MASTER

U. S. DEPARTMENT OF
ENERGY FUELS FROM
BIOMASS PROGRAM

OPTIMIZATION OF STEAM
EXPLOSION PRETREATMENT

BY
IOTECH CORPORATION
OTTAWA, ONTARIO

APRIL 1980

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DOE/ET/23050--1

MASTER

CONTRACT NO. DE-AC02-79ETZ3050

FINAL REPORT

OPTIMIZATION OF STEAM
EXPLOSION PRETREATMENT

Submitted to

The U.S. Department of Energy
Fuels from Biomass Program

April 1980

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ABSTRACT

OPTIMIZATION OF THE IOTECH STEAM EXPLOSION PRETREATMENT PROCESS

Different operating conditions are required to optimize the yield from each of the various fractions in the substrate. Xylose recovery is maximized at short cooking times whereas maximum lignin recovery requires much longer cooking times. Peak glucose yield and rumen digestibility occur at intermediate times. If process conditions are set for maximum glucose yield we have achieved a yield of 68% of the theoretical, based on an average of a dozen substrates tested. Individual results ranged from 46-87%. If the process is optimized for maximum total sugars (i.e. glucose plus xylose) we have obtained an average yield of 60%, with a range of 31-75%. With rumen microflora, the average value of the in-vitro cellulose digestibility was 82%, with a range of 41-90%. The optimum operating conditions for total sugars are a pressure of 500-550 psig with a cooking time of 40-50 seconds and 35% starting moisture content. Particle size is not a significant factor, nor is pre-steaming or use of a constricting die in the gun nozzle. High quality lignin can be extracted with 80% yield. The Iotech lignin is very soluble, has a low molecular weight and is reactive. The unique properties of the lignin derive from the explosion at the end of the pretreatment. A lignin formaldehyde resin has been successfully formulated and tested. It represents a high value utilization of the lignin byproduct with immediate market potential. A detailed engineering design of the process gives an estimated operating cost of \$7.50/OD ton of biomass. At this low cost, the Iotech process achieves many important pretreatment goals in a single step. The substrate has been sterilized; it has been pulverized into a powder; the cellulose has been made accessible; and a highly reactive lignin fraction can be recovered and utilized.

TABLE OF CONTENTS

	<u>Page</u>
List of Figures	ii
List of Tables	xii
List of Photographs	xviii
1.0 Abstract	
2.0 Executive Summary	1
3.0 Effect of Pressure	12
4.0 Effect of Particle Size of Starting Material	34
5.0 Effect of Starting Moisture Content	55
6.0 Effect of Pre-conditioning	87
7.0 Effect of Die Configurations	98
8.0 Investigation of Different Substrates	107
9.0 Extraction of Lignin	201
10.0 Molecular Weight of Iotech Lignin	234
11.0 Reactivity of Lignin	238
12.0 Lignin Resin Investigation	245
13.0 Preliminary Material Balance	257
14.0 Process Economics	289
15.0 Microscopic Examination of Exploded Wood	300
APPENDIX A Sources and Preparation of Aspen and Other Substrates	306
APPENDIX B Procedure for Gun Experiments	309
APPENDIX C Materials and Method of Enzyme Hydrolysis Assay	313
APPENDIX D Method of Rumen Digestibility Assay	330
APPENDIX E Method of Sugar Assay by High Pressure Liquid Chromatography	337
APPENDIX F Yeast Growth Studies	342
APPENDIX G Theoretical Products from Hydrolysis of Aspen Wood	354
APPENDIX H Weight Loss Correction Chart	357

LIST OF FIGURES

<u>Number</u>	<u>Title</u>	<u>Page</u>
<u>Section 2:</u>		
2.1	Effect of Cooking Time on Various Yields	3
<u>Section 3:</u>		
3.1	Effect of Pressure on Aspen as Shown by Enzymatic Digestion	17
3.2	Effect of Pressure on Aspen as Shown by Enzymatic Digestion	18
3.3	Summary of Effect of Pressure on Aspen as Shown by Enzymatic Digestion	19
3.4	Effect of Pressure on Aspen as Shown by Rumen Digestion	20
3.5	Effect of Pressure on Aspen as Shown by Rumen Digestion	21
3.6	Summary of Effect of Pressure on Aspen as Shown by Rumen Digestion	22
3.7	Relationship Between Assay Peaks and Pressure	23
3.8	Effect of Pressure on Yields	24
3.9	Economic Gain (or Loss) from Operation at Various Pressures	25
<u>Section 4:</u>		
4.1.1	Effect of Size of Input Chips at 550 psig as Shown by Enzymatic Digestion	37
4.1.2	Effect of Size of Input Chips at 550 psig as Shown by Enzymatic Digestion	38
4.2	Effect of Size of Input Chips at 640 psig as Shown by Rumen Digestion	39

