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Publication Date

1978-09-01

Energy and Environment Division
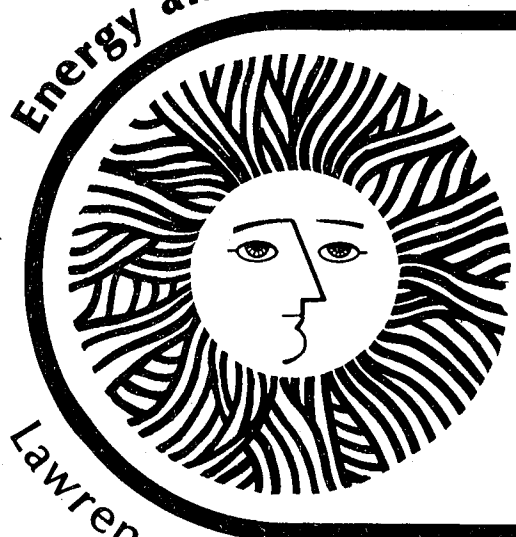
Effect of Nitrogen Oxide Pretreatments on Enzymatic Hydrolysis of Cellulose

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

(M. S. Thesis)

September 1978

Lawrence Berkeley Laboratory   University of California/Berkeley

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ON ENZYMATIC HYDROLYSIS OF CELLULOSE

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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 acces-
sibility 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 (liter/gmole)(hr.$^{-1}$) and 0.03 hr.$^{-1}$, respectively. However, the rate of formation is much greater (20.4 (liter/gmole)(hr.$^{-1}$)) 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.

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.

NO AIR WATER T.*viride* CELLULASE SOLUTION

WHEAT STRAW → NO$_x$ REACTION → SOLID → EXTRACTION → SOLID → ENZYMATIC HYDROLYSIS → SOLID

LIQUOR

HYDROLYSATE

-3-
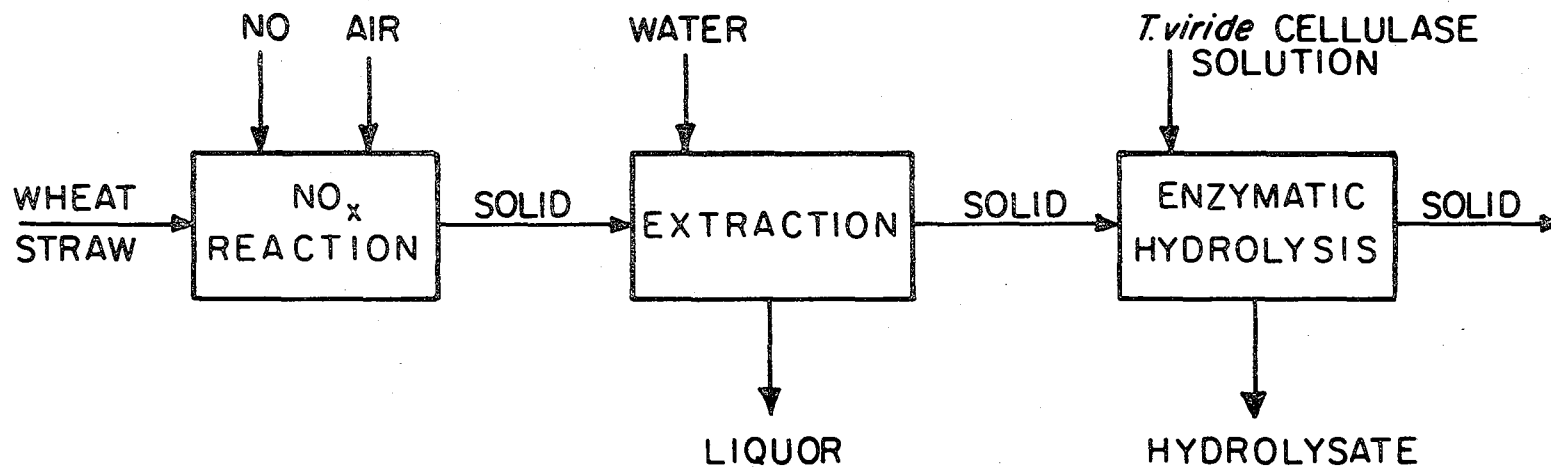
XBL 789-5768

Fig. 1.1 Overall process flow diagram for enzymatic hydrolysis of
lignocellulose employing a 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 countires, 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 pretreat-
ments 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 carbo-
hydrates 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.

	100 g wheat straw	ACID TREAT	liquid 37.6 g	62.4 g solid	ENZYMATIC HYDROLYSIS	liquid 18.2 g	44.2 g solid
Glucose	36.5		2.5	32.8		12.5	19.1
Xylose	19.2		9.2	6.0		1.9	3.6
Arabinose	2.4		1.5	0.4		0.1	0.3
Mannose	0.8		--	--		--	--
Galactose	2.4		--	--		--	--
Poly. Glu.	--		1.2	--		1.2	--
Poly. Xyl.	--		1.7	--		0.5	--
Poly. Arab.	--		0.5	--		0.0	--
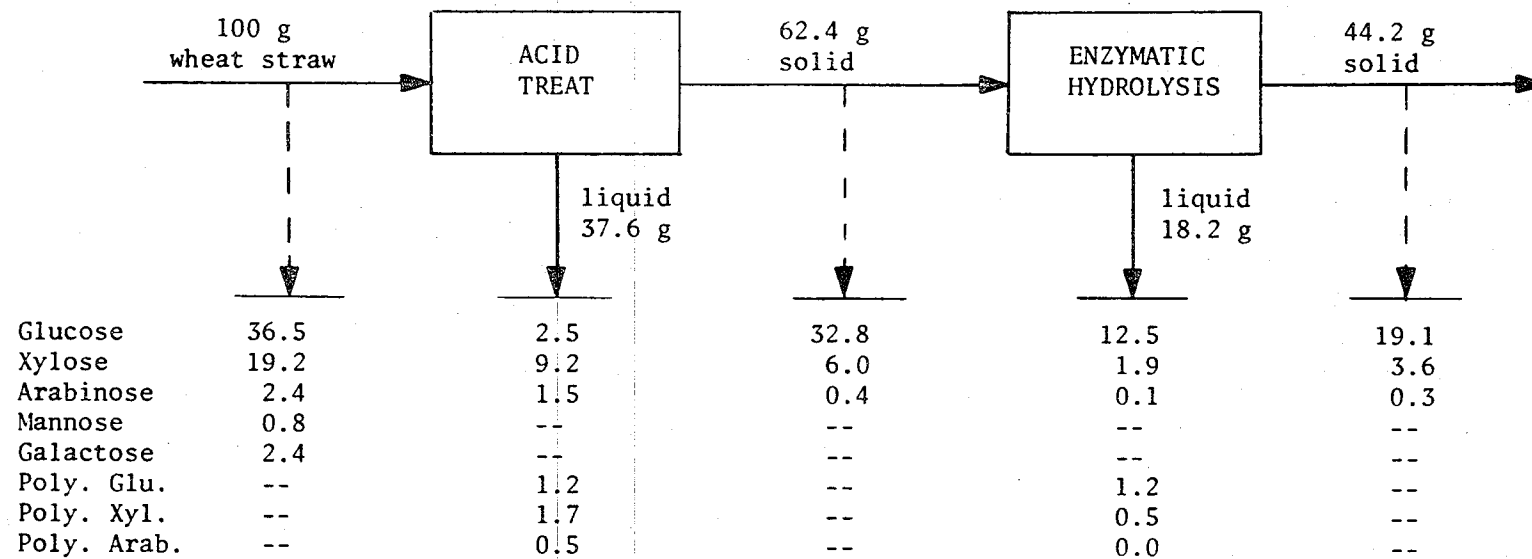
Fig. 3.1 Results obtained by enzymatic hydrolysis of dilute acid
pretreated wheat straw. Pretreatment involves contacting
straw with 0.09 $\underline{M}$ H_2SO_4 for 5.5 hours at 100°C.

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.

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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

Crystallite 600 Å | Porous Region

(a)

100 Å
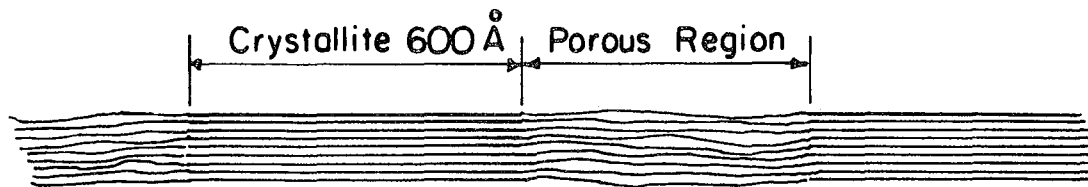
30 Å
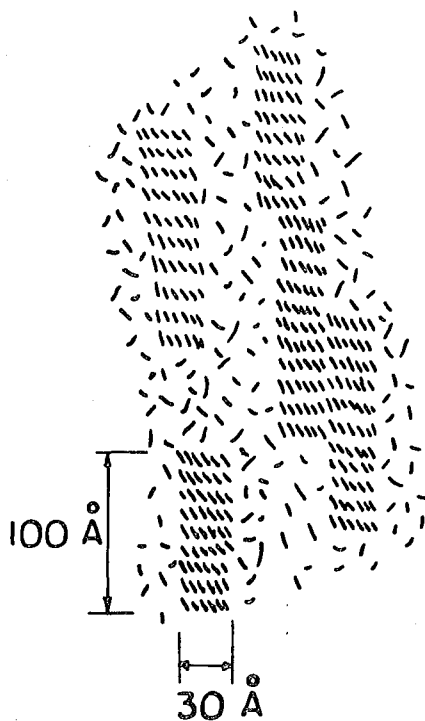
(b)

XBL 788-5680

Fig. 4.1 Proposed micellar theory of cellulose structure.[3]
(a) longitudinal view, (b) cross sectional view.
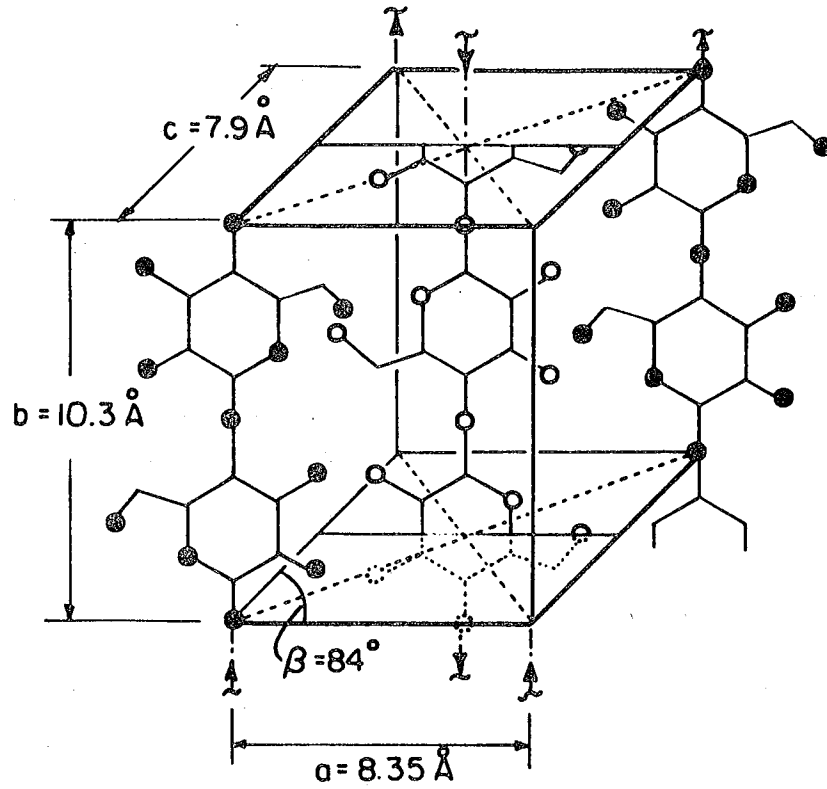
Fig. 4.2 The monoclinic elementary cell of cellulose.[4]

