SACCHARIFICATION OF LIGNOCELLULOSIC MATERIALS

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Abstract — Pyrolytic reactions and enzymatic hydrolysis have been investi-
gated for solving the problems of saccharification and conversion of ligno-
cellulosic materials to sugar derivatives.

Pyrolysis of cellulose provides a tar that contains mainly levoglucosan
and its condensation products and can be readily hydrolyzed to glucose.
This reaction proceeds rapidly at 400-450°C to provide up to 85% yield of
tar from pure cellulose, depending on the substrate. However, best yields
are obtained from cellulose samples that have been acid treated. Wood and
raw cellulosic materials provide poor yields, unless they are treated with
inorganic acids. Based on these data a process has been devised for
saccharification of softwoods to fermentable sugars. In this process
softwood is hydrolyzed to remove the hemicelluloses (mainly glucomannans
and galactoglucomannans). The remaining lignocellulose is pyrolyzed to a
tar that is hydrolyzed to reducing sugars. This leaves a char, derived
mainly from lignin, that could be used as fuel. In this process a common
softwood such as Douglas fir can be converted to 14% char and 42% hexoses.
This amounts to 59% recovery of the hexoses, 32% from prehydrolysis and
27% from pyrolysis.

Acid catalyzed pyrolysis of cellulosic materials, particularly waste paper
provides a pyrolyzate that contains levoglucosenone (1,6-anhydro-3,4-
dideoxy-β-$\underline{D}$-glycero-hex-3-enopyranos-2-ulose) as the major component and
2-furaldehyde, 1,4:3,6-dianhydro-α-$\underline{D}$-glucopyranose and 5-(hydroxymethyl)-
2-furaldehyde as the minor components. The former compound could be
separated by distillation and used as a synthetic intermediate.

Enzymatic hydrolysis is inhibited by the crystallinity of cellulose and
the restraining effect of lignin. It has been shown that simultaneous
wet milling, in an attritor containing stainless steel beads, results in
substantial enhancement of enzymatic hydrolysis. Attrition is more effec-
tive than ball milling because it provides a continuous and effective
method for delamination and comminution of the substrate with apparent
formation of new reactive sites. Organosolv pulping eliminates the re-
straining effect of lignin and further increases the rate of saccharifica-
tion up to 2.5 times, within the first 6 hours, depending on the extent of
delignification.

INTRODUCTION

Biomass or lignocellulosic materials could be converted to fuels, chemicals and even animal
feed. Although the feasibility of such conversions have been long established, their com-
mercialization or economic applications remain in doubt or marginal even at the currently
high costs of fuel and chemicals. This situation prevails despite numerous pilot plant
studies that until recently were being considered as a short cut for demonstrating feasibil-
ity of biomass utilization. In view of numerous attempts at up-dating the old technology
and processing methods, the prospects for future large scale utilization of biomass as a
renewable source of fuel and chemicals lies in development of modern and innovative conversion
methods. Inline with the present or future state of science and technology, integrated opera-
tions must produce not only fuel but also chemical by-products of higher value, and new
applications and markets especially suited for the products of biomass conversion. We
cannot expect to produce and market these products competitively, using the basic technology
and applications that existed at the turn of the century or were developed since then for the
petrochemical industry. These considerations have significant implications. They indicate
that the highly complex and technical problems of biomass conversion cannot be solved at
short notice and require sustained efforts in basic research rather than pilot plant work.

The major problem in conversion of cellulosic materials is saccharification of the poly-
saccharides. This could be achieved by acid hydrolysis that has been extensively investiga-
ted or through enzymatic hydrolysis and thermochemical processes which are still at the
early stages of their development. The thermochemical method, although very rapid and
efficient is best suited for gasification and production of fuel. For chemical conversions
it suffers from a lack of specificity and formation of too many products due to a series of
consecutive and concurrent reactions. Extensive basic studies on pyrolysis however, have
shown that these reactions could be controlled and catalyzed to minimize the side reactions
and increase the yield of individual compounds. In contrast to the thermochemical conver-
sion, enzymatic hydrolysis is highly specific but is hindered by the inaccessibility of the
cellulose molecule to the enzyme system. This problem is further aggravated by the constrain-
ing effect of lignin in the commonly available lignocellulosic materials. This article pro-
vides a general discussion of the progress that has been made for solution of these problems.

PRIMARY AND SECONDARY PYROLYTIC REACTIONS

Heating of cellulose at temperatures below 300°C results in reduction of the degree of poly-
merization (DP), elimination of water, evolution of carbon dioxide and carbon monoxide, and
formation of a charred residue containing trapped free radicals. The mechanisms and kinetics
of these reactions have been investigated and reported before (1,2). Of special interest at
this time is the reduction in DP on isothermal heating of cellulose. This has been measured
by the vicosity method within the temperature range of 150-190°C in both air and nitrogen
(see Fig. 1). The resulting data have been correlated with the rate of bond scission and
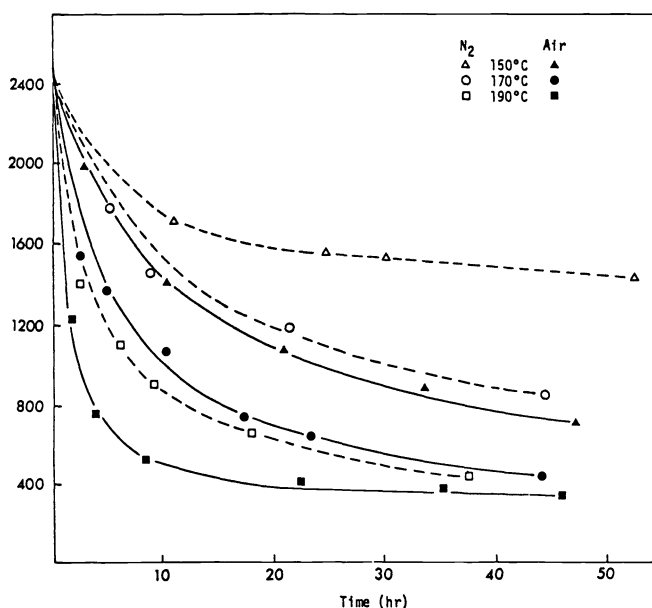used for determining the kinetics of thermal depolymerization.

Figure 1.   Vicosity average degree of polymerization (P_v) of cellulose
heated in air or nitrogen at 150, 170 and 190°C.