List of Figures

<u>Number</u>	<u>Title</u>	<u>Page</u>
4.3.1	Effect of Pressure on Large Aspen Chips as Shown by Enzymatic Digestion	40
4.3.2	Effect of Pressure on Large Aspen Chips as Shown by Enzymatic Digestion	41
4.3.3	Summary of Effect of Pressure on Large Aspen Chips as Shown by Enzymatic Digestion	42
4.3.4	Effect of Pressure on Large Aspen Chips as Shown by Rumen Digestion	43
4.3.5	Effect of Pressure on Large Aspen Chips as Shown by Rumen Digestion	44
4.3.6	Summary of Effect of Pressure on Large Aspen Chips as Shown by Rumen Digestion	45

Section 5:

5.1.1	Effect of Moisture Content at 650 psig on the Glucose Production. Aspen	57
5.1.2	Effect of Moisture Content at 650 psig on the Xylose Production. Aspen	58
5.1.3	Effect of Moisture Content at 650 psig as Shown by Rumen Digestion. Aspen	59
5.2.1	Effect of Moisture Content at 560 psig on the Glucose Production. Aspen	60
5.2.2	Effect of Moisture Content at 560 psig on the Xylose Production. Aspen	61
5.2.3	Effect of Moisture Content at 560 psig as Shown by Rumen Digestion. Aspen	62
5.3.3	Effect of Moisture Content at 470 psig as Shown by Rumen Digestion. Aspen	63
5.4.1	Effect of Moisture Content at 360 psig as Shown by Glucose Production. Aspen	64
5.4.2	Effect of Moisture Content at 360 psig on Xylose Production. Aspen	65

List of Figures

<u>Number</u>	<u>Title</u>	<u>Page</u>
5.4.3	Effect of Moisture Content at 360 psig as Shown by Rumen Digestion. Aspen	66
5.5.3	Effect of Moisture Content at 200 psig as Shown by Rumen Digestion	67
5.6.1	Effect of Varying Pressures on Aspen Chips with 50% Average Moisture	68
5.6.2	Effect of Pressure on Aspen Chips with 35% Average Moisture	69
5.6.3	Effect of Pressure on Aspen Chips with 22% Average Moisture	70
5.6.4	Effect of Pressure on Aspen Chips with 7% Average Moisture	71
5.7	Summary of Peak Activities of Glucose, Xylose, Holocellulose and IVCD with Different Moisture Content	72

Section 6:

6.1.1	Effect of Presteamng at 150 psig for 12 seconds on Enzymatic Digestion. Aspen at 650 psig	88
6.1.2	Effect of Presteamng at 150 psig for 12 seconds on Rumen Digestion. Aspen at 650 psig	89
6.2.1	Effect of Presteamng Barley Straw at 100 psig for 20 seconds as Shown by Enzymatic Digestion. 550 psig	90
6.2.2	Effect of Presteamng Barley Straw at 550 psig for 20 seconds as Shown by Rumen Digestion	91
6.3.1	Effect of Presteamng with 150 psig Steam on Green, Air-dried and Frozen Chips. Aspen at 650 psig for 55 seconds	92
6.3.2	Effect of Presteamng with 150 psig Steam on Green, Air-dried and Frozen Chips. Aspen at 650 psig for 55 seconds	93

List of Figures

<u>Number</u>	<u>Title</u>	<u>Page</u>
<u>Section 7:</u>		
7.1	Star Die	100
7.2	Sieve Die	101
7.3	Circular Die	102
7.4	Effect of Using Different Dies as Shown by Enzymatic Digestion. Aspen at 650 psig	103
7.5	Effect of Using Different Dies as Shown by Rumen Digestion. Aspen at 650 psig	104
<u>Section 8:</u>		
8.1.1	Effect of Pressure on Birch Chips as Shown by Enzymatic Digestion	110
8.1.2	Effect of Pressure on Birch Chips as Shown by Enzymatic Digestion	111
8.1.3	Summary of Effect of Pressure on Birch Chips as Shown by Enzymatic Digestion	112
8.1.4	Effect of Pressure on Birch Chips as Shown by Rumen Digestion	113
8.1.5	Effect of Pressure on Birch Chips as Shown by Rumen Digestion	114
8.1.6	Summary of Effect of Pressure on Birch Chips as Shown by Rumen Digestion	115
8.2.1	Effect of Pressure on Maple Chips as Shown by Enzymatic Digestion	116
8.2.2	Effect of Pressure on Maple Chips as Shown by Enzymatic Digestion	117
8.2.3	Summary of Effect of Pressure on Maple Chips as Shown by Enzymatic Digestion	118