of neighboring glucose molecules ($< 10 \overset{\circ}{A}$) 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 4-0-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
O-Acetyl-4-O-methylglucurono-xylan	Hardwoods	20-35	β-D-Xylp	10	1 → 4
			4-O-Me-α-D-GlupA	1	1 → 2
			O-Acetyl		
Glucomannan	Hardwoods	3-5	β-D-Manp	1-2	1 → 4
			β-D-Glup	1	1 → 4
Arabino-4-O-methylglucurono-xylan	Softwoods	10-15	β-D-Xylp	10	1 → 4
			4-O-Me-α-D-GlupA	2	1 → 2
			-Araf	1,3	1 → 3
Galactoglucomannan (water-soluble)	Softwoods	5-10	β-D-Manp	3	1 → 4
			β-D-Glup	1	1 → 4
			α-D-Galp	1	1 → 6
			O-Acetyl	0.24	
Galactoglucomannan (alkali-soluble)	Softwoods	10-15	β-D-Manp	3	1 → 4
			β-D-Glup	1	1 → 4
			α-D-Galp	0.1	1 → 6
			O-Acetyl	0.24	
Arabinogalactan	Larchwood	10-20	β-D-Galp	6	1 → 3, 1 → 6
			L-Araf	2/3	1 → 6
			β-L-Arap	1/3	1 → 3
			β-D-GlupA	Few	1 → 6
Pectin	All woods	1	α-D-GalpA	Most	1 → 4
			D-Galp	Few	
			L-Araf	Few	
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 guiacylpropane units. Hardwood and grass lignins contain, as a first
approximation, the same amounts of both guiacylpropane and syringyl-
propane units. Grasses also contain a significant amount of para-
hydroxyphenyl 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
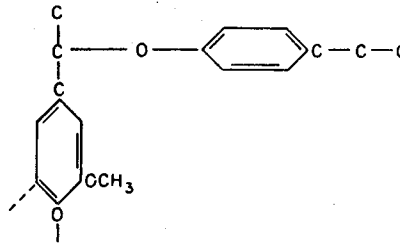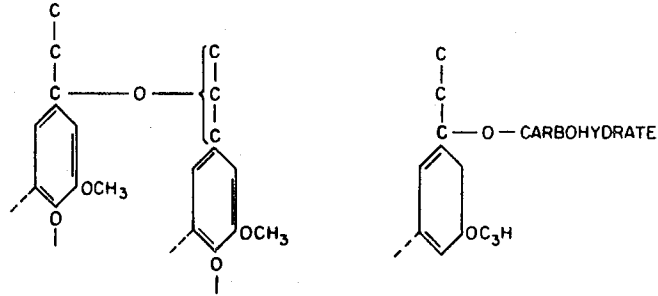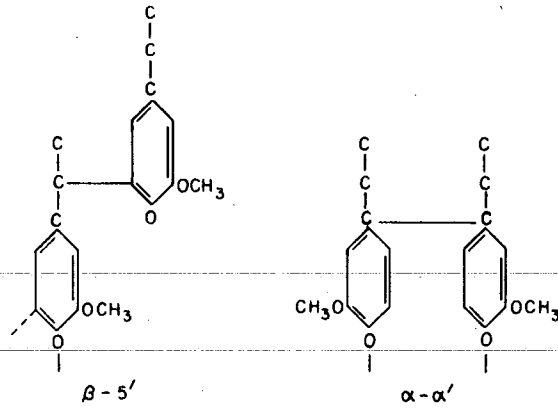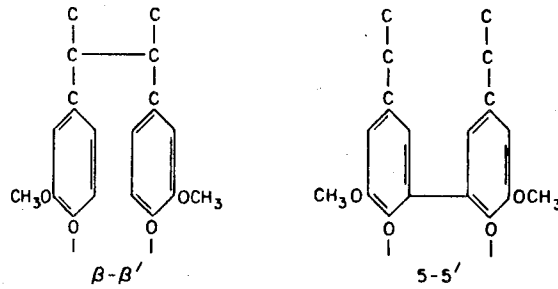
Fig. 4.3 Postulated intermonomeric linkages in lignin.(8)

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_x Reactions

When exposed to an environment containing nitrogen oxides, cellulose is oxidized. Studies conducted employing NO_2 and N_2O_4 have shown that the oxidation is more or less specific to the primary hydroxyl group of carbon 6 (9). However, studies with NO_2 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_2 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 β-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 β-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
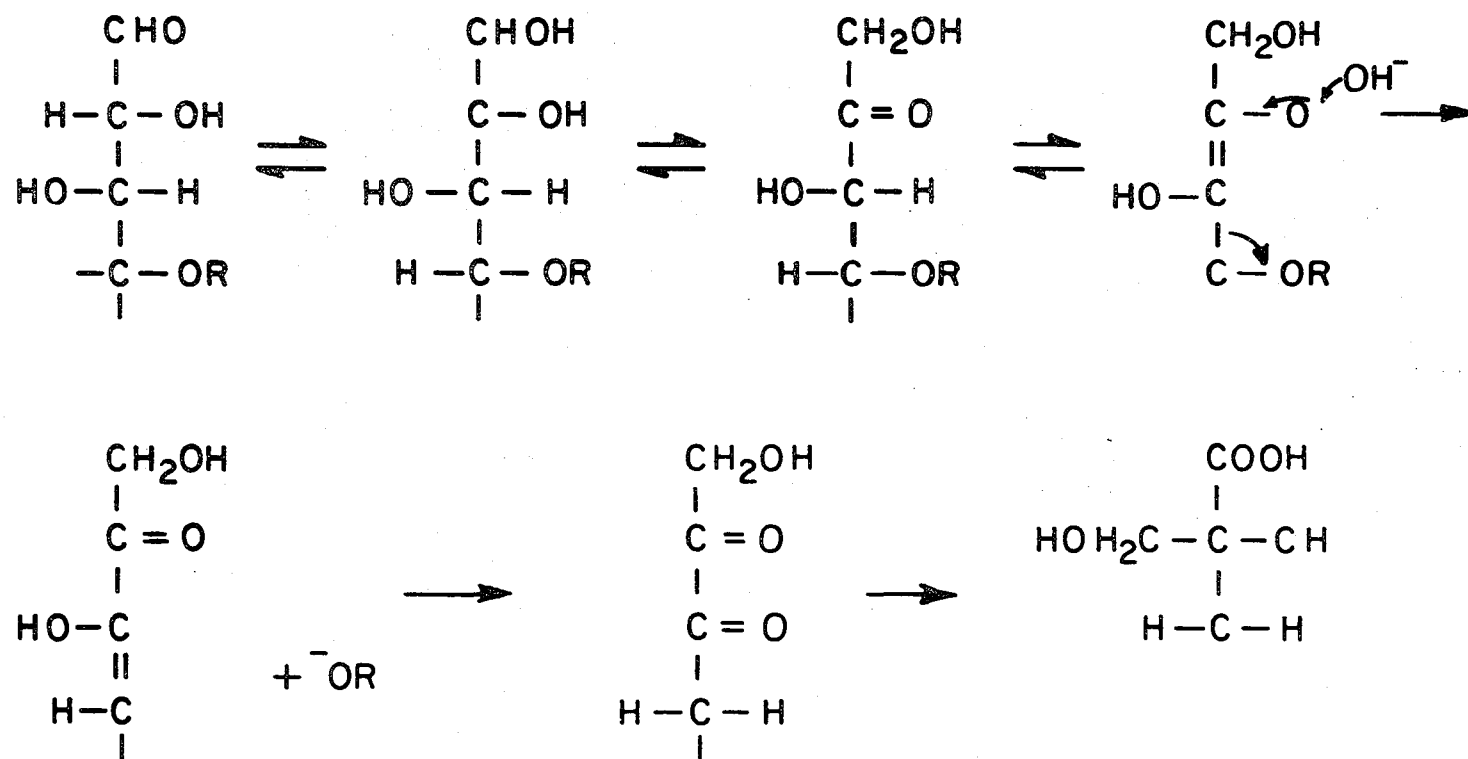
Fig. 4.4 Mechanism of primary peeling.

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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 occuring 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.
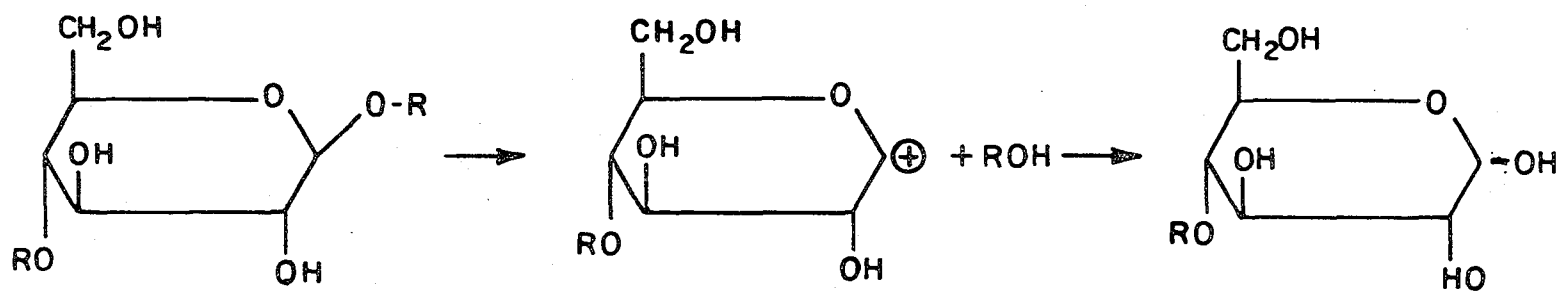
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Fig. 4.5 Proposed acid hydrolysis mechanism.

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→B→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-β-glucan glucanhydrolase (also called C_x); 2) 1,4-β-glucan

cellobiohydrolase (also called C_1); and 3) -glucosidase.

C_x enzymes are endoglucanases which randomly attack the amor-

phous 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 exoglu-

canase. 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, β-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_2, 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:

1) $HNO_3 + H^+ \rightarrow H_2O^+NO_2 \rightarrow H_2O + NO_2^+$

2) $HNO_3 + HNO_2 \rightarrow N_2O_4 + H_2O \rightarrow NO^+ + NO_3^- + H_2O$
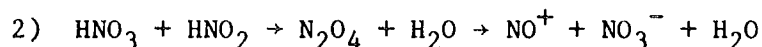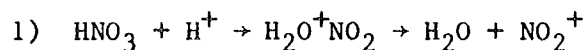
Due to the rapid rate of protonation of nitric acid and $H_2ONO_2^+$ the rate of formation of the electrophiles is slow. In an environment of nitrogen oxides the rate of electrophile production is rapid. For this reason, nitrogen oxides more selectively attack lignin than

AROMATIC SUBSTITUTION:

$$X^{\oplus} + \underset{OR}{\overset{C_3}{\bigcirc}}OMe \longrightarrow X\underset{OR}{\overset{C_3}{\bigcirc}}OMe + H^{\oplus}$$
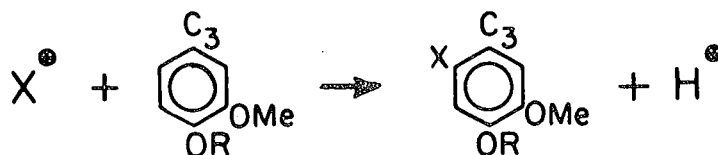
ELECTROPHILIC DISPLACEMENT:

$$X^{\oplus} + \underset{OR}{\overset{\overset{R}{|}\,CHOH}{\bigcirc}}OMe \longrightarrow \overset{R}{\underset{}{\overset{|}{CHO}}} + \underset{OR}{\overset{X}{\bigcirc}}OMe + H^{\oplus}$$

ALIPHATIC SUBSTITUTION:

$$X^{\oplus} + H\overset{|}{\underset{|}{C}}-CO- \longrightarrow X\overset{|}{\underset{|}{C}}-CO- + H^{\oplus}$$

ADDITION:

$$X^{\oplus} + RO\bigcirc_{OMe}CH=CH- \longrightarrow RO\bigcirc_{OMe}^{\oplus}\overset{X}{\overset{|}{CH}}-CH- \overset{B^{\ominus}}{\longrightarrow} RO\bigcirc_{OMe}\overset{X}{\underset{B}{\overset{|}{CH}}}CH-$$

OXIDATION:

$$X^{\oplus} + HO\overset{|}{C}H- \underset{-H^{\oplus}}{\longrightarrow} XO\overset{|}{C}H- \underset{-HX}{\longrightarrow} O\overset{|}{C}-$$

$$X^{\oplus} + O=CH- \longrightarrow XO\overset{\oplus}{C}H- \overset{OH^{\ominus}}{\longrightarrow} XO\overset{OH}{\underset{}{\overset{|}{C}}}H- \underset{-HX}{\longrightarrow} O\overset{OH}{\underset{}{\overset{|}{C}}}-$$
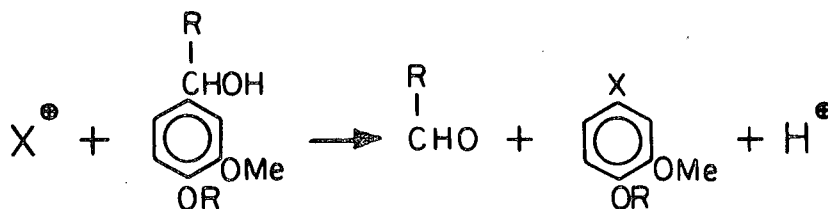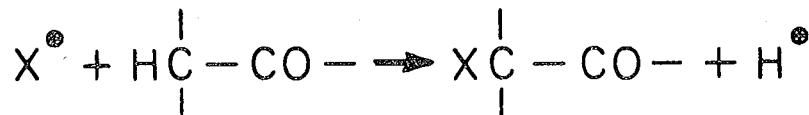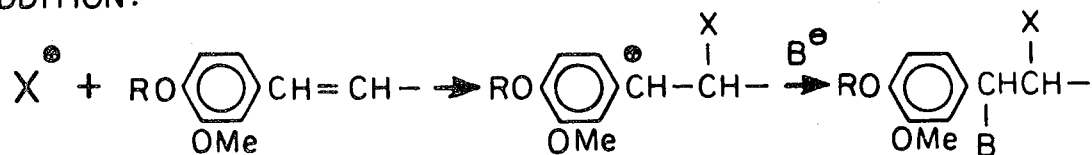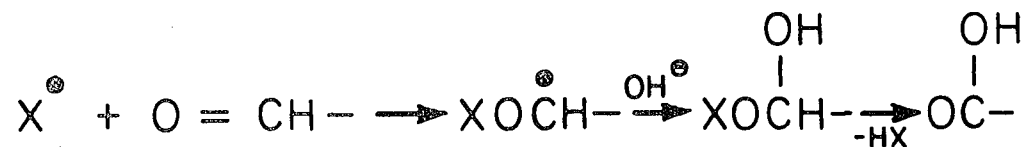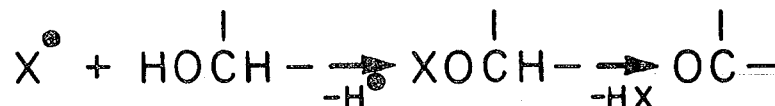
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Fig. 4.6 Summary of electrophilic reactions occurring during
the nitration of lignin. $X^{\oplus}$ denotes electrophile.
R denotes alkyl substituent.(13)

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).