At temperatures above 300°C, cellulose molecules gather sufficient flexibility, by depoly-
merization and breaking of hydrogen bonds (transition) so that they can readily undergo
conformational changes and intramolecular transglycosylation to form anhydrosugar derivatives
that collect in the tarry pyrolyzate. This process is accompanied by the char forming re-
actions that start at lower temperatures but as the temperature is increased, tar formation
rapidly accelerate and char production is reduced. The primary reactions are apparently
followed by further intra- and intermolecular transglycosylation of the anhydrosugars result-
ing in the production of levoglucosan (1,6-anhydro-β-D-glucopyranose), its furanose isomer
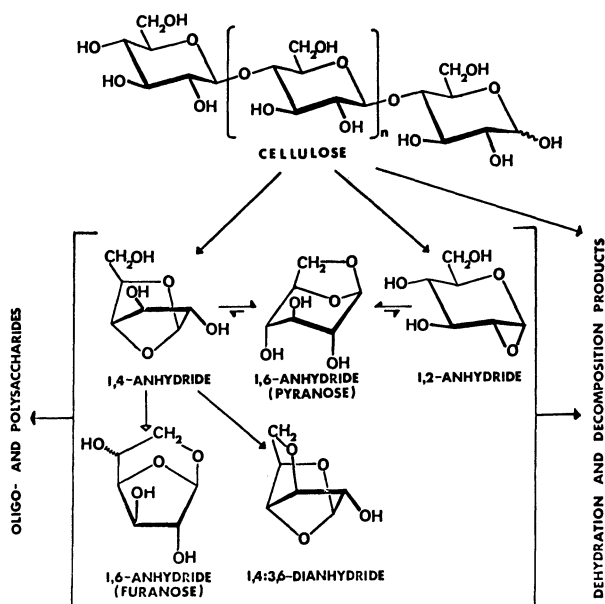(1,6-anhydro-β-D-glucofuranose) and randomly linked oligosaccharides as shown in Fig. 2.

Fig. 2.   Transglycosylation of cellulose to anhydro sugars.

The inter- and intramolecular transglycosylations shown in this figure are accompanied by a series of secondary reactions involving dehydration, fission and disproportionation reactions in the gas phase and further decomposition,   condensation and aromatization in the solid phase. The secondary reactions are enhanced by inroganic materials, particularly acidic reagents, which catalyze the dehydration and condensation reaction and alkaline reagents which catalyze the rearrangement and fission reactions. The net result is substantial variations in the composition and yield of different products under different conditions of heat and mass transfer, and inorganic catalysis. The products and mechanism of these reactions (2-6), except for the aromatization and properties of the char (7-9) have been extensively investigated and reported before. The g.l.c. analysis of the tarry pyrolyzate, produced from pyrolysis of CF 11 cellulose powder under vacuum at 360-385°C, after trimethylsilylation of the free hydroxyl group (4,6)is shown in Fig. 3. The pyrolyzate contains levoglucosan, and its furanose isomer and their transglycosylation or condensation products as the
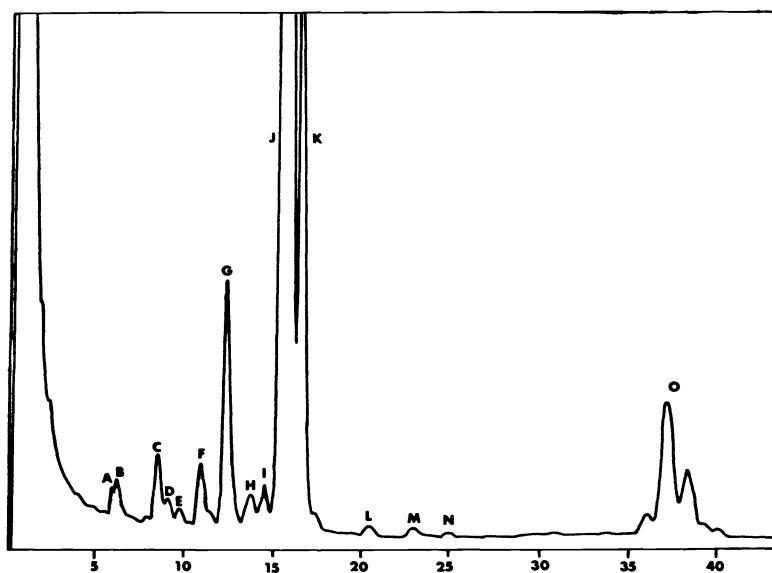

Fig 3.   GLC of trimethylsilylated tar from pyrolysis of cellulose at 350°C in vacuum.   Peaks: A, 5-hydroxymethyl-2-furaldehyde; B, 1,4:3,6-dianhydro-α-D-glucopyranose;   C, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; D, unknown; E, 3,5-dihydroxy-2-methyl-4H-pyran-4-one; F, unknown; G, 1,5-anhydro-4-deoxy-D-glycero-hex-1-en-3-ulose; H and I, unknown; J, levoglucosan; K, 1,6-anhydro-β-D-glucofuranose; L, α-D-glucose; M, β-D-glucose; N, 3-deoxy-D-erythro-hexosulose; O, O-D-glucosyl-levoglucosans.

major components and 5-(hydroxymethyl)-2-furaldehyde, 1,4:3,6-dianhydro-α-D-glucopyranose, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 3,5-dihydroxy-2-methyl-4H-pyran-4-one, 1,5-anhydro-4-deoxy-D-glycero-hex-1-en-3-ulose as the minor components.

In addition to these compounds the pyrolyzate contains levoglucosenone (1,6-anhydro-3,4-dideoxy-β-D-glycero-hex-3-enopyranos-2-ulose) and other volatile compounds lacking free hydroxyl groups. Pyrolyzates from wood also contain furan and phenolic derivatives formed from the hemicelluloses and lignin. Rapid formation and analysis of these products provides the basis for various methods of analytical pyrolysis (10). This includes Curie point pyrolysis and field ionization mass spectroscopy. This method is particularly suited for characterization or finger printing small amounts of lignocellulosic materials because it gives distinguishing mass ion peaks for lignin, carbohydrates or other materials to the extent that they may be present (see Fig. 4). This method however, could not distinguish between the molecular ions of isomeric compounds such as pyranose or furanose forms of levoglucosan, or levoglucosenone and 5-(hydroxymethyl)-2-furaldehyde.
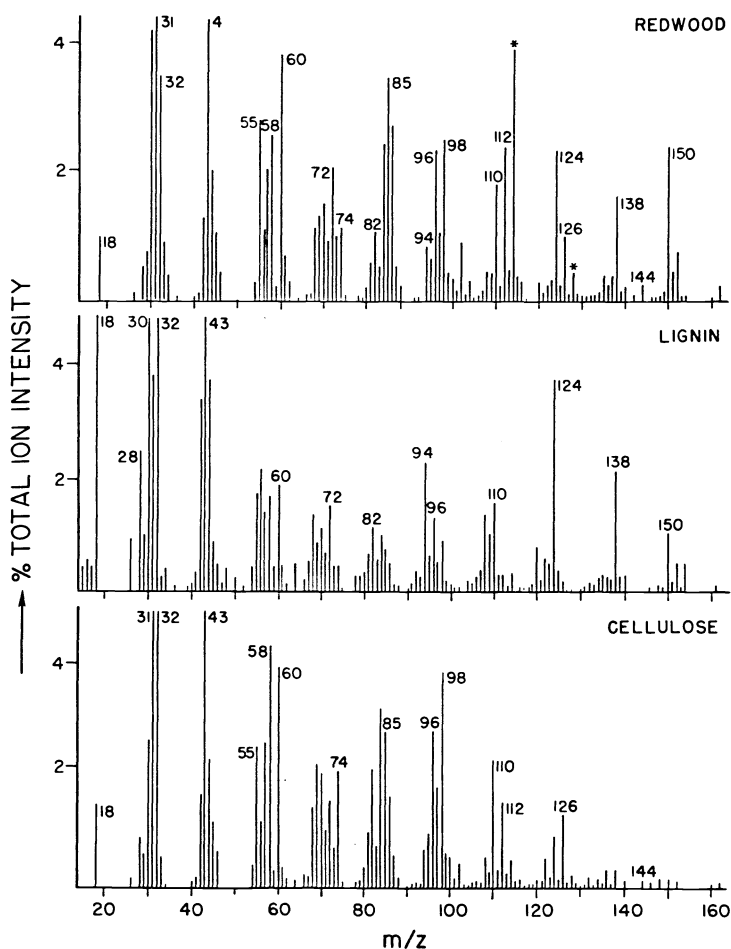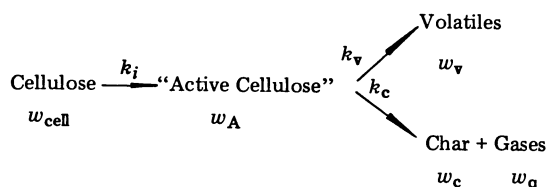

Fig. 4.   Analytical pyrolysis of cellulose, lignin and wood by Curie point
           pyrolysis and field ionization mass spectroscopy.

