ , which converts to \$225,000 per year.

#### Appendix. References

1. K.M. Guthrie, Chem. Eng., 77, No. 13, 140 (1970).
2. B.H.J. Bell, Chem. and Ind., p. 724 (1959).
3. Chemical Marketing Reporter, 214, No. 3 (1978).
4. Anon., Chem. Eng., 63, No. 1, 274 (1956).
5. M.S. Peters and K.D. Timmerhaus, Plant Design and Economics for Chemical Engineers, 2nd Edition, McGraw-Hill, New York, 1968.
6. T.H. Chilton, The Manufacture of Nitric Acid by the Oxidation of Ammonia, Chem. Eng. Prog. Monograph Series, 56, No. 3 (1960).

Table A.1 Major Items of Equipment for NO<sub>x</sub> Reaction Stage

<u>Item</u>	<u>Unit Specification</u>	<u>Number of units</u>	<u>cost/unit \$</u>
Evacuation Chamber	15,000 gal., carbon steel, vacuum vessel	1	47,000
NO Impregnation Chamber	15,000 gal., stainless steel	1	52,000
Vacuum Pump	85 HP, centrifugal	1	24,000
Solid Feeder	belt conveyor, 54 tons/hour	5	11,000
NO <sub>x</sub> Reaction Silo	1.47 x 10 <sup>6</sup> gallons, stainless steel	3	547,000
Off-Gas Scrubber	10 ft. diameter, 40 ft. high, 35 plates	1	*
Compressor	22,000 cu.ft./min. capacity, 120 psig	1	175,000
Total Equipment Cost			\$1,994,000

\*cost included under nitric oxide production



This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

TECHNICAL INFORMATION DEPARTMENT  
LAWRENCE BERKELEY LABORATORY  
UNIVERSITY OF CALIFORNIA  
BERKELEY, CALIFORNIA 94720