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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 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 atmos-
pheric pressure. The second pretreatment step is an aqueous extraction
of the solid material from the 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
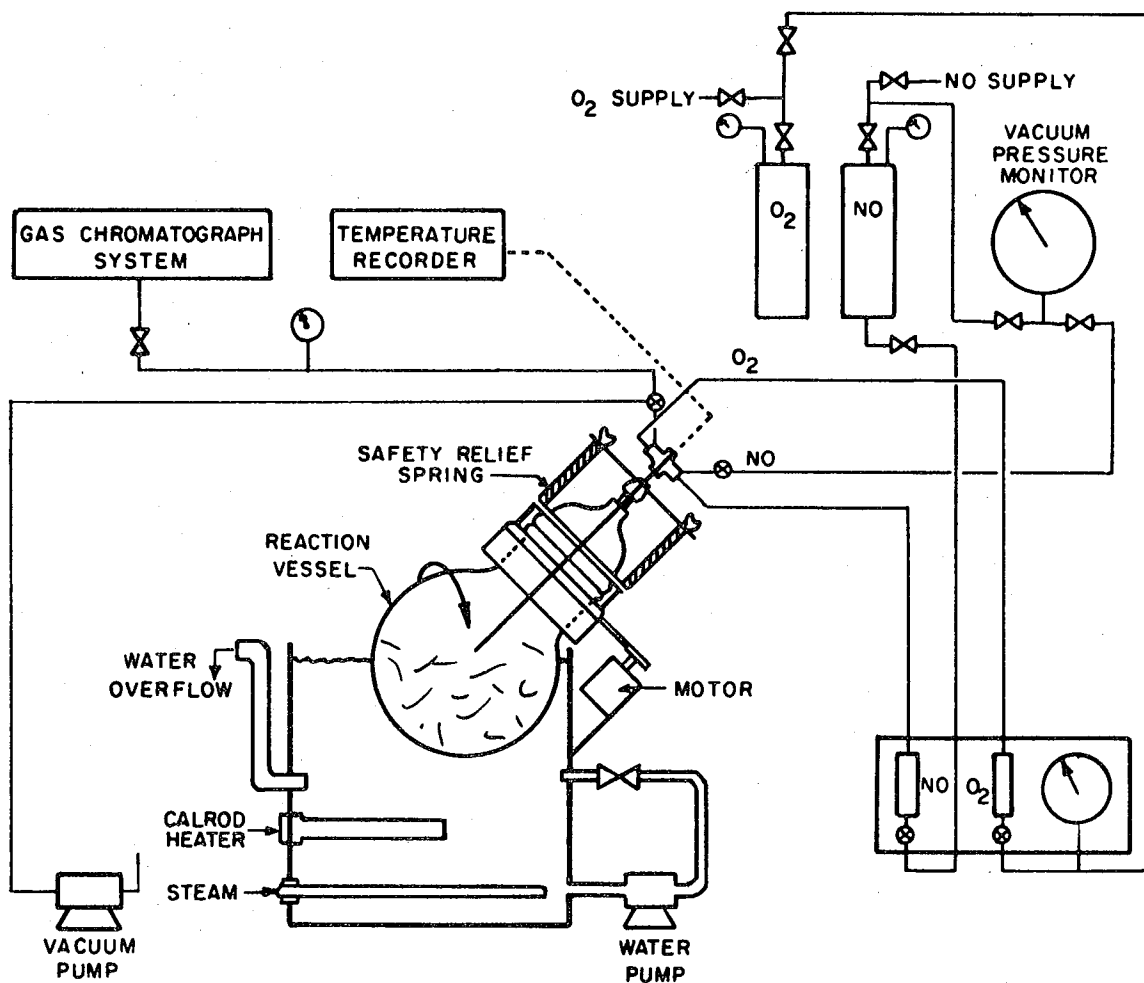
Fig. 5.1 Schematic diagram of gas-phase reaction system.(1)

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 experi-
   ment 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 (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

Ehrlenmeyer 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°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°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/1 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 supernatent 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 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 incu-

bation 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 α-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/

```
                        SOLID MATERIAL
                              |
                              | Wiley mill,
                              | sieve,
                              | dry at 105 °C
                              ▼
                    40-60 MESH FRACTION

                              | Benzene/Ethanol Extraction
                              ▼
                    EXTRACTED MATERIAL

                              | Exhaustive Hydrolysis
         _____|_____
        |                                                  |
     RESIDUE                                            LIQUOR
        |                                                  |
        | dry at 105 °C,                                   | Gas-Liquid
        | weigh                                            | Chromatography
        ▼                                                  ▼
   LIGNIN + ASH                                       CARBOHYDRATE
        |                                                CONTENT
        | 600 °C
        ▼
       ASH
```
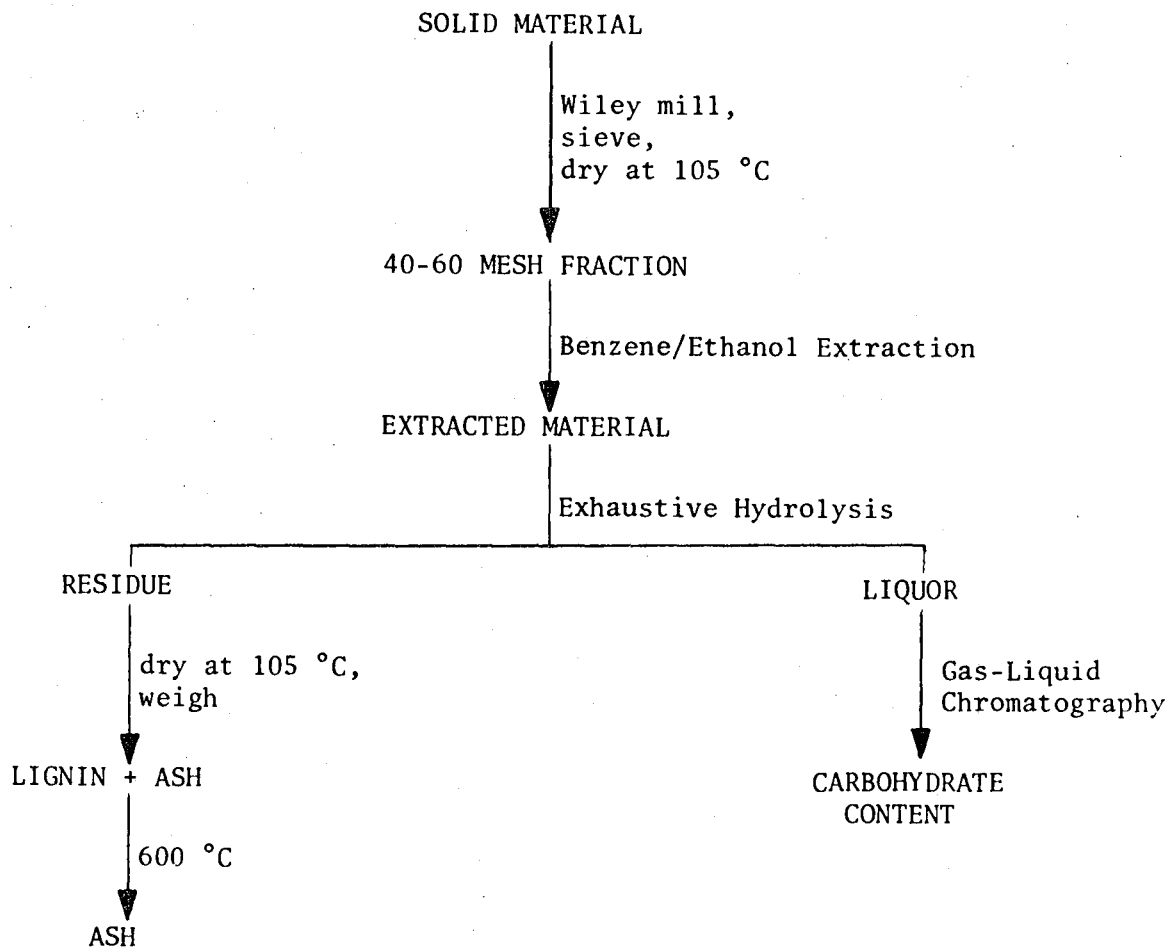
Fig. 5.2 Solid analysis procedure.

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_2, N_2, NO, and CO were detected when eluting from the second column onto the reference side of the detector, whereas N_2O and CO_2 eluted from the first column onto the sample side of the detector.

The NO_2 absorption flask was a 50 ml round bottom flask to which 10 ml of a solution containing glacial acetic acid and N-1-napthyl-ethylenediamine dihydrochloride was added (7).

The acetone-dry ice bath functioned to prevent NO_2 and water vapor from entering the column.

The procedure was as follows:

1) The sample line and NO_2 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_2 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_2 absorbance was also converted

to concentration by the method of Lin.

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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.

	100 g wheat straw	liquor 24.6 g	75.4 g solid
Glucose	36.6	7.3	29.1
Xylose	19.2	1.7	17.6
Arabinose	2.4	0.1	1.9
Mannose	0.8	--	0.0
Galactose	2.4	1.2	1.2
Cellobiose	--	0.0	--
Lignin	14.5	0.3	14.2
Ash	9.6	4.0	5.6
Extractives	7.2	4.5	2.7
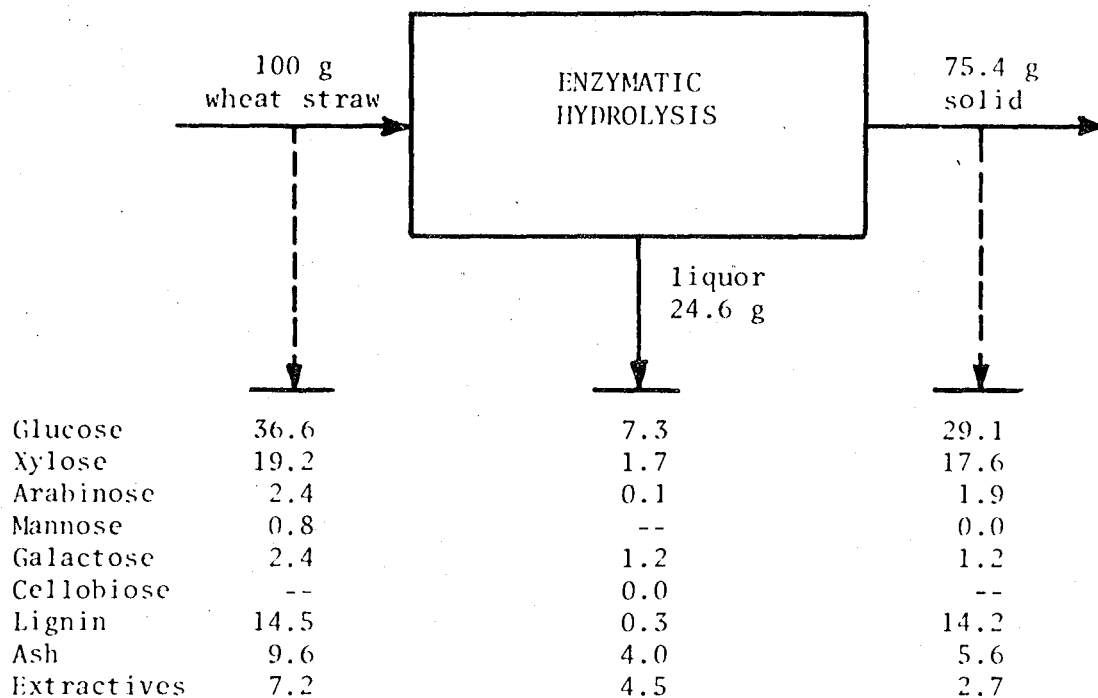
Fig. 6.1 Experiment 1. Enzymatic hydrolysis of untreated wheat straw.

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 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).

	100 g Wheat Straw	NO$_x$ Reaction → 102.0 g solid	Extraction Stage: Liquor 36.9 g pH 1.7	Extraction Stage → 65.1 g solid	Enzymatic Hydrolysis: Liquor 30.3 g	→ 34.8 g solid
Glucose	36.6		0.2	36.2	17.8	13.4
Xylose	19.2		4.1	9.3	4.2	2.3
Arabinose	2.4		--	0.3	0.1	0.2
Mannose	0.8		--	--	--	--
Galactose	2.4		--	--	--	--
Cellobiose	--		0.0	0.0	2.8	0.0
Lignin	14.5		4.6	9.9	2.8	7.1
Ash	9.6		3.0	6.6	4.2	2.4
Extractives	7.2		5.4	1.8	2.1	0.0

Reaction Conditions

NO$_x$ stage-- Sequentially add 5 g of NO and 25.8 g of air to straw. React at 25 °C and approx. atm. pressure for 24 hr.

Extraction-- Add 790 ml of water to 102 g of straw. Extract at 100 °C for 2 hr. at pH 1.7

% Yield Data

Glucose + CB $= \dfrac{20.9}{36.6} = 57.1$

Xylose $= \dfrac{8.3}{19.2} = 43.2$

Overall $= \dfrac{29.2}{61.4} = 47.6$

Fig. 6.2 Experiment 2. Results of 24-hour NO$_x$ reaction at 25°C followed by extraction for 2 hours at 100°C and pH 1.7.

	100 g Wheat Straw		102.0 g solid		61.3 g solid		22.3 g solid

Flow: 100 g Wheat Straw → NO$_x$ Reaction → 102.0 g solid → Extraction Stage → 61.3 g solid → Enzymatic Hydrolysis → 22.3 g solid

Extraction Stage → Liquor 40.7 g pH 7.8

Enzymatic Hydrolysis → Liquor 39.0 g

	Wheat Straw	Liquor 40.7 g pH 7.8	61.3 g solid	22.3 g solid
Glucose	36.6	0.0	36.0	18.7
Xylose	19.2	0.0	11.1	7.2
Arabinose	2.4	0.0	0.8	0.3
Mannose	0.8	0.0	0.0	--
Galactose	2.4	0.0	--	--
Cellobiose	--	0.0	--	3.9
Lignin	14.5	7.6	6.9	--
Ash	9.6	1.4	8.2	--

Reaction Conditions

NO$_x$ stage-- Sequentially add 5 g of NO and 25.8 g of air to straw. React at 25 °C and approx. atm. pressure for 24 hr.