At the higher temperatures the tar-forming reactions accelerate rapidly and overshadow the production of char and gases. The data in Table 1 shows the production of diminishing amounts of char and increasing amounts of tar (containing anhydrosugar derivatives that could be hydrolyzed to reducing sugar) as the oven temperature is raised from 300° to 500°C (11). At this time, it should be pointed out that evaporation of levoglucosan and the volatile pyrolysis products is highly endothermic. Thus, the increased oven temperature could raise the rate of heat transfer but not necessarily the temperature of the ablating substrate (12) until the rapid evaporation and the accompanied change in entropy is over. In other words, at the higher temperatures the pyrolysis process could be controlled by the rate of heat transfer rather than the kinetics of the chemical reaction. Material transport also has a profound effect, because if the products of primary reactions are not

removed, they could undergo further decomposition reactions. Pyrolysis under vacuum gives more tar and less char than pyrolysis under nitrogen at atmospheric pressure because under vacuum the primary volatile products are removed before they could further react and form condensation products and char (4). The latter reactions are catalyzed by inorganic mater- ials. In view of these considerations, the chemical kinetics of cellulose pyrolysis have been investigated within the limited temperature range of 260-340° and under vacuum in order to obtain chemically meaningful data. Under these conditions, the chemical kinetics of cellulose pyrolysis could be represented by the three reaction model shown in Fig. 5 (12). In this model, it is assumed that the initiation reactions discussed before lead to the formation of an active cellulose, which subsequently decomposes by two competitive first order reactions, one yielding anhydrosugars (transgylcosylation products) and the other char and a gaseous fraction.

$$\text{Cellulose} \xrightarrow{k_i} \text{``Active Cellulose''} \begin{array}{c} \xrightarrow{k_v} \text{Volatiles} \quad w_v \\ \searrow^{k_c} \\ \text{Char + Gases} \\ w_c \quad w_q \end{array}$$

$$w_{\text{cell}} \qquad\qquad w_A$$

where

$$\frac{-d(w_{\text{cell}})}{dt} = k_i[w_{\text{cell}}]$$

$$\frac{d(w_A)}{dt} = k_i[w_{\text{cell}}] - (k_v + k_c)[w_A]$$

$$\frac{d(w_c)}{dt} = 0.35 k_c[w_A]$$

Fig. 5. Kinetic Model for pyrolysis of pure cellulose under vacuum. The rate constants $k_i = 1.7 \times 10^{21} e^{-(58.000/RT)}$ min^{-1} $k_v = 1.9 \times 10^{16} e^{-(47.000/RT)}$ min^{-1} and $k_c = 7.9 \times 10^{11} e^{-(36.000/RT)}$ min^{-1}

TABLE 1. Effect of temperature on the products from pyrolysis of cellulose powder[a] under vacuum.

Oven Temperature (°C)	Pyrolysis Time (min)	Percent Yield from Cellulose				
		Char	Tar	Levoglucosan	1,6-Anhydro-β-D-glucofuranose	Reducing Sugar[b]
300	180	21	60	34	4	47
325	60	10	70	38	-	54
350	30	8	70	38	4	52
375	10	6	70	38	-	59
400	5	5	77	39	4	60
425	4	4	78	40	4	59
450	3	4	78	39	4	57
475	3	3	80	38	4	58
500	3	3	81	38	4	57

[a]Whatman CF 11 powder

[b]Increase in reducing sugar after tar hydrolysis

As discussed before, these reactions are followed by a series of secondary reactions both in the solid phase and the gas phase, which are subject to catalysis. As in aqueous solutions the dehydration reactions are strongly catalyzed by acidic reagents. The g.l.c. analysis of the pyrolyzate obtained from acid catalyzed pyrolysis of cellulose at ~350°C under nitrogen current is shown in Fig. 6 (13). The pyrolyzate contains levoglucosenone instead of levo- glucosan as the major component and 1,4:3,6-dianhydro-α-D-glucopyranose, 2-furaldehyde and 5-(hydroxymethyl)-2-furaldehyde as minor components (13, 14). Levoglucosenone, formed by dehydration reactions shown in Fig. 7, could be separated by fractional distillation.
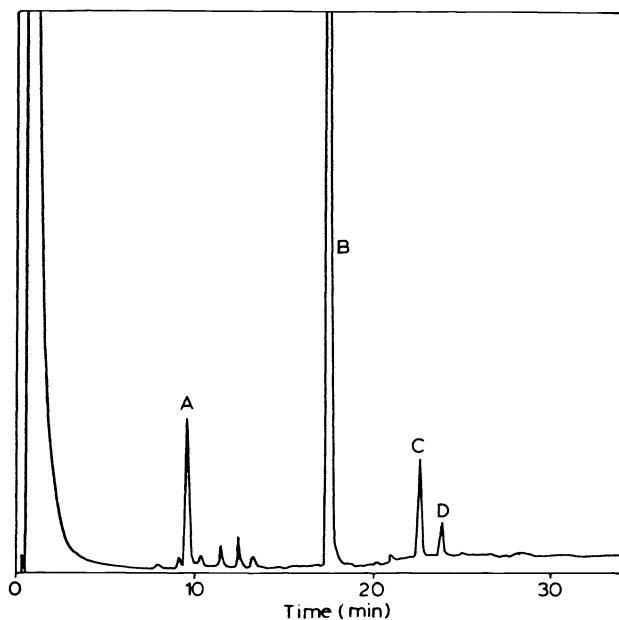
Fig. 6.   GLC analysis of the pyrolyzate from cellulose + 2% phosphoric acid at 350°C. Peaks: A, furaldehyde; B, levoglucosenone; C, 1,4:3,6-dianhydro-α-$\underline{D}$-glucopyranose; D, 5-(hydroxymethyl)-2-furaldehyde.
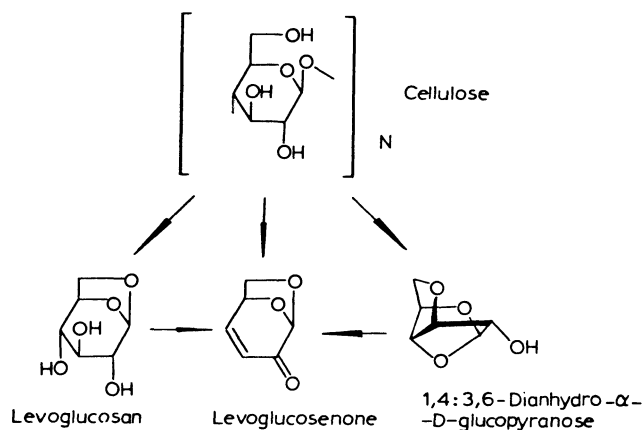
Fig. 7.   Formation of levoglucosenone by dehydration reactions.