Extraction-- Add 740 ml of 1.1 w% NaOH solution to 102 g of straw. Extract at 100 °C for 2 hr.

% Yield Data

$$\text{Glucose} + \text{CB} = \frac{22.6}{36.6} = 61.7$$

$$\text{Xylose} = \frac{7.2}{19.2} = 37.5$$

$$\text{Overall} = \frac{30.1}{61.4} = 49.0$$

Fig. 6.3 Experiment 3. Results of 24-hour NO$_x$ reaction at 25°C followed by extraction for 2 hours at 100°C and pH 7.8.

	100 g Wheat Straw	NO$_x$ Reaction	102.0 g solid	Extraction Stage	Liquor 59.8 g pH 12.1	42.2 g solid	Enzymatic Hydrolysis	Liquor 28.3 g	13.9 g solid
Glucose	36.6				0.0	31.3		10.3	9.2
Xylose	19.2				0.0	5.8		2.8	1.1
Arabinose	2.4				0.0	0.5		0.5	0.1
Mannose	0.8				0.0	0.0		--	--
Galactose	2.4				0.0	--		--	--
Cellobiose	--				0.0	--		7.9	--
Lignin	14.5				12.3	2.2		0.5	1.7
Ash	9.6				5.9	3.7		2.4	1.3

Reaction Conditions

NO$_x$ stage-- Sequentially add 5 g of NO and 25.8 g of air to straw. React at 25 °C and approx. atm. pressure for 24 hr.

Extraction-- Add 740 ml of 2.2 w% NaOH to 102 g of straw. Extract at 100 °C for 1 hr.

% Yield Data

Glucose= $\dfrac{18.2}{36.6}$ = 49.7
+ CB

Xylose = $\dfrac{2.8}{19.2}$ = 14.6

Overall= $\dfrac{21.5}{61.4}$ = 35.0

Fig. 6.4 Experiment 4. Results of 24-hour NO$_x$ reaction at 25°C followed for 1 hour at 100°C and pH 12.1.

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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
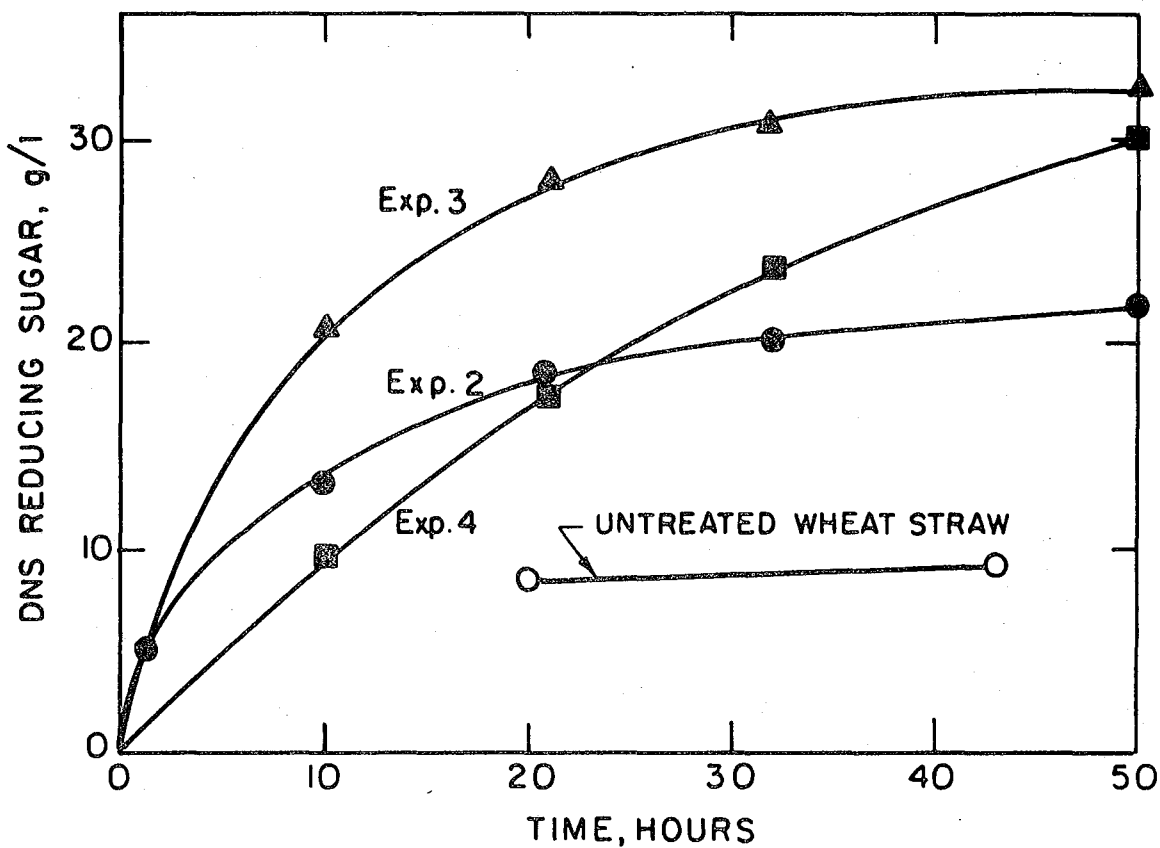
XBL788-5684

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 NO_x 24-hour
reacted straw was extracted at 100°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 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.

	100 g Wheat Straw	Liquor 31.5 g pH 6-7	70.5 g solid	Liquor 33.7 g	36.8 g solid
Glucose	36.6	0.1	33.8	13.7	11.8
Xylose	19.2	0.7	12.8	7.0	5.3
Arabinose	2.4	0.6	1.3	0.5	0.6
Mannose	0.8	--	0.3	--	--
Galactose	2.4	--	2.2	2.1	0.0
Cellobiose	--	0.0	--	1.3	--
Lignin	14.5	5.4	9.1	0.7	8.4
Ash	9.6	5.1	4.5	3.6	6.0

Flow: 100 g Wheat Straw → NO_x Reaction → 102.0 g solid → Extraction Stage → 70.5 g solid → Enzymatic Hydrolysis → 36.8 g solid

Reaction Conditions

NO_x stage-- Sequentially add 5 g of NO and 25.8 g of air to straw. React at 25 °C and approx. atm. pressure for 24 hr.

Extraction-- Add 1065 ml of water and 1.8 g of NaOH to straw to adjust pH to 6-7. Extract at 100 °C for 1 hr. keeping pH between 6 and 7 with NaOH (an additional 0.5 g of NaOH).

% Yield Data

$$\text{Glucose + CB} = \frac{15.1}{36.6} = 41.3$$

$$\text{Xylose} = \frac{7.7}{19.2} = 40.1$$

$$\text{Overall} = \frac{26.0}{61.4} = 42.3$$

Fig. 6.7   Experiment 5. Results of 24-hour NO_x reaction at 25°C followed by extraction for 1 hour at 100°C and pH 6 to 7.

```
      100 g                    102.0 g                 70.5 g                  44.0 g
   Wheat Straw       NO_x       solid    Extraction     solid    Enzymatic     solid
                   Reaction              Stage                   Hydrolysis
                      |                              |
                      |                  Liquor      |           Liquor
                      |                  29.5 g      |           26.5 g
                      ▼                  ▼ pH 5.1     ▼           ▼
Glucose      36.6                          0.0        32.6        10.9
Xylose       19.2                          0.0        10.0         5.7
Arabinose     2.4                          0.0         1.0         0.3
Mannose       0.8                          0.0         0.1         0.1
Galactose     2.4                          0.0         2.4         1.8
Cellobiose    --                           0.0         --          1.9
Lignin       14.5                          3.7        10.8         --
Ash           9.6                          5.0         4.6         --
```

Reaction Conditions   % Yield Data

NO_x stage-- Sequentially add 5 g of NO and 25.8 g of air to straw.   Glucose= $\frac{12.8}{36.6}$ = 35.0
               React at 25 °C and approx. atm. pressure for 24 hr.   + CB

Extraction-- Add 1040 ml of water to straw. Mix at 22 °C for 1 hr. (pH 1.8). Xylose = $\frac{5.7}{19.2}$ = 29.7
             Filter water from saving and analyzing leachate. Add an addi-
             tional 810 ml of water to straw (pH --- 5.1), then extract at   Overall= $\frac{20.7}{61.4}$ = 33.7
             100 °C for 1 hr.

Fig. 6.8 Experiment 6. Results of 24-hour NO_x reaction at 25°C followed by water leaching,
          then extraction for 1 hour at 100°C and pH 5.1.

	100 g Wheat Straw	NOₓ Reaction → 102.0 g solid	Extraction Stage → Liquor 12.8 g pH 5.3	89.2 g solid	Enzymatic Hydrolysis → Liquor 40.1 g	49.1 g solid
Glucose	36.6		0.0	34.3	11.1	21.0
Xylose	19.2		0.0	16.9	7.3	9.3
Arabinose	2.4		0.0	2.0	0.7	0.7
Mannose	0.8		0.0	0.8	0.6	0.2
Galactose	2.4		0.0	2.4	1.9	0.5
Cellobiose	--		0.0	--	0.2	--
Lignin	14.5		5.2	9.3	1.0	8.3
Ash	9.6		3.9	5.5	1.9	3.6

Reaction Conditions

NOₓ stage-- Sequentially add 5 g of NO and 25.8 g of air to straw. React at 25 °C and approx. atm. pressure for 24 hr.

Extraction-- Add 1390 ml of water and 0.6 g of NaOH to 102 g of straw (pH 5.3). Immediately filter liquor from straw.

% Yield Data

$$\text{Glucose} + \text{CB} = \frac{11.3}{36.6} = 30.9$$

$$\text{Xylose} = \frac{7.3}{19.2} = 38.0$$

$$\text{Overall} = \frac{21.8}{61.4} = 35.5$$

Fig. 6.9 Experiment 7. Results of 24-hour NOₓ reaction followed by neutralization to pH 5.3, but not employing extraction.

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

	100 g Wheat Straw	101.5 g solid		63.6 g solid		34.0 g solid

Reaction boxes: NO$_x$ Reaction → Extraction Stage → Enzymatic Hydrolysis

Extraction Stage: Liquor 37.9 g, pH 1.7

Enzymatic Hydrolysis: Liquor 29.6 g

Glucose	36.6	0.2	17.6
Xylose	19.2	3.9	3.9
Arabinose	2.4	0.0	0.1
Mannose	0.8	--	--
Galactose	2.4	--	--
Cellobiose	--	0.0	3.2

Reaction Conditions

NO$_x$ stage-- Sequentially add 5 g of NO and 6 g of oxygen to straw. React at 25 °C and approx. atm. pressure for 24 hr.

Extraction--Add 715 ml of water to 101.5 g of straw. Extract at 100 °C for 5 hr.

% Yield Data

Glucose = $\frac{21.0}{36.6}$ = 57.4
+ CB

Xylose = $\frac{7.8}{19.2}$ = 40.6

Overall = $\frac{28.9}{61.4}$ = 47.1

Fig. 6.10 Experiment 8. Results of 24-hour NO$_x$ 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:

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

which upon integration gives:

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

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 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 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 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 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:

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

and:

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

where:

$\qquad [H^+] =$ hydrogen ion concentration

The values of k_1' calculated from Equation (6.4) are 4.96, 4.53, and 4.40 (liter/gmole)(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.	initial xylose as xylan, g [a]	liquor volume, ml	pH	time, hours	xylose conc., g/l	xylose in solution, g	total xylose -initial xylose, g [X]	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
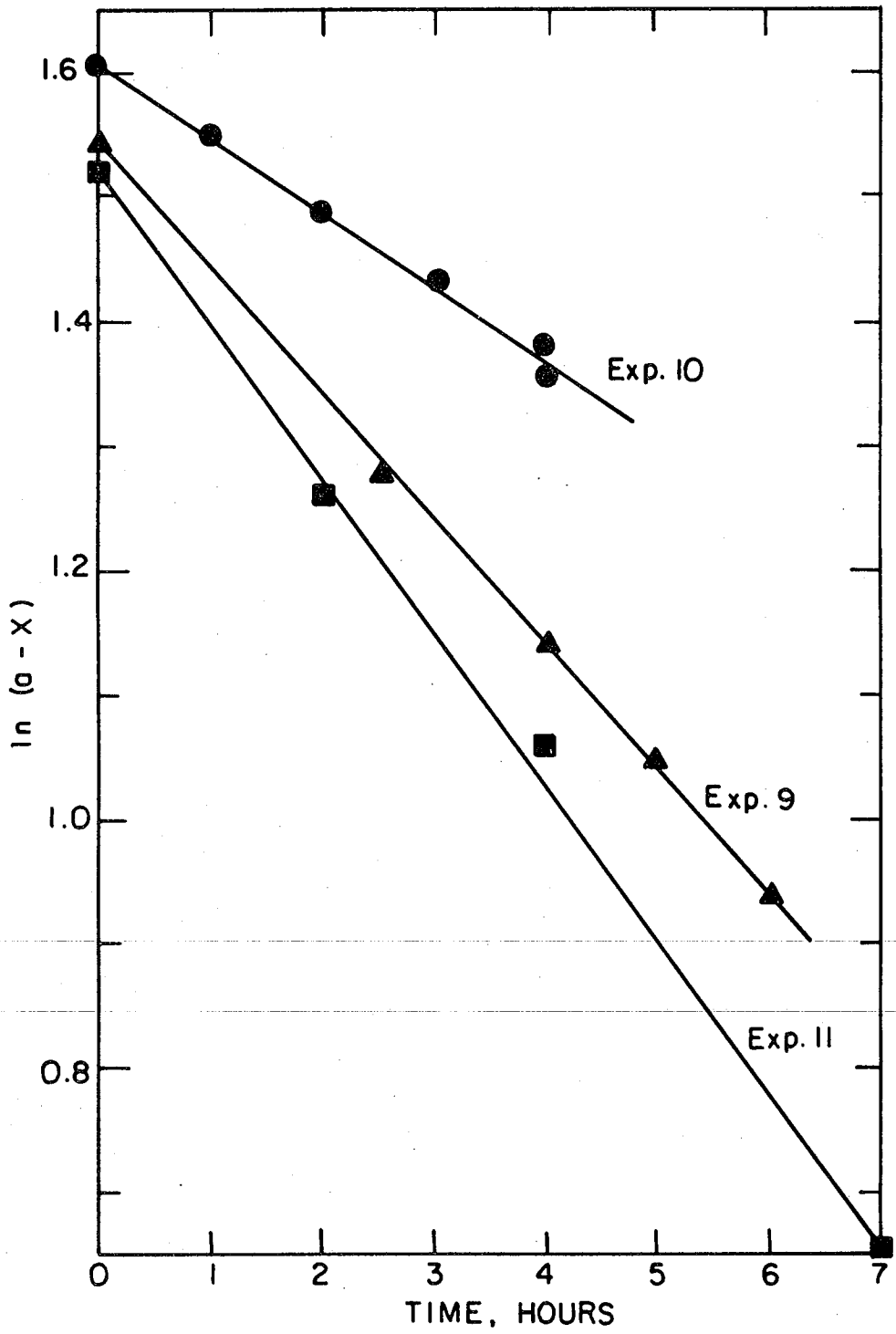
Fig. 6.11 Xylose formation kinetic experiments
in extraction liquor at 100°C.

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:

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

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.$^{-1}$

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^{-1} 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^{-1}, 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
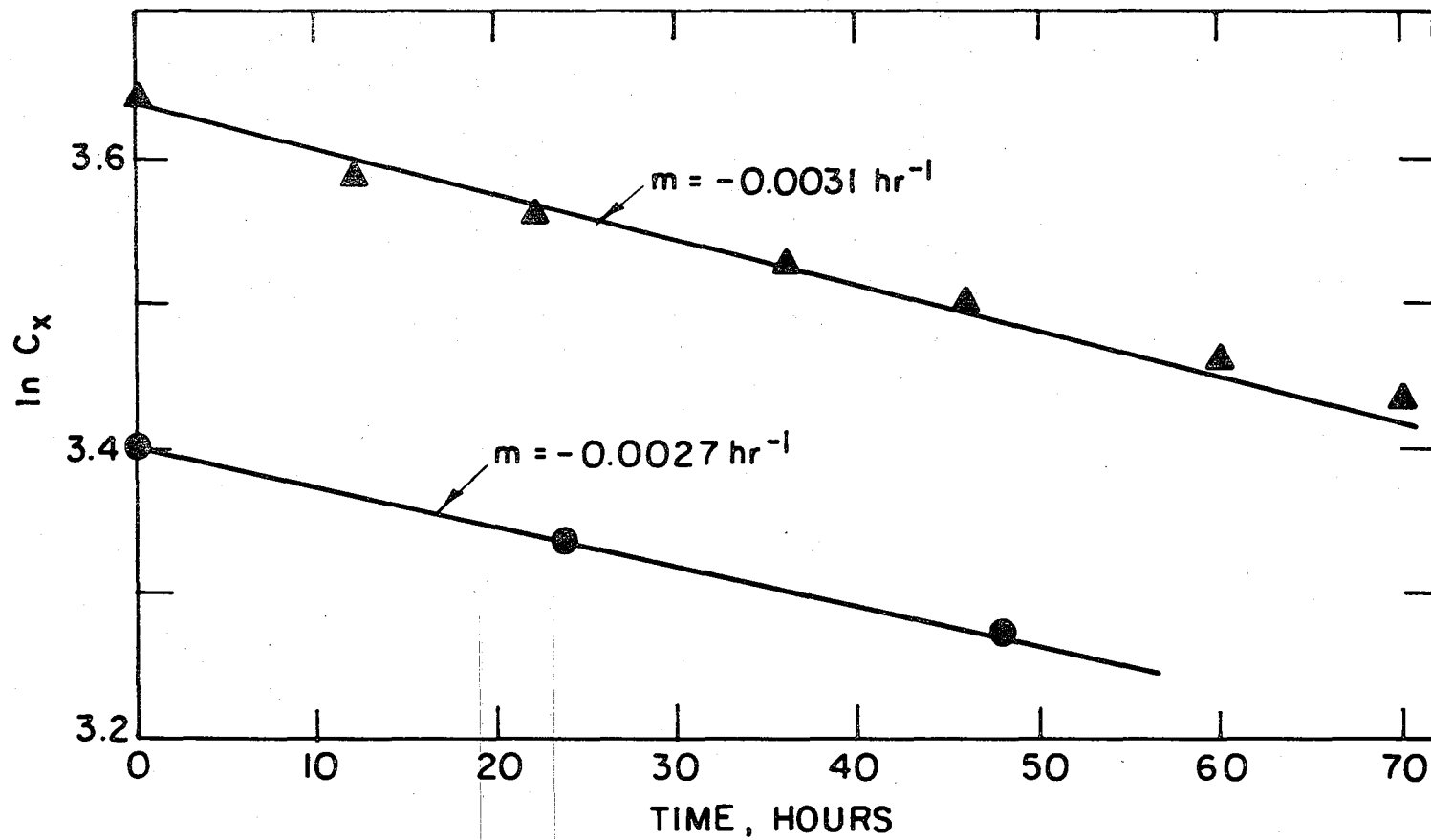
Fig. 6.12 Stability of xylose in acidic extraction liquor.
▲ this work, ● data of Freitas (4).

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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:

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

and occurs at time:

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

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:

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

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

6.5 The Effect of Varying Reaction Time of 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

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

| | 100 g Wheat Straw | → | NOx Reaction | → | 107.5 g solid | → | Extraction Stage | → | 66.4 g solid | → | Enzymatic Hydrolysis | → | 42.9 g solid | → |

Liquor 41.1 g pH 1.7

Liquor 23.5 g

Glucose	36.6		1.3	13.8
Xylose	19.2		6.0	3.2
Arabinose	2.4		2.0	0.1
Mannose	0.8		--	0.1
Galactose	2.4		--	0.6
Cellobiose	--		0.0	0.6

Reaction Conditions

% Yield Data

NOx stage-- Sequentially add 5 g of NO and 25.8 g of air to straw. React at 25 °C and approx. atm. pressure for 20 min.

Extraction-- Add 830 ml of water to 107.5 g of straw. Extract at 100 °C for 5 hr.

$$\text{Glucose} + \text{CB} = \frac{15.7}{36.6} = 42.9$$

$$\text{Xylose} = \frac{9.2}{19.2} = 47.9$$

$$\text{Overall} = \frac{27.5}{61.4} = 44.8$$

Fig. 6.14 Experiment 12. Results for 20-minute NOx reaction at 25°C followed by extraction for 5 hours at 100°C and pH 1.7.

```
     100 g                     106.7 g                    63.8 g                    41.5 g
  Wheat Straw      ┌─────────┐  solid   ┌──────────┐ solid   ┌──────────┐ solid
 ───────────────→ │   NO_x  │ ───────→ │Extraction│ ──────→ │ Enzymatic│ ─────────→
                  │ Reaction │          │  Stage   │          │Hydrolysis│
          │       └─────────┘          └──────────┘          └──────────┘
          │                                 │ Liquor              │ Liquor
          │                                 │ 42.9 g              │ 22.3 g
          ▼                                 ▼ pH 1.7              ▼
```

Glucose	36.6	1.9	12.9
Xylose	19.2	6.7	2.8
Arabinose	2.4	2.3	0.1
Mannose	0.8	--	0.1
Galactose	2.4	--	0.1
Cellobiose	--	0.0	0.1

Reaction Conditions

NO$_x$ stage-- Sequentially add 5 g of NO and 25.8 g of air to straw.
React at 25 °C and approx. atm. pressure for 1 hr.

Extraction-- Add 840 ml of water to 106.7 g of straw. Extract at
100 °C for 5 hr.

% Yield Data

Glucose= $\dfrac{14.9}{36.6}$ = 40.7
+ CB

Xylose = $\dfrac{9.5}{19.2}$ = 49.5

Overall= $\dfrac{27.2}{61.4}$ = 44.3

Fig. 6.15 Experiment 13. Results of 1 hour NO$_x$ reaction at 25°C followed by extraction for
5 hours at 100°C and pH 1.7.

```
   100 g                        105.6 g                    63.0 g                        41.5 g
   Wheat Straw    ┌─────────┐   solid    ┌───────────┐     solid    ┌────────────┐       solid
   ──────────────▶│   NOx   │───────────▶│ Extraction│─────────────▶│  Enzymatic │──────────────▶
             │    │ Reaction│            │   Stage   │              │ Hydrolysis │
             │    └─────────┘            └───────────┘              └────────────┘
             │                                 │ Liquor                   │ Liquor
             │                                 │ 42.6 g                   │ 21.5 g
             │                                 ▼ pH 1.7                   ▼
             ▼
```

Glucose	36.6	1.4	15.0
Xylose	19.2	11.6	2.6
Arabinose	2.4	2.0	0.0
Mannose	0.8	0.8	0.0
Galactose	2.4	0.1	0.9
Cellobiose	--	0.0	1.3

Reaction Conditions

NOx stage-- Sequentially add 5 g of NO and 25.8 g of air to straw.
            React at 25 °C and approx. atm. pressure for 2 hr.

Extraction-- Add 740 ml of water to 105.6 g of straw. Extract at
             100 °C for 7 hr.