The solid phase reactions provide a highly reactive and pyrophoric char (7-9, 15, 16). The cellulosic chars are characterized by a high surface area and a high concentration of free spins (trapped free radicals) which change on heating and peak at about 550°C HTT (heat treatment temperature), as shown in Figs. 8 and 9 (7). The reactivity of the char could be measured by the rates of oxygen chemisorption, combustion, and gasification. The oxygen chemisorption are measured by thermogravimetry (TG). The process follows Elovich Kinetics, which are based on the assumption that the rate of reaction (dq/dt) declines, as the more reactive sites are quenched, according to the equation (7, 15, 16):

$$dq/dt = a\,\exp(-bq)$$

where, q is the amount of oxygen chemisorbed at time t and a and b are constants.
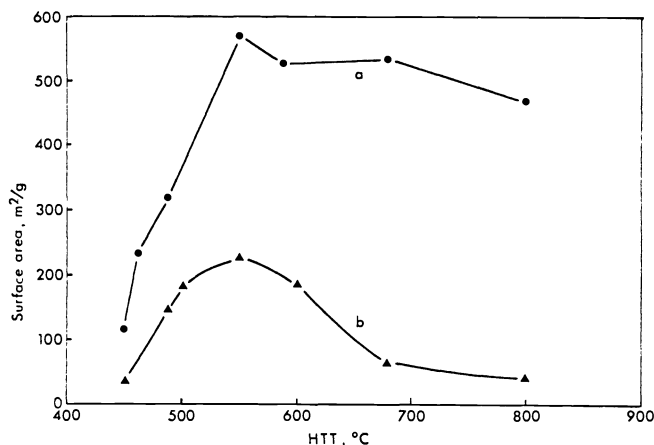
Fig. 8.   Comparison between total surface area calculated from CO_2 adsorption at 25°C (curve a) and that occupied by surface oxides calculated from oxygen chemisorption at 230°C (curve b).
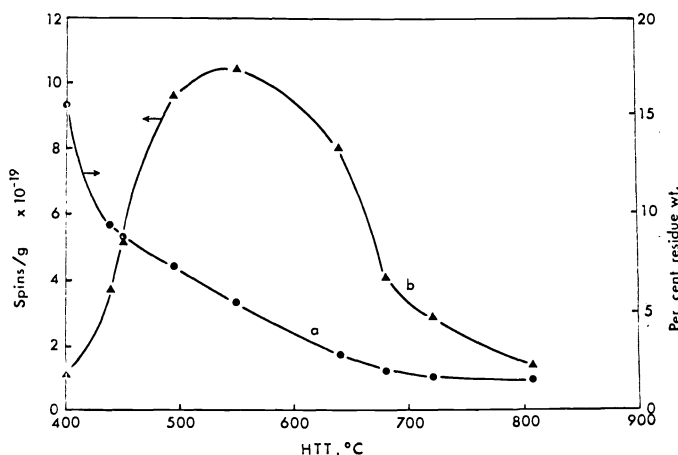

Fig. 9.   Comparison between char residue weight (curve a) and free spin concentration (curve b) as a function of char HTT.

The rate of combustion is measured by the formation of carbon monoxide and carbon dioxide or heat release that follow the first order Arrhenius kinetics (9, 11).

The relationship between the char characteristics, catalysis, and the rates of combustion and heat release are shown in Table 2. The kinetics of the gasification of char with steam and carbon dioxide has been studied by measurement of the combustible gases. As shown in Fig. 10 these reactions are strongly catalyzed by alkaline compounds (17).

Development of aromaticity in char could be investigated by permanganate oxidation that gives benzene polycarboxylic acid derived from the aromatic nuclei present. These compounds could be methylated by diazomethane, separated by GLC, (Fig. 11) and related to the aromaticity of the char. Formation of these and other functionalities could also be studied by FT-IR and CP/MS (cross polarization magic angle spinning) ^{13}C-NMR spectroscopy as shown in Fig. 12 (8).

The yield of the pyrolysis products, particularly the tar and its levoglucosn content is further affected by the composition of the substrate and its inorganic or ash content. The lignin component provides mainly char, and pentosans give more furan derivatives (18,19).
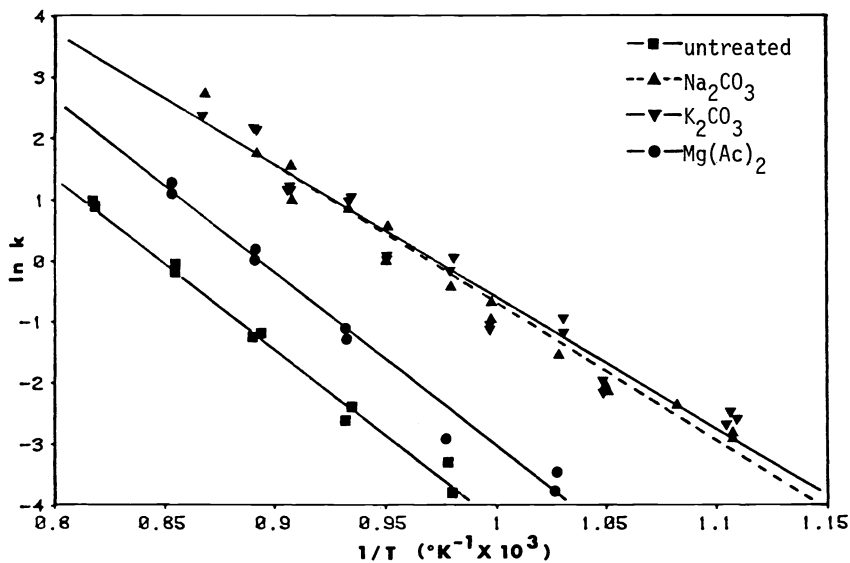
Fig. 10. Arrhenius plots for the CO_2 gasification of chars (HTT 1000°C)
          prepared from untreated and treated Douglas fir (33% CO_2 in N_2).
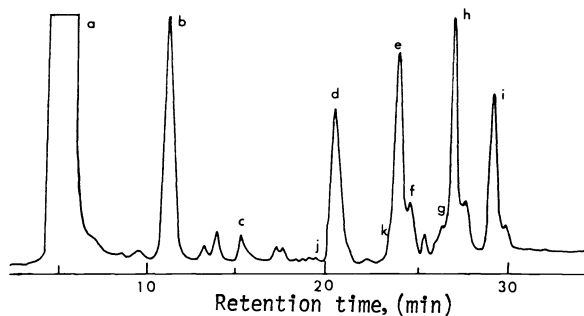

Fig. 11. Typical gas chromotogram of methylated benzene carboxylic acids.
          a) solvent; b) internal standard; c) benzene dicarboxylic acid,
          B2C; d) benzene tricarboxylic acid, B3C; e) benzene tetracarboxylic
          acid, B4C; f, g) monohydroxy benzene tetracarboxylic acid, HB4C;
          h) benzene pentacarboxylic acid, B5C; i) benzene hexacarboxylic
          acid, B6C; j) monohydroxy benzene dicarboxylic acid, HB2C; k) mono-
          hydroxy benzene tricarboxylic acid, HB3C.