% Yield Data

Glucose= $\frac{17.7}{36.6}$ = 48.3
+ CB

Xylose = $\frac{14.2}{19.2}$ = 74.0
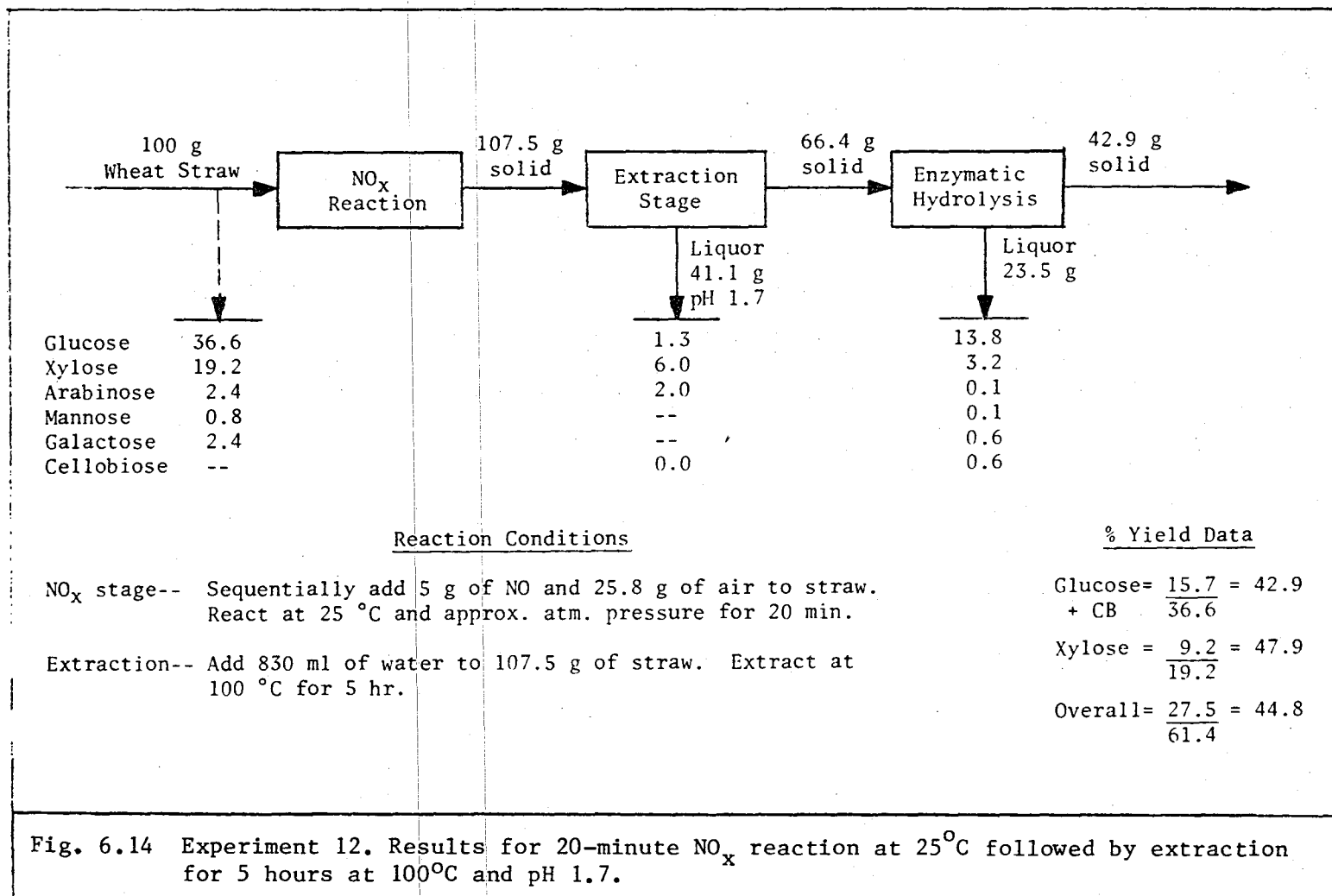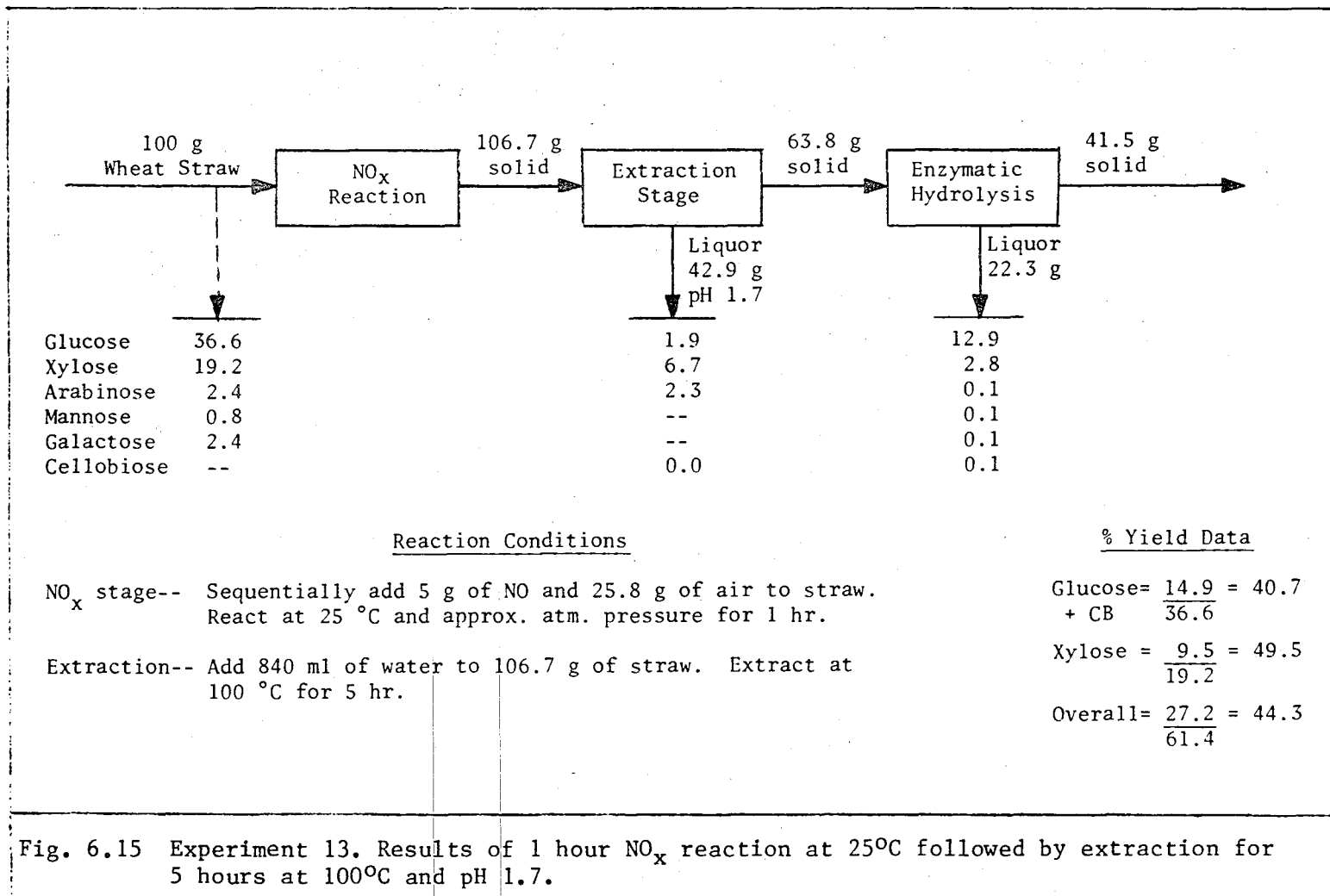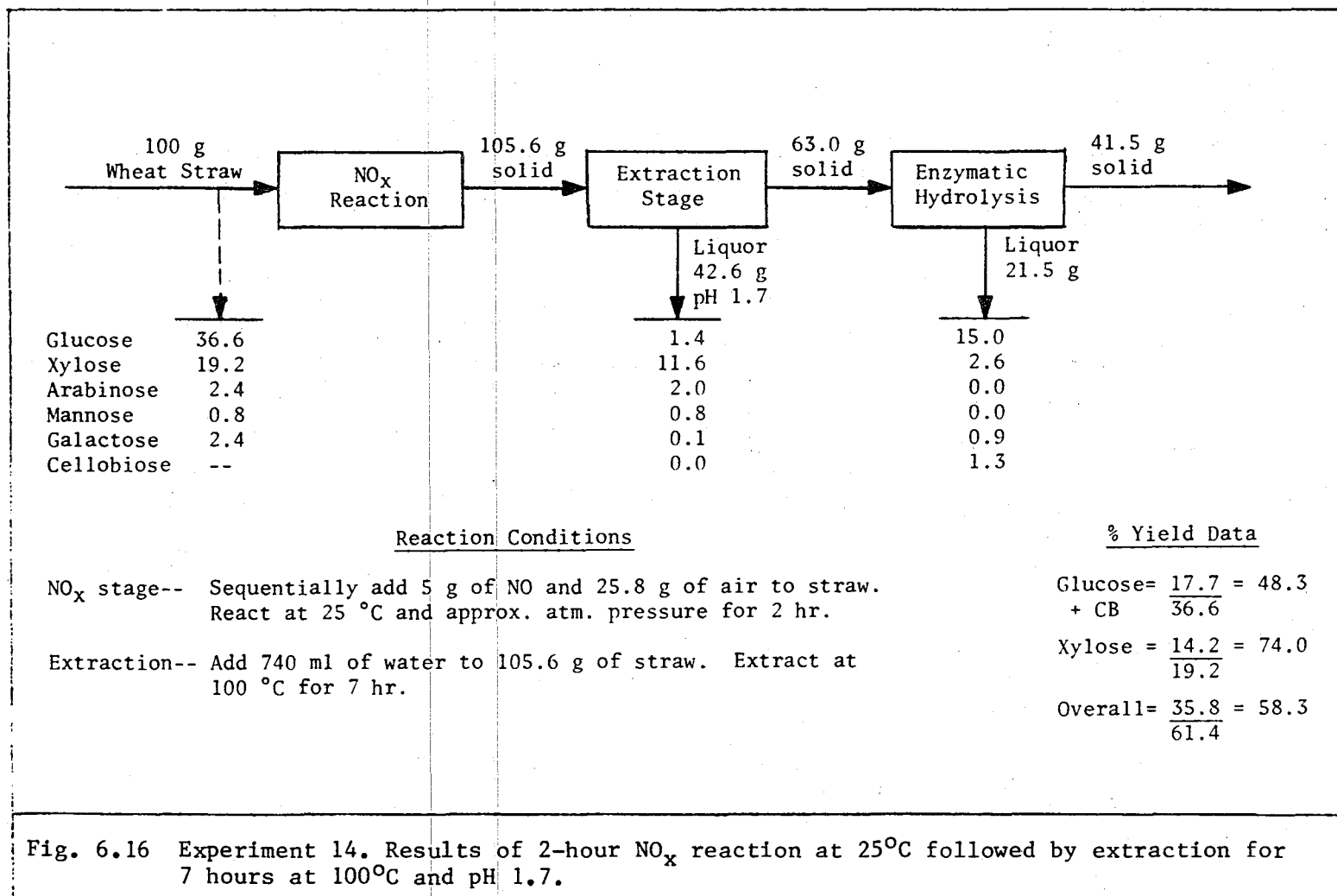
Overall= $\frac{35.8}{61.4}$ = 58.3

Fig. 6.16   Experiment 14. Results of 2-hour NO_x reaction at 25°C followed by extraction for
            7 hours at 100°C and pH 1.7.

these experiments it can be seen that substantially more xylose is formed during the extraction stage for the two hour NO_x 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_x 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
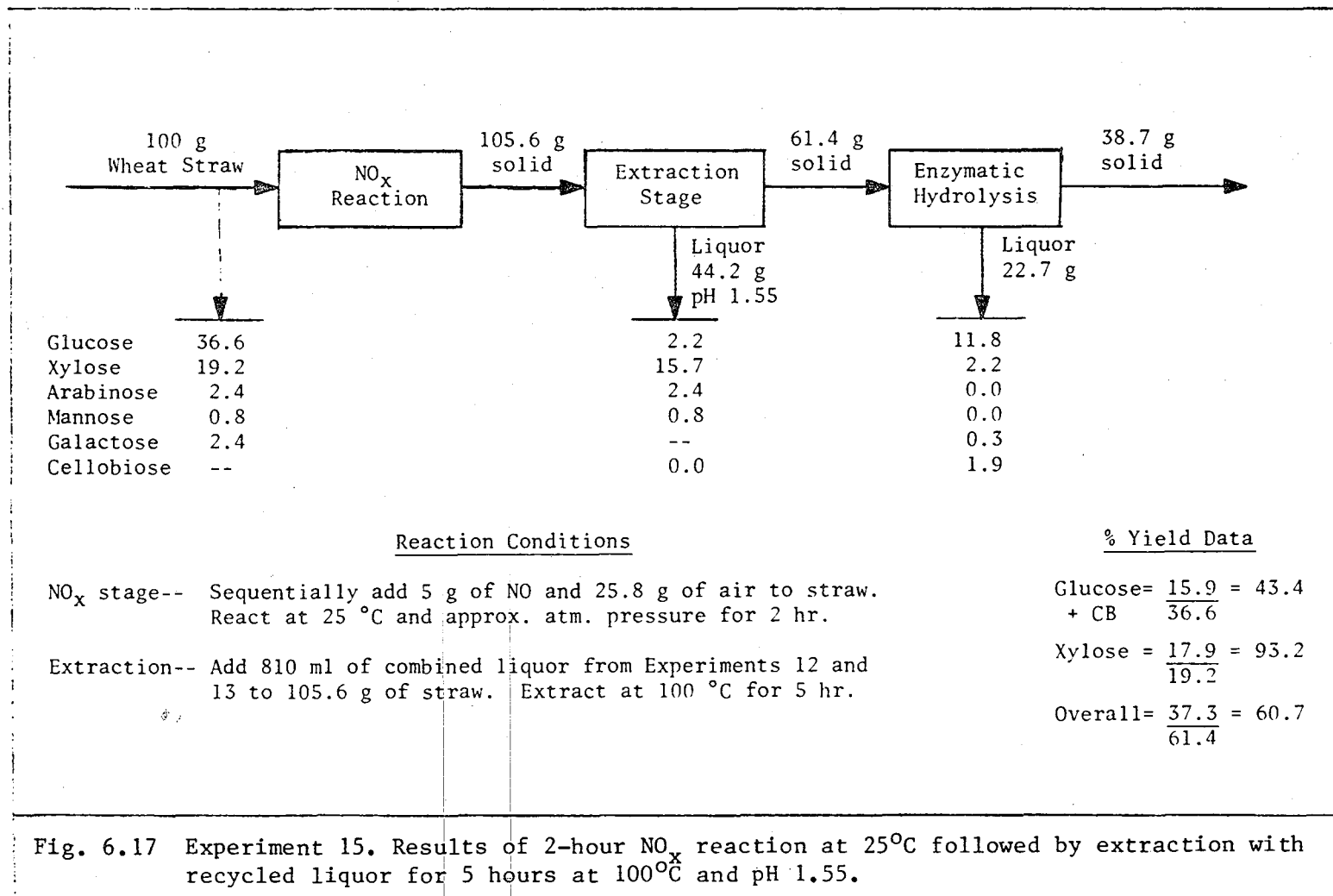
the rate of hydrolysis of the xylan.

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 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 hr.$^{-1}$ This corresponds to a k_1' value (defined in Equation (6.4)) of 20.4 (liter/gmole)(hr^{-1}), 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

| | 100 g Wheat Straw → | NOₓ Reaction | → 105.6 g solid | Extraction Stage | → 61.4 g solid | Enzymatic Hydrolysis | → 38.7 g solid |

Liquor 44.2 g pH 1.55

Liquor 22.7 g

Glucose	36.6	2.2	11.8
Xylose	19.2	15.7	2.2
Arabinose	2.4	2.4	0.0
Mannose	0.8	0.8	0.0
Galactose	2.4	--	0.3
Cellobiose	--	0.0	1.9

Reaction Conditions

NOₓ stage-- Sequentially add 5 g of NO and 25.8 g of air to straw. React at 25 °C and approx. atm. pressure for 2 hr.

Extraction-- Add 810 ml of combined liquor from Experiments 12 and 13 to 105.6 g of straw. Extract at 100 °C for 5 hr.

% Yield Data

Glucose= $\frac{15.9}{36.6}$ = 43.4
+ CB

Xylose = $\frac{17.9}{19.2}$ = 93.2

Overall= $\frac{37.3}{61.4}$ = 60.7

Fig. 6.17 Experiment 15. Results of 2-hour NOₓ reaction at 25°C followed by extraction with recycled liquor for 5 hours at 100°C and pH 1.55.

```
  100 g              102.0 g            68.9 g             33.2 g
Wheat Straw    NOx    solid  Extraction  solid  Enzymatic  solid
              Reaction       Stage              Hydrolysis

                                    Liquor            Liquor
                                    33.1 g            35.7 g
                                    pH 1.6
```

Glucose	36.6	2.0	19.6
Xylose	19.2	15.0	3.5
Arabinose	2.4	2.3	0.1
Mannose	0.8	--	0.1
Galactose	2.4	0.8	--
Cellobiose	--	0.0	0.0

Reaction Conditions

NO_x stage-- Sequentially add 5 g of NO and 25.8 g of air to straw.
React at 25 °C and approx. atm. pressure for 24 hr.

Extraction-- Add 1400 ml of liquor from Experiment 10 to 102 g of
straw. Extract at 100 °C for 5 hr.