TABLE 2. Properties and reactivity of chars from untreated and additive-treated
          cellulose (HTT 550°C).

Additive	Char Yield %	Surface Area $m^2 \cdot g^{-1}$	Unpaired Spin Concentration ratio	Gasification $(kCO_2+kCO)750°C$ min^{-1}	Elovich Kinetic Parameters* a	b	Rate of Heat Release (750°C) $(kcal.mole.min^{-1})$
NaCl	13.3	359±81	2.05	1.29	1.31+0.41	2.96+0.04	-83.5
$Na_2B_4O_7$	33.3	371±13	1.23	1.24	1.26∓0.28	3.50∓0.39	-64.3
Untreated	5.7	352±49	1.00	1.04	0.87+0.19	2.20+0.14	-54.2
$(NH_4)_2HPO_4$	21.8	324±21	0.41	0.74	0.38∓0.09	1.76∓0.08	-27.3
H_3BO_3	18.7	351±50	0.58	0.61	0.41+0.06	2.34+0.11	-27.5

*Chemisorption of O_2 at 230°C.

Fig. 12. CP/MAS ^{13}C-NMR Spectra of cellulose after different heat treatments. The spectra for untreated cellulose, (a) gives sharp peaks numbered 1 to 6 corresponding to the carbon atoms in the polymeric units. On heating after 5 min. at 350°C, (b) four new peaks associated with: aliphatic carbons located at 0-60 ppm; olefinic and aromatic carbons at about 100-160 ppm; carboxylic acid and ester carbons at about 160-180 ppm; and carbonyl carbons at 190-220 ppm are observed. On heating at 500°C, (c) olefinic and aromatic carbons increase whereas the other three types decrease.
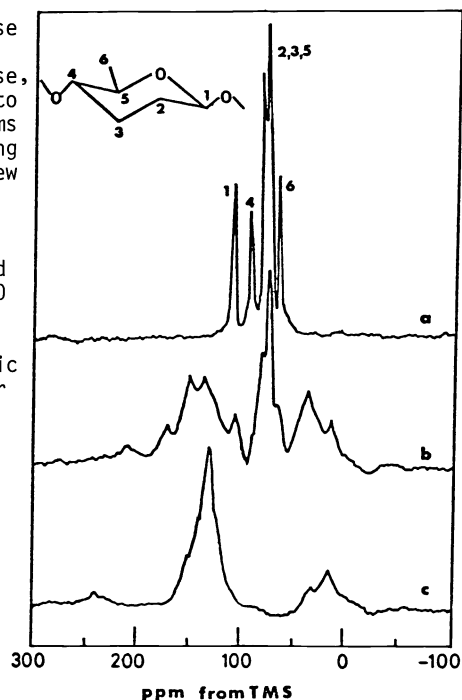
PRODUCTION OF SUGAR DERIVATIVES

There have been numerous laboratory and industrial experiments for preparation of levogluco-san by pyrolysis but the yield from commonly available cellulosic material has been almost negligible and from isolated cellulose has been variable, expensive and controversial (11,20). The data in Table 3 show that the yields for the tar, levoglucosan and reducing sugar formed on hydrolysis of tar could vary greatly according to the type of cellulose that has been used (11). Materials which have been cleaned, acid treated or partially hydrolyzed provide higher yields of tar and levoglucosan. For instance, hydrocellulose provides 85% tar that contains 58% levoglucosan. Further experiments listed in Table 4 show that this conclusion also applies to hardwood (Cottonwood), lignocellulose obtained by acidic prehydrolysis of the hemicellulose components and newsprint. The yields from the original substrates are almost negligible, but could be increased substantially after washing with acid or prehydrolysis of the hemicelluloses.

These data provide the basis for a new process for wood saccharification (21). In this process the hemicelluloses which are more amenable to hydrolysis are removed by a relatively mild prehydrolysis and the remaining lignocellulose is pyrolyzed to a tar, and a char residue that could be used as fuel. The tar contains levoglucosan as well as its condensation products, all of which are easily hydrolyzable to glucose. Therefore the amount of free sugar obtained from the hydrolysis of the tar is more than what would be expected from the hydrolysis of levoglucosan alone. This constitutes one of the attractive features of this process. Thus, possible options for utilizing the tar include separating the levoglucosan from the tar by crystallization and using it as an anhydro-sugar, or hydrolyzing the tar to glucose for fermentation to alcohol or other applications. Since isolation of levoglucosan from the tar by crystallization is a relatively difficult and low yield process and does not utilize the condensation products of levoglucosan, the latter alternative may be preferred.

The process is particularly suitable for saccharification of softwoods because the hemicellu-lose component contains mainly glucomannans and galactoglucomannans, which on hydrolysis give fermentable sugars. Therefore, the tar can be hydrolyzed by addition to the acidic prehydrolysis liquors, and the resulting glucose used along with the other hexoses (mannose, galactose and glucose), obtained from the prehydrolysis. This avoids the necessity of separate   neutralization and processing of the fermentable sugars. This combination is possible because the tar could be hydrolyzed under milder conditions than those of hemicellu-loses (21). The yields obtained in small scale laboratory experiments are shown in Fig. 13. and the general process envisaged for production of alcohol by this method is shown in Fig. 14.

The enhancement in production of levoglucosan on pyrolysis of acid washed or treated sub-strates could be due to the removal of inorganic impurities, the presence of trace amounts of acid that may catalyze the transglycosylation reaction or both. This was investigated
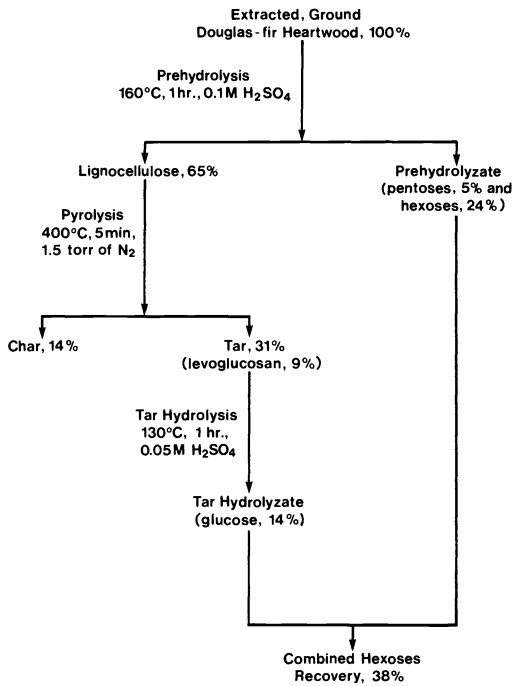
Fig. 13

Fig. 14

Fig. 13.   Saccharification of Douglas-fir heartwood by prehydrolysis
            pyrolysis and tar hydrolysis. Percentages are weight percent.
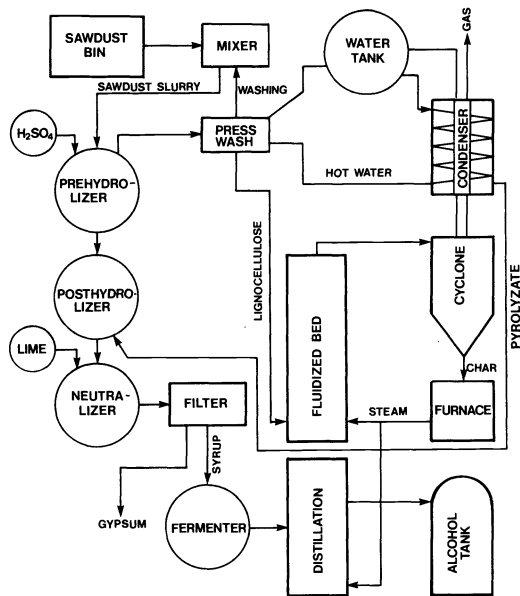
Fig. 14.   A pyrolytic process for production of alcohol from softwoods.

through several experiments with samples of CF-11 cellulose, Douglas-fir wood and its holo-
cellulose and lignocellulose (prehydrolyzed) products. These samples were used as such,
after being washed with acid followed with distilled water and finally with and without 0.1%
added sulfuric acid. The results, summarized in Table 5, show that removal of the ash
content is most important, especially when a larger amount of it is present as could be seen
from the experiments with holocellulose before and after reduction of the ash content by
washing with acid. However, the positive effect of added acid was also apparent with ligni-
fied materials (wood and lignocellulose having a low ash content to begin with. As shown in

TABLE 3. Products from Pyrolysis of Celluloses at 400°C under Vacuum.

Substrate	Char	Tar	Levoglucosan Isomers pyranose	furanose	Reducing Sugar
Cotton Hydrocellulose	2	85	58	6	77
Baker Microcrystalline	3	83	51	5	-
Avicel Microcrystalline	3	84	49	5	-
Whatman CF 11, Lot A	5	69	39	4	49
Whatman No. 41 F P	5	69	38	4	-
Cotton Linters	5	64	30	3	-
Whatman CF 11, Lot B	7	58	29	3	-
Cotton Fabric	9	46	14	1	-

TABLE 4. Pyrolysis of Wood-Derived Materials at 400°C under Vacuum.

		Percent Yield from Substrate (or from Cellulose Portion, shown in parentheses)			Levoglucosan Isomers		Reducing[a]
Substrate	Pretreatment	Ash	Char	Tar	Pyranose	Furanose	Sugars
Cottonwood	Untreated[b]	0.59	16	47	3(6)	trace	5(12)
	1% H_2SO_4	<0.05	12	52	9(20)	0.6(1.5)	14(31)
Cottonwood Lignocellulose	Untreated [c]	<0.05	18	71	34(57)	3(6)	46(78)
Cottonwood Holocellulose	Untreated[d]	0.87	21	21	1(1)	trace	1(2)
	1% NaOH then 1% H_2SO_4	<0.05	9	65	29(51)	3(6)	40(71)
Newsprint	Untreated	-	12	39	2	0.3	-
	1% H_2SO_4	-	19	43	15	2	-
	1% HCl	-	14	56	19	2	-

[a] After hydrolysis; [b] Extracted and washed with water; [c] Filtered from dilute sulfuric acid reaction mixture and washed with water to neutrality; [d] Washed with water only.

TABLE 5. Product Yields from the Pyrolysis of Various Cellulosic Substrates

Substrate	Washing	Ash	Char	Tar	Levoglucosan
CF-11	acid		5	68	36
CF-11 + H_2SO_4[a]	acid		7	63	35
Holocellulose	none	0.6	17	26	1
Holocell. + H_2SO_4	none	0.6	17	27	1
Holocellulose	acid	0.05	8	66	26
Holocell. + H_2SO_4	acid	0.05	0	57	23
Lignocellulose	water	<0.02	24	44	12
Lignocell. + H_2SO_4	water	<0.02	24	51	26
Wood	water	0.1	17	45	7
Wood + H_2SO_4	water	0.1	17	48	14
Wood	acid	<0.02	16	51	9
Wood + H_2SO_4	acid	<0.02	17	50	19

[a] All H_2SO_4 added as 0.1% of substrate.

Fig. 15 the amount of acid was critical. The addition of trace amounts of acid (up to 0.0.%) had very little effect on the pyrolysis of the lignocellulose, while the addition of 0.1% sulfuric acid doubled the yield of levoglucosan. When the acid concentration was further increased to 1.0%, the yield dropped back to the uncatalyzed level.
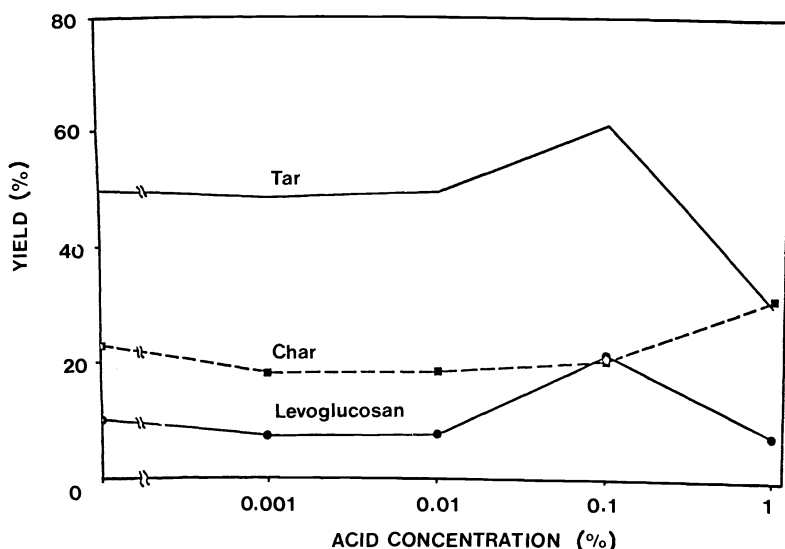

Fig. 15.   Product yields from Douglas-fir lignocellulose pyrolysis, expressed
            as a percent of the lignocellulose.

To demonstrate the effect of small amounts of sulfuric acid, the lignocellulose was carefully extracted with water for 8 hours in a Soxhlet extractor and the process was carried out with and without added acid. The results showed that without the addition of acid to the ligno-cellulose, 22% of the wood was recovered as free hexoses from the prehydrolysis, and 10% from the tar hydrolysis, amounting to an overall yield of 45% of the hexose content in the wood. When 0.1% acid was added back to the lignocellulose, the recovery of hexoses following tar hydrolysis was 19%, and this increased the overall hexose yield to 59%.

The process could be readily adapted to hardwoods and agricultural residues, which contain substantial amounts of xylan (acetyl-4-$\underline{O}$-methyl-$\underline{D}$-glucuronoxylan) as the principal hemicellu-lose component. In this case (22) the prehydrolyzate could be used for production of furfur-al, xylitol or fermentation products of xylose which are currently the subject of extensive investigation. Fig. 16 shows the diagram for a laboratory-scale adaptation of this process to 100 g of cottonwood, containing about 44% cellulose, 25% lignin, 21% acetyl-4-$\underline{O}$-methyl-glucuronoxylan, and 10% glucomannan and other components (18).