% Yield Data

Glucose= $\frac{21.6}{36.6}$ = 59.0
+ CB

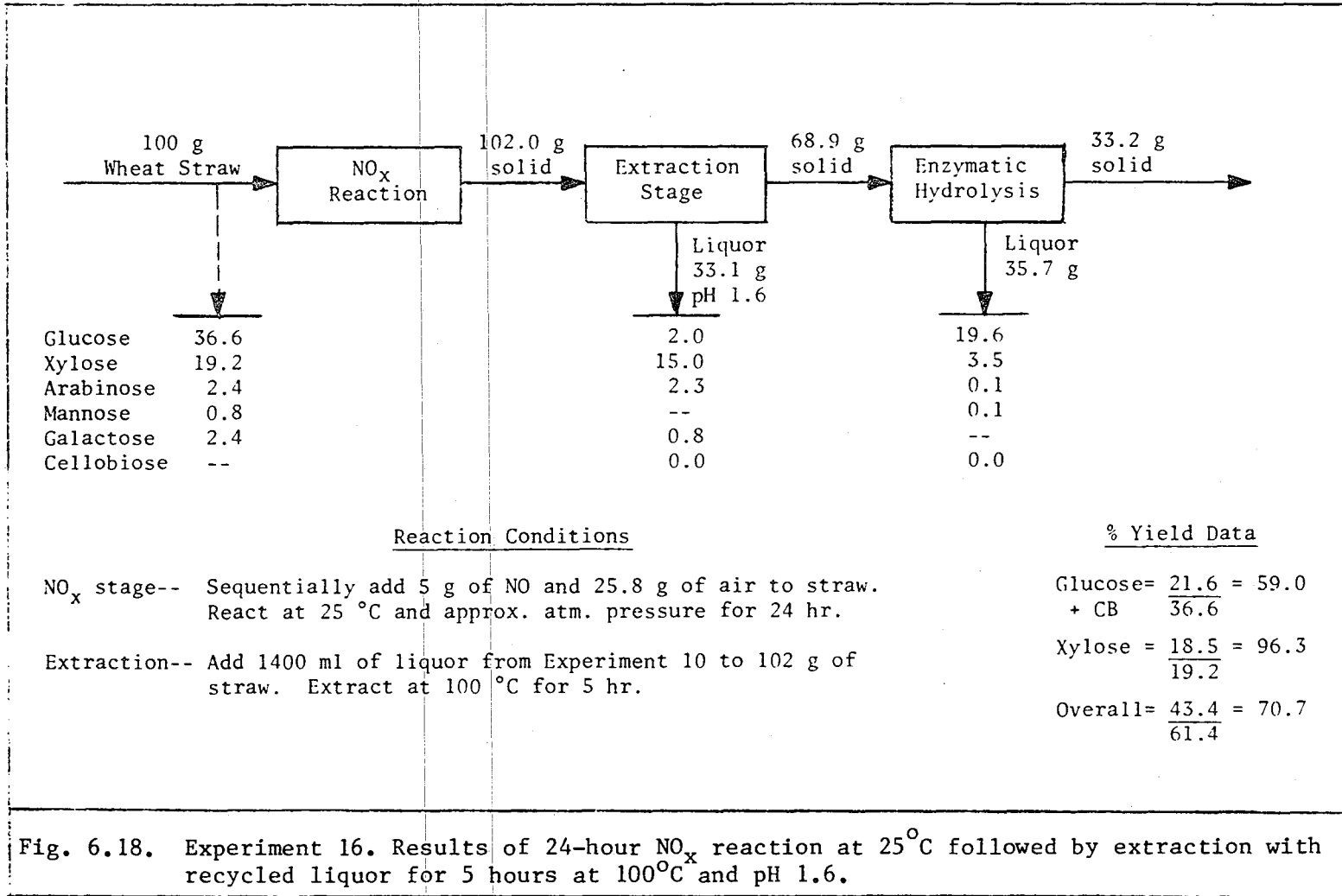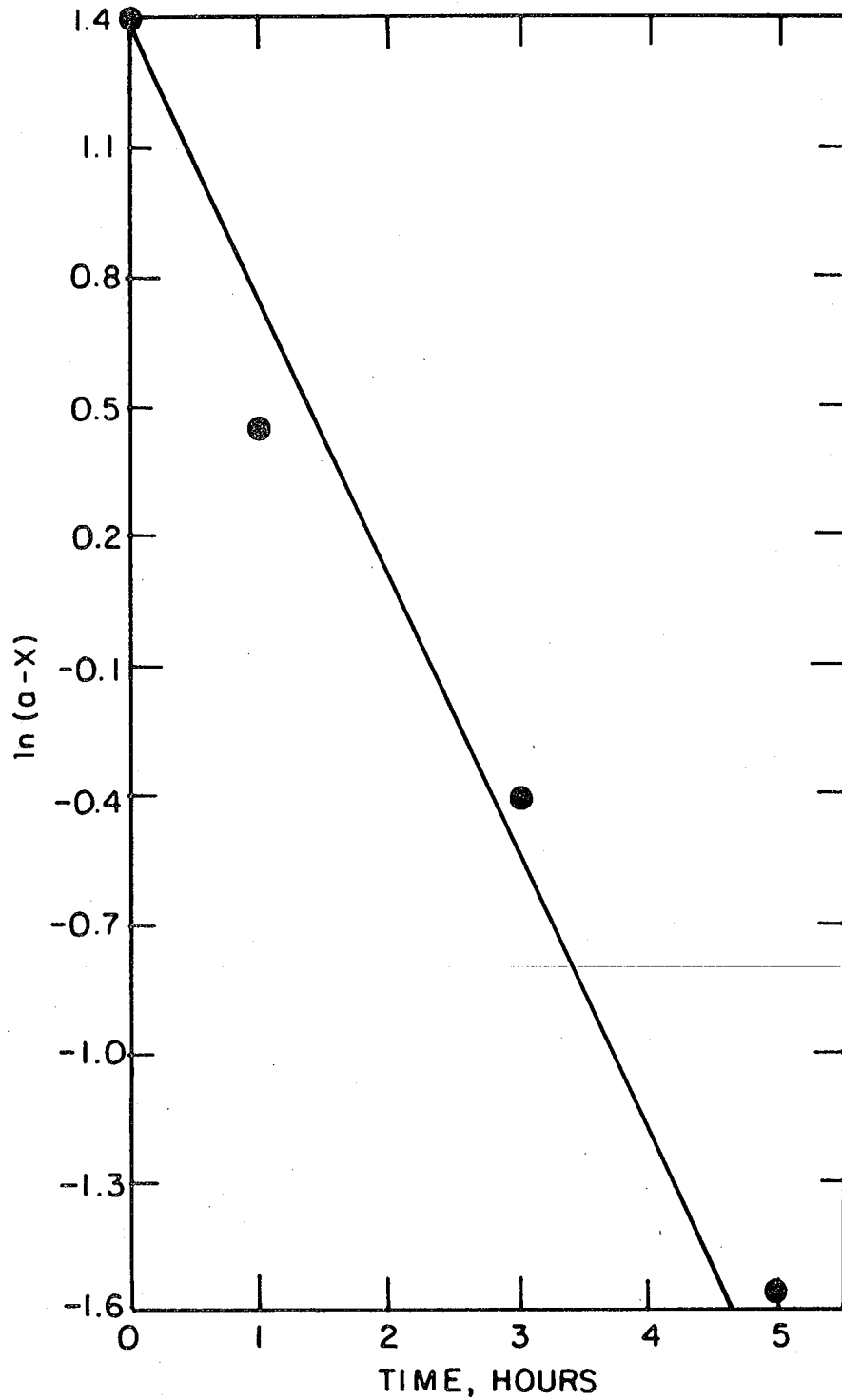Xylose = $\frac{18.5}{19.2}$ = 96.3

Overall= $\frac{43.4}{61.4}$ = 70.7

Fig. 6.18. Experiment 16. Results of 24-hour NO_x reaction at 25°C followed by extraction with recycled liquor for 5 hours at 100°C and pH 1.6.

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

XBL 788-5686

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 cor-

responds to a yield of 96.4%. The glucose yield is 59%.

6.7 NO_x 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_x gas phase reaction with cellulase are signifi-

cantly different at 80°C than at 25°C. Satisfactory pulping was

obtained only at the lower temperature. For this reason, an investiga-

tion 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_x 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

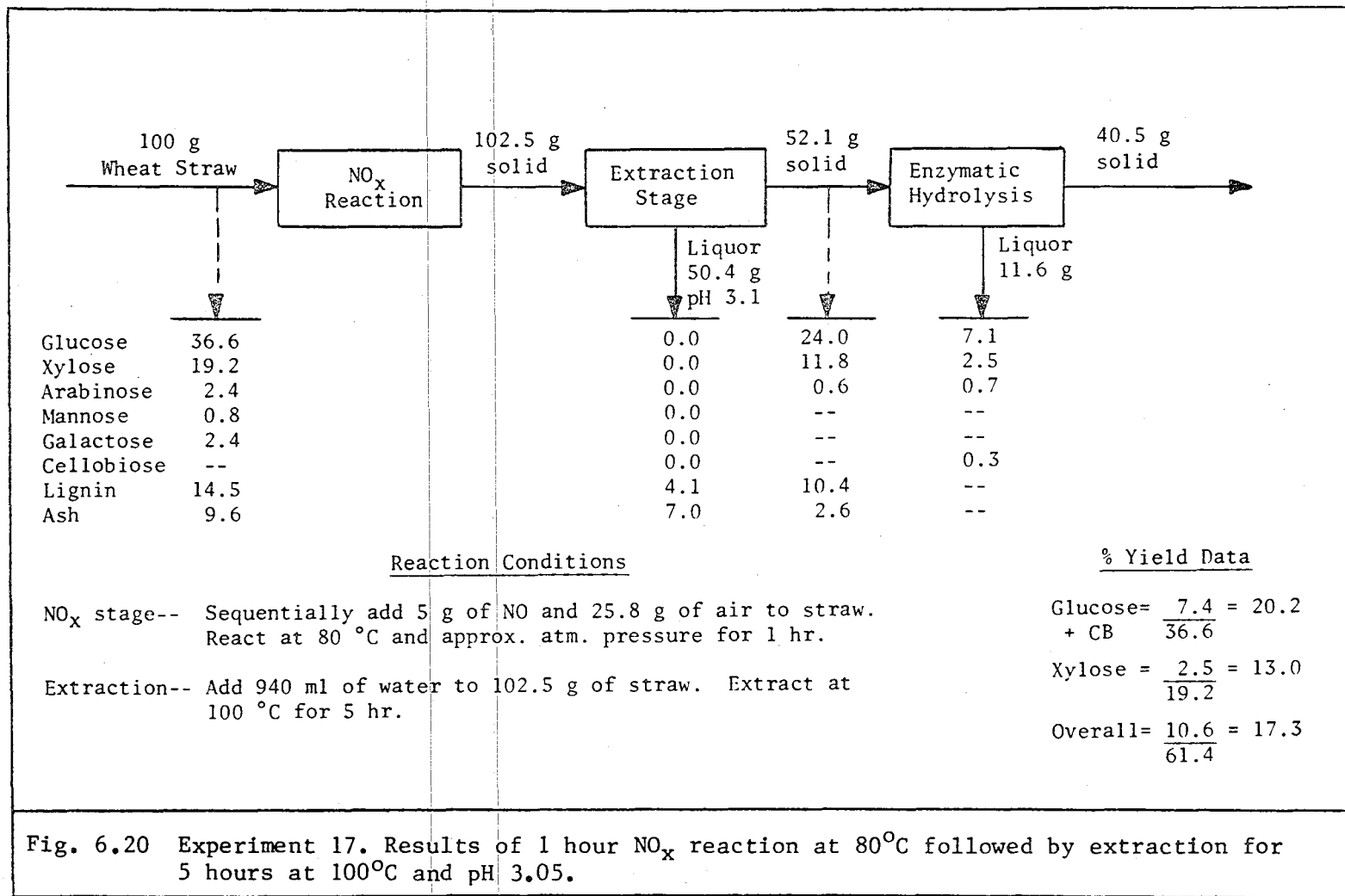value of 3 is not conductive to acid hydrolysis.

A NO_x reaction at 80°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°C

would be futile.

6.8   Composition of Off-Gas from NO_x Stage

In several experiments the composition of the gases found in the

head space of the NO_x gas phase reactor was determined. This was

done by the procedures outlined in 5.2.5. The results for NO_x reactions

at 25°C are shown in Table 6.3.

The results are somewhat surprising in the fact that no N_2O, NO,

CO_2, or 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 NO_2 was found to range between 1 and 2% of

the total gases. Clearly, this NO_2 must be removed in an absorber.