The principles and techniques developed in this process have also been used for laboratory preparation of 1,6-anhydro-β-$\underline{D}$-mannopyranose (a stereo-isomer of levoglucosan) from pyroly-sis of ivory-nut meal (23). This material obtained from a South American palm tree (Phytelephas macrocarpa), contains about 52% mannan (essentially a linear polymer of 1 → 4 linked β-$\underline{D}$-mannopyranosyl units), 13% glucan, 6% hydrolysis residue (Klason lignin), 1.3% ash and 30% unaccounted for organics. On pyrolysis this material provides considerable char and a tar that contains very little of the expected anhydro-sugar. The g.l.c. analyses of the pyrolyzates revealed, in addition to the 1,5-anhydro-β-$\underline{D}$-mannopyranose, small proportions of levoglucosan, 1,6-anhydro-4-deoxy-$\underline{D}$-glycero-hex-1-en-3-ulose, and a component presumed to be 1,5-anhydro-β-$\underline{D}$-mannofuranose. Levoglucosan is evidently formed from the $\underline{D}$-glucose con-tent of the nut meal, whereas the anhydro hexulose derivative can be produced from both the $\underline{D}$-mannosyl and $\underline{D}$-glucosyl residues. The ivory-nut meal was subjected to a variety of pre-treatments with hydrochloric acid (see Table 6) in attempts to improve the yield of the anhydromannose. Boiling with 1$\underline{M}$ hydrochloric acid for 10 min, followed by thorough washing with distilled water (which lowered the ash content from 1.29 to 0.12%) was the most success-ful treatment. Vacuum pyrolysis provided a 27% yield of the anhydromannose which constituted 55% of the tarry pyrolyzate.

For the laboratory scale preparation, an acetone solution of the tar was treated with anhy-drous copper (II) sulfate. This gave the 2,3-isopropylidene acetals of the mannose deriva-tives, which could then be extracted by chloroform; leaving the unsubstituted by-products in the aqueous phase. Recrystallization of the crude extract from 2-propanol gave 1,6-anhydro-2,3-$\underline{O}$-isopropylidene-β-$\underline{D}$-mannopyranose (in 17% yield from ivory-nut meal, ~32% based on a ~52% content of $\underline{D}$-mannan) that could be readily hydrolyzed in 0.05$\underline{M}$ sulfuric acid solution to 1,6-anhydro-β-$\underline{D}$-mannopyranose.
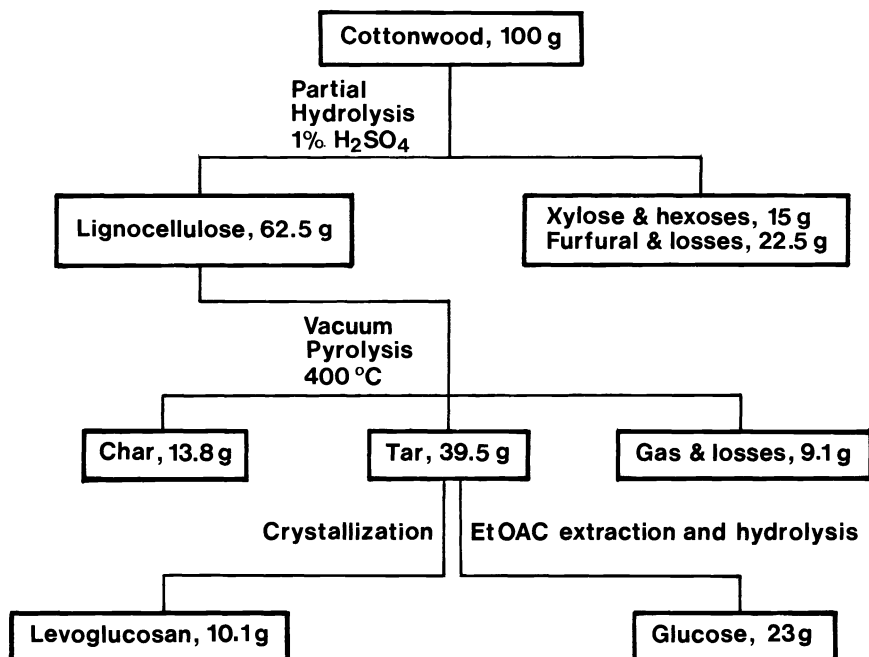
Fig. 16.   Production of levoglucosan or glucose by pyrolysis of cottonwood.

TABLE 9.   Pyrolytic Production[a] of 1,6-anhydro-β-D-mannopyranose (1),
            1,6-anhydro-β-D-mannofuranose (5), and 1,6-anhydro-β-D-
            glucopyranose (3) from Ivory-nut Meal.

Pretreatment	No. of Water Washes	Percent Yield[b]						
		Char	Tar	1	3	5	Ash	% 1 in tar
Untreated	0	32	20	5	0.5	0.4	1.29	25
1M HCl, 20°, 15h	1	30	41	16	1.8	1.2		39
	2	26	43	21	2.0	1.5		49
	3	26	43	21	2.0	1.3		49
0.5M HCl, 20°, 8h	3	27	47	22	2.1	1.4		46
1M HCl, 60°, 20 min	3	29	40	17	1.8	1.2		42
1M HCl, boiling, 10 min	3	22	49	27	3.0	1.9	0.12	55
Conc. HCl, boiling 3 min	3	27	49	15	7.0	1.0		31

[a]Pyrolysis of 1 g samples at 1.5 torr in an oven preheated to 370°.

[b]Based on dry-weight of nut-meal.

It should be noted that 1,5-anhydro-4-deoxy-D-glycero-hex-1-en-3-ulose, which is an interesting enone, is also present in the cellulose pyrolyzate and can be isolated by column chromotography in 1.4% yield (6).

As mentioned before, acid catalyzed pyrolysis of cellulose and cellulosic materials provides a pyrolyzate that contains mainly levoglucosenone and smaller amounts of 2-furaldehyde, 1,4:3,6-dianhydro-α-D-glucopyranose, and 5-(hydroxymethyl)-2-furaldehyde (Figures 6 and 7). For production of levoglucosenone the catalyst could be an Arrhenius acid, a Lewis acid, or even a compound such as diammonium phosphate that could produce acidic decomposition products on pyrolysis (24,25). However, phosphoric acid is preferred because of its low vapor

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pressure (13). Investigation of the effects of pressure, temperature, acid concentration, and the scale of production, which are conceived as the principal variables in the production of this compound indicate that better yields of levoglucosenone are obtained on acid-catalyzed pyrolysis at atmospheric pressure (13). This is in contrast to the production of levogluco-san, which is enhanced under vacuum. The pressure, however, has little or no effect on the total amount of tar and char, so that the increased production of levoglucosenone under atmos-pheric conditions is at the expense of levoglucosan. Moreover, it was noted that increasing the acid concentration from 0.5 to 5% has little effect on the yield of levoglucosenone from pyrolysis, and that the use of an intermediate vaccum (150 torr) is of no advantage. Further-more, increasing the oven temperature from 300 to 400°C decreases the yield of char and in-creases the yields of tar and dianhydroglucose, but has little effect on the yield of levo-glucosenone, which is consistently within the range of 7 to 8%. Thus it appears that, at higher temperatures, any increased synthesis of levoglucosenone is offset by its increased degradation and conversion into char and other tar components.

Yields of 11% are obtained with smaller size samples but as the sample size increases the yield of levoglucosenone falls off rapidly. Considering the fact that levoglucosenone is decomposed under the same conditions that it is formed, the inverse relationship between the yield and the sample size seems to be due to the poor heat transfer in the substrate, result-ing in slower evaporation, increased decomposition and charring of the product. This obser-vation indicates that the yields of levoglucosenone could be further improved by better handling of the problems of heat and mass transfer. For industrial and even laboratory pre-parations, waste paper is the preferred substrate because it is readily available and easily processed. Paper products, however, contain some pentosan which results in increased produc-tion of 2-furaldehyde.