The composition is relatively constant after the intitial addition of
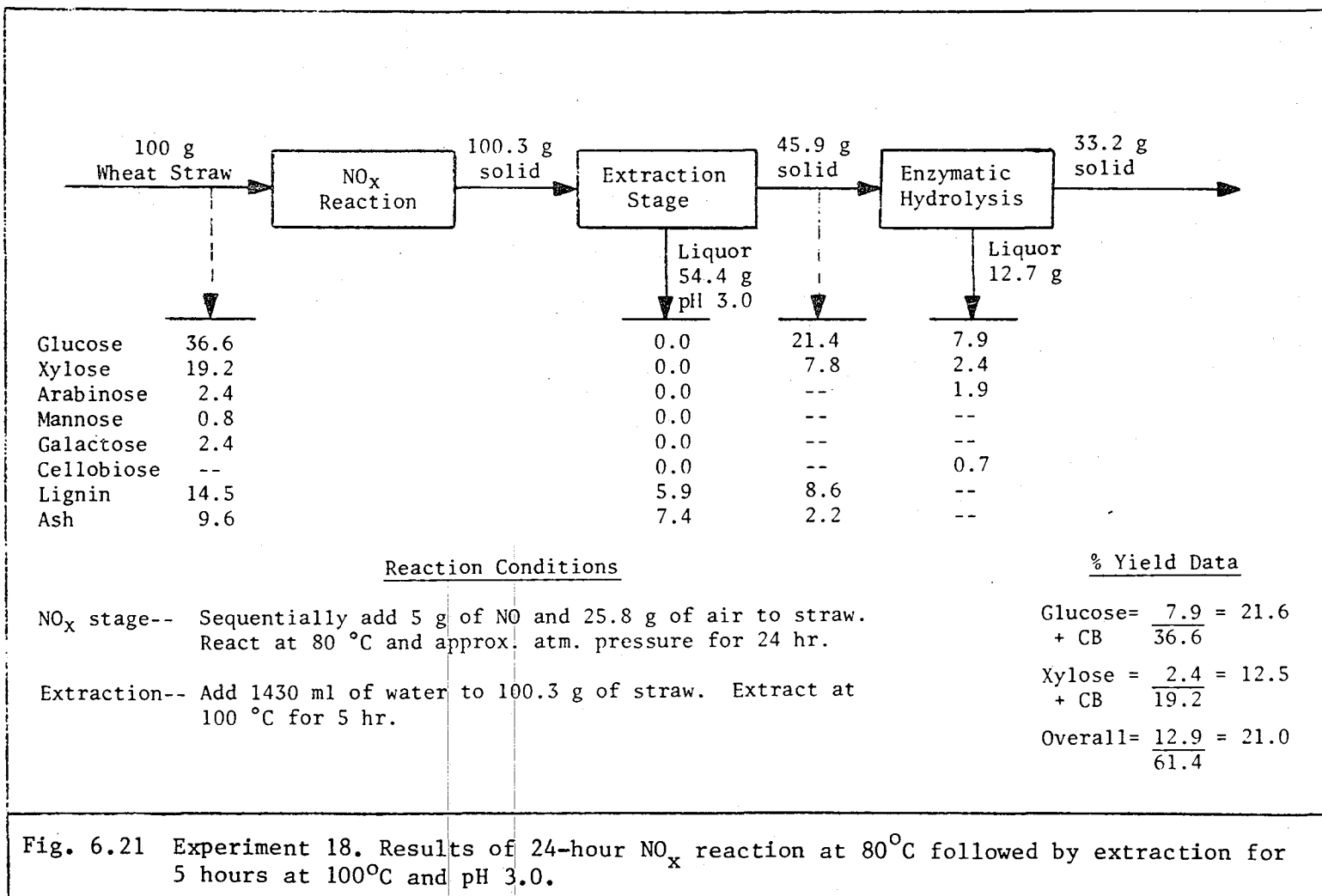
air.

	100 g Wheat Straw		102.5 g solid		52.1 g solid		40.5 g solid

Flow: 100 g Wheat Straw → NO$_x$ Reaction → 102.5 g solid → Extraction Stage → 52.1 g solid → Enzymatic Hydrolysis → 40.5 g solid

Extraction Stage → Liquor 50.4 g pH 3.1
Enzymatic Hydrolysis → Liquor 11.6 g

	100 g Wheat Straw	Liquor 50.4 g pH 3.1	52.1 g solid	Liquor 11.6 g
Glucose	36.6	0.0	24.0	7.1
Xylose	19.2	0.0	11.8	2.5
Arabinose	2.4	0.0	0.6	0.7
Mannose	0.8	0.0	--	--
Galactose	2.4	0.0	--	--
Cellobiose	--	0.0	--	0.3
Lignin	14.5	4.1	10.4	--
Ash	9.6	7.0	2.6	--

Reaction Conditions

NO$_x$ stage-- Sequentially add 5 g of NO and 25.8 g of air to straw. React at 80 °C and approx. atm. pressure for 1 hr.

Extraction-- Add 940 ml of water to 102.5 g of straw. Extract at 100 °C for 5 hr.

% Yield Data

$$\text{Glucose} + \text{CB} = \frac{7.4}{36.6} = 20.2$$

$$\text{Xylose} = \frac{2.5}{19.2} = 13.0$$

$$\text{Overall} = \frac{10.6}{61.4} = 17.3$$

Fig. 6.20 Experiment 17. Results of 1 hour NO$_x$ reaction at 80°C followed by extraction for 5 hours at 100°C and pH 3.05.

	100 g Wheat Straw		NO$_x$ Reaction	100.3 g solid	Extraction Stage	45.9 g solid	Enzymatic Hydrolysis	33.2 g solid

Liquor 54.4 g pH 3.0

Liquor 12.7 g

Glucose	36.6	0.0	21.4	7.9
Xylose	19.2	0.0	7.8	2.4
Arabinose	2.4	0.0	--	1.9
Mannose	0.8	0.0	--	--
Galactose	2.4	0.0	--	--
Cellobiose	--	0.0	--	0.7
Lignin	14.5	5.9	8.6	--
Ash	9.6	7.4	2.2	--

Reaction Conditions

NO$_x$ stage-- Sequentially add 5 g of NO and 25.8 g of air to straw. React at 80 °C and approx. atm. pressure for 24 hr.

Extraction-- Add 1430 ml of water to 100.3 g of straw. Extract at 100 °C for 5 hr.

% Yield Data

Glucose = $\frac{7.9}{36.6}$ = 21.6
+ CB

Xylose = $\frac{2.4}{19.2}$ = 12.5
+ CB

Overall = $\frac{12.9}{61.4}$ = 21.0

Fig. 6.21 Experiment 18. Results of 24-hour NO$_x$ reaction at 80°C followed by extraction for 5 hours at 100°C and pH 3.0.

Table 6.3 Off-Gas Analysis Results for NO_x Reaction

	COMPOSITION (mole fraction)				
GAS	0.25 hr.	1 hr.	4 hr.	12 hr.	24 hr.
N_2	0.874	0.880	0.885	0.930	0.943
O_2	0.116	0.110	0.099	0.054	0.037
NO_2	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_2	0.707	0.714	0.718	0.720	0.715
O_2	0.094	0.089	0.080	0.042	0.028
NO_2	0.008	0.009	0.013	0.012	0.015
TOTAL	0.809	0.812	0.811	0.774	0.758

Chapter 6. References

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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 NO_x reaction temperature and time were 25°C and 24 hours, respectively. The extraction stage is designed for a solid residence time of 5.5 hours at 100°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 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 900-1000°C with a very short contact time to produce nitric oxide and water. The equation is:

$$4 \ NH_3 + 5O_2 \rightarrow 4NO + 6H_2O \qquad (7.1)$$
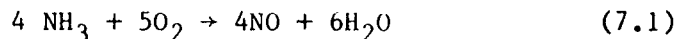
Product gases from the nitric oxide converter are then cooled to 50°C, condensing the water. Below 100°C, NO_2 is formed by the reaction between nitric oxide and excess oxygen:
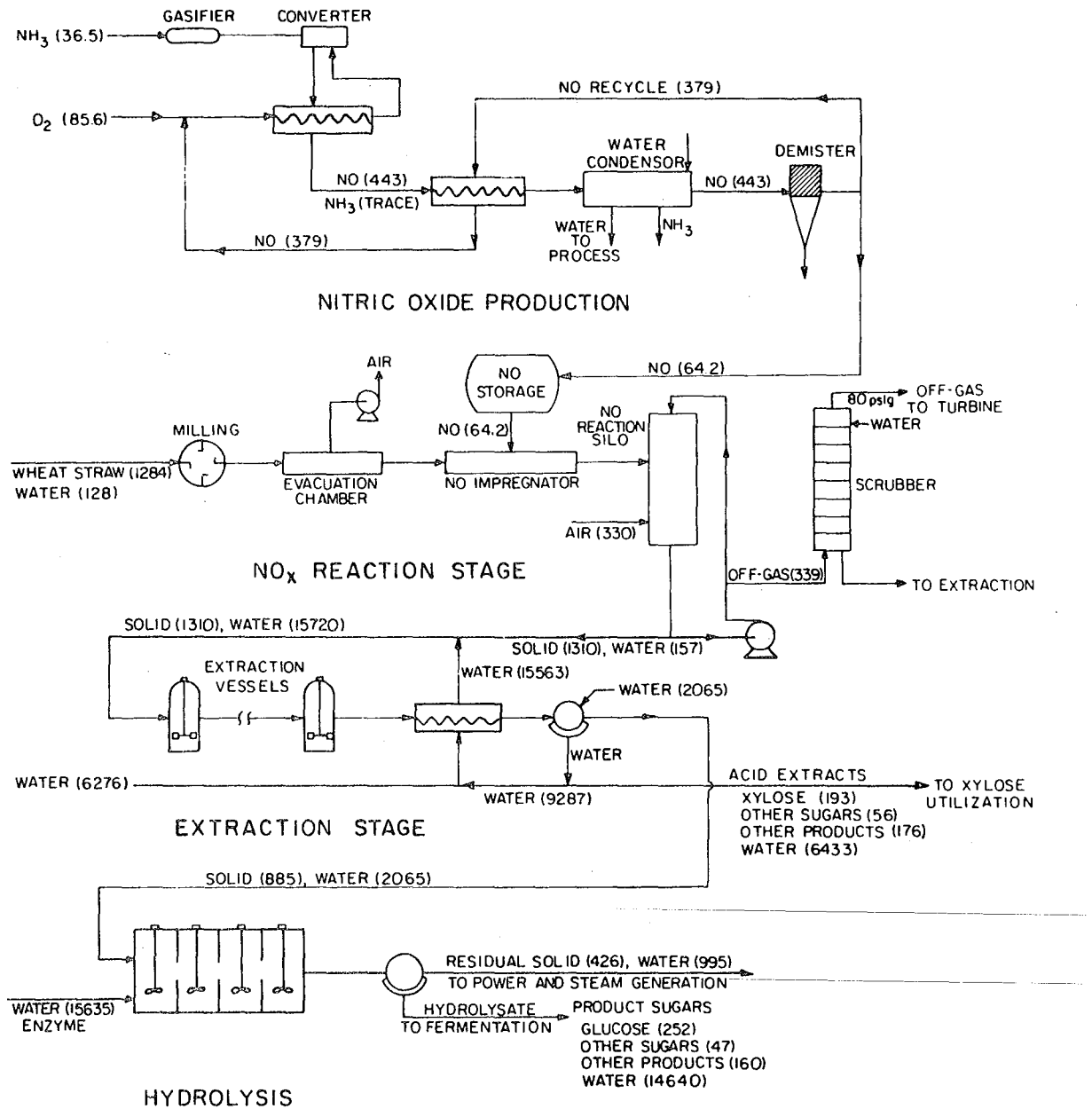
Fig. 7.1 Material balance flow diagram for processing scheme.

$$2NO + O_2 \rightarrow 2NO \qquad (7.2)$$

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:

$$3NO_2 + H_2O \rightarrow 2HNO_3 + NO \qquad (7.3)$$
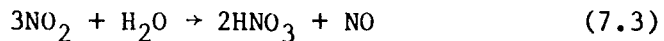
The nitric oxide formed at each plate reacts with oxygen, forming nitrogen dioxide, which is absorbed on the next plate.

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 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_x 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_2 passes to the absorber. 1310 tons per day of NO_x 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$_x$ 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$_x$ 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 con-
ducted 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 concentra-
tion 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 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_x 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_x 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_x 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_x 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_x 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_x Pretreatment vs. Dilute-Acid Pretreatment

GLUCOSE COST ¢/lb.	ASSUMING NO RAW MATERIAL COST	RAW MATERIAL AT $30/ton	TOTAL
NO_x 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_x 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).

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6. K.M. Guthrie, Chem. Eng., 77, No. 13, 140 (1970).

APPENDIX

COST CALCULATIONS FOR NITRIC OXIDE PRODUCTION AND NO_x 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_x reaction stage. Compressed oxygen is used instead of air.

The overall equation for nitric acid formation is:

$$NH_3 + 2O_2 \rightarrow HNO_3 + H_2O \qquad \text{(A.1)}$$
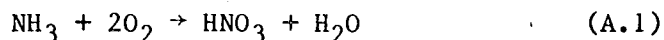
From Equation (A.1) it can be calculated that 46.2 tons of nitric oxide correspond to 135 tons/day of nitric acid. From Guthrie (1) the total capital investment required for a nitric acid plant of this size is $2,730,000. Assuming a Lange Factor of 3.65 (= 3.1/.85) gives the equipment cost to be $748,000.

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_x 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:

$$4NH_3 + 5O_2 \rightarrow 4NO + 6H_2O \qquad (A.2)$$
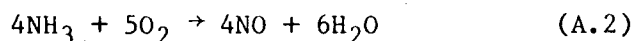
$$\frac{\text{raw material cost}}{\text{lb. NO}} = \frac{4(6.5)(17 \text{ lb./mole}) + 5(2.0)(32 \text{ lb./mole})}{(4 \text{ moles NO})(30 \text{ lb./mole})}$$

$$= 6.35¢/\text{lb.} = \$127/\text{ton}$$

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

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$_x$ Reaction Stage

As discussed in Chapter 7, the following major items of equipment

are required for the NO$_x$ reaction stage: air evacuation chamber, nitric

oxide impregnator, NO$_x$ 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 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) $\underline{NO_x \text{ Reaction Silo}}$ -- 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, 1.47×10^6 gallons, will be used. These

cost $547,000 each (stainless steel).

6) $\underline{\text{Off-Gas Scrubber}}$ -- 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 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 simlar 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 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 NO_x reaction stage may be taken as that required to power the compressor. This has been reported as 160 kw.hr/ton of HNO_3, which converts to $225,000 per year.

Appendix. References

1. K.M. Guthrie, Chem. Eng., 77, No. 13, 140 (1970).

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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_x Reaction Stage

Item	Unit Specification	Number of units	cost/unit $
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_x Reaction Silo	1.47×10^6 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

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