Levoglucosenone is a highly reactive enone that could be converted to a variety of vinyl, substituted and addition compounds, through Diels-Alder addition, Michael addition, Grignard reaction and various types of oxidation and reduction that have been investigated in our laboratory (13,25-30).

The 1,4:3,6-dianhydro-α-D-glucopyranose formed as a by-product on the acid catalyzed pyrolysis of cellulose could be isolated by column chromatography as a crystalline product in 0.5% yield.

The above data shows the possible applications and potential value of pyrolytic reactions for commercial and laboratory scale production of sugar derivatives from various types of biomass. It is hoped that the basic information that has been generated could lead to further scien-tific and industrial development of this approach.

          ENZYMATIC HYDROLYSIS OF CELLULOSE

Biological methods hold considerable promise for conversion of biomass to various fermenta-tion products, single cell protein and readily usable fuels such as ethanol and methane. However, in these processes a major hurdle is conversion of biomass to sugar. As noted before, this process is hindered by inaccessibility of cellulose macromolecule and the con-straining effect of lignin. Various physical and chemical methods have been used to break down the substrate and expose the cellulose to the enzymic system. Our initial approach to this problem was lowering the DP by thermolysis (Fig. 1) and increasing the reactive sites As shown in Fig. 17, contrary to our expectation this treatment was not very effective, but when cellulose was treated with 0.5% phosphoric acid and then heated at 106° under nitrogen for 1 hour, there was a major improvement (31), that unfortunately was hard to control or reproduce. Therefore, this approach was abandoned in favor of a more systematic investiga-tion. These investigations indicated that simultaneous attrition and enzymatic hydrolysis of cellulosic materials results in substantial enhancement of the enzymatic hydrolysis (32-34). The attrition exerts two opposing effects, continuous delamination and comminution of the substrate with formation of new active sites and a gradual denaturation and inactivation of the enzyme.

The data presented in Fig. 18 indicate that despite the latter effect, simultaneous hydroly-sis of CF-11 cellulose in the attritor is more rapid and extensive than hydrolysis in a shaking flask containing glass or stainless-steel beads. Furthermore, both methods are far more effective than stationary hydrolysis with or without prior dry ball milling (as a pre-treatment), which has been selected as a standard for comparison. The attritor apparently provides a more efficient grinding mechanism for generating or exposing reactive sites for association with the enzyme (33). Furthermore, the simultaneous formation of new sites allows the saccharification to continue very rapidly at first and level off to about 70% after 12 hours. This method was also effective for cottonwood containing 25% lignin and 44% cellulose (18), although the hydrolysis was slower and levelled off to about 50% saccharifi-cation after 12 hours, due to the restraining effect of the lignin content. This effect was investigated by comparing the hydrolysis of whole cottonwood, the corresponding holocellulose and three organosolv pulps containing 12.4, 9.9 and 4% residual lignin, respectively. The

results shown in Fig. 19 indicate that the rate of hydrolysis of the organosolv pulp contain-
ing 4% lignin was similar to the rate of hydrolysis of cottonwood holocellulose. This pulp
yielded 1.4 times the amount of sugar as the sample with 12.4% lignin after 6 hours hydroly-
sis. Overall, the organosolv fibers yielded 1.8 to 2.5 times as much sugar as whole cotton-
wood. An additional 6 hour attrition-hydrolysis of the fibers produced about 15% additional
saccharification. These data show that simultaneous attrition and organosolv pulping are
very effective pretreatments for enzymatic hydrolysis of cellulosic materials.
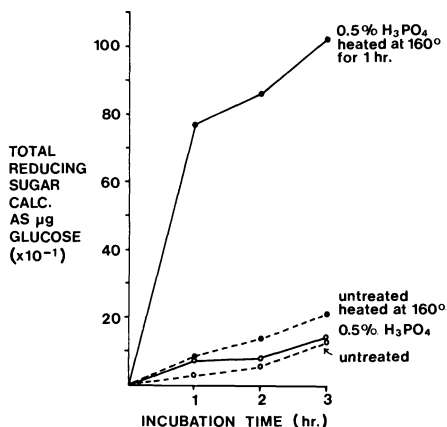
Fig. 17.

Hydrolysis of Cellulose Powder by Cellulase
Enzyme at 45°C After Various Pretreatments
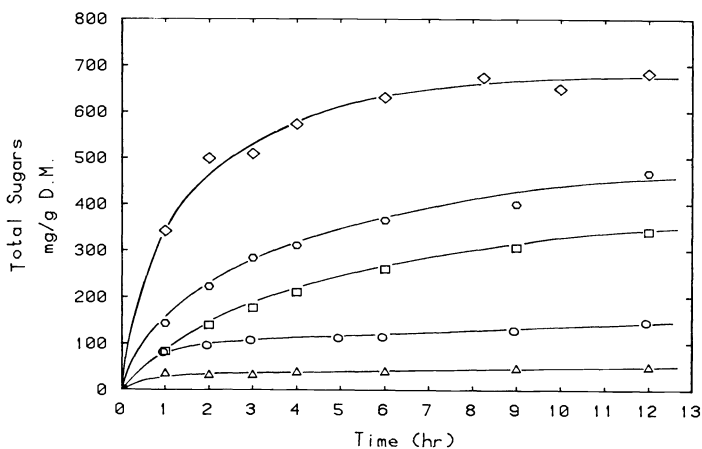
Fig. 18.   Comparison of shaking flask and attrition methods for enzymatic hydrolysis
            of Whatmann CF-11 cellulose. Unlimited control (△), ball milled (○),
            60 g of glass beads (□), 136 g of stainless steel beads (◯), all with a
            shaker speed of 200 rpm (◇). Attrition at 200 rpm. PP 158: 1 IU/ml and
            2% substrate.
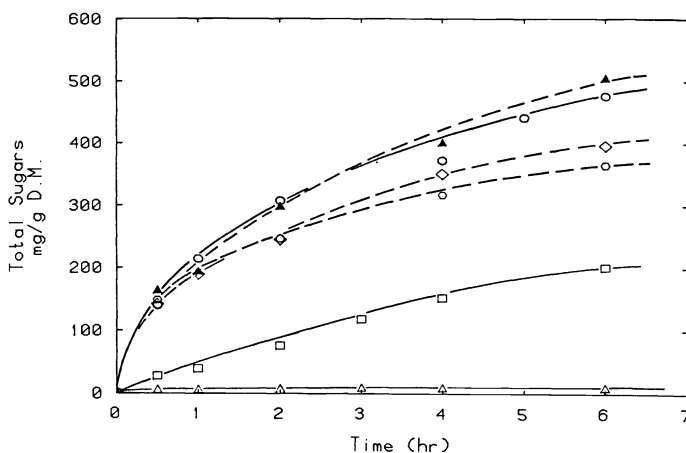

Fig. 19.   Enzymatic hydrolysis of organoslov fibers and cottonwood substrates.
            Unmilled cottonwood control (△), cottonwood 44% α-cellulose (□),
            organosolv fibers Nos. 1 (○), 2 (◇) and 3 (▲), holocellulose 58%
            α-cellulose (◯). Attritor: 200 rpm, PP 158: 1 IU/ml and 4% substrate.

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