List of Figures

<u>Number</u>	<u>Title</u>	<u>Page</u>
8.2.4	Effect of Pressure on Maple Chips as Shown by Rumen Digestion	119
8.2.5	Effect of Pressure on Maple Chips as Shown by Rumen Digestion	120
8.2.6	Summary of Effect of Pressure on Maple Chips as Shown by Rumen Digestion	121
8.3.1	Effect of Pressure on Barley Straw as Shown by Enzymatic Digestion	122
8.3.2	Effect of Pressure on Barley Straw as Shown by Enzymatic Digestion	123
8.3.3	Summary of Effect of Pressure on Barley Straw as Shown by Enzymatic Digestion	124
8.3.4	Effect of Pressure on Barley Straw as Shown by Rumen Digestion	125
8.3.5	Effect of Pressure on Barley Straw as Shown by Rumen Digestion	126
8.3.6	Summary of Effect of Pressure on Barley Straw as Shown by Rumen Digestion	127
8.4.1	Effect of Pressure on Oat Straw as Shown by Enzymatic Digestion	128
8.4.2	Effect of Pressure on Oat Straw as Shown by Enzymatic Digestion	129
8.4.3	Summary of Effect of Pressure on Oat Straw as Shown by Enzymatic Digestion	130
8.4.4	Effect of Pressure on Oat Straw as Shown by Rumen Digestion	131
8.4.5	Effect of Pressure on Oat Straw as Shown by Rumen Digestion	132
8.4.6	Summary of Effect of Pressure on Oat Straw as Shown by Rumen Digestion	133
8.5.1	Effect of Pressure on Wheat Straw as Shown by Enzymatic Digestion	134

List of Figures

<u>Number</u>	<u>Title</u>	<u>Page</u>
8.5.2	Effect of Pressure on Wheat Straw as Shown by Enzymatic Digestion	135
8.5.3	Summary of Effect of Pressure on Wheat Straw as Shown by Enzymatic Digestion	136
8.5.4	Effect of Pressure on Wheat Straw as Shown by Rumen Digestion	137
8.5.5	Effect of Pressure on Wheat Straw as Shown by Rumen Digestion	138
8.5.6	Summary of Effect of Pressure on Wheat Straw as Shown by Rumen Digestion	139
8.6.1	Effect of Pressure on Bagasse as Shown by Enzymatic Digestion	140
8.6.2	Effect of Pressure on Bagasse as Shown by Enzymatic Digestion	141
8.6.3	Summary of Effect of Pressure on Bagasse as Shown by Enzymatic Digestion	142
8.6.4	Effect of Pressure on Bagasse as Shown by Rumen Digestion	143
8.6.5	Effect of Pressure on Bagasse as Shown by Rumen Digestion	144
8.6.6	Summary of Effect of Pressure on Bagasse as Shown by Rumen Digestion	145
8.7.1	Effect of Pressure on Corn Stover as Shown by Enzymatic Digestion	146
8.7.2	Effect of Pressure on Corn Stover as Shown by Enzymatic Digestion	147
8.7.3	Summary of Effect of Pressure on Corn Stover as Shown by Enzymatic Digestion	148
8.7.4	Effect of Pressure on Corn Stover as Shown by Rumen Digestion	149
8.7.5	Effect of Pressure on Corn Stover as Shown by Rumen Digestion	150
8.7.6	Summary of Effect of Pressure on Corn Stover as Shown by Rumen Digestion	151

List of Figures

<u>Number</u>	<u>Title</u>	<u>Page</u>
8.8.1	Effect of Pressure on Sunflower Stalks as Shown by Enzymatic Digestion	152
8.8.2	Effect of Pressure on Sunflower Stalks as Shown by Enzymatic Digestion	153
8.8.3	Summary of Effect of Pressure on Sunflower Stalks as Shown by Enzymatic Digestion	154
8.8.4	Effect of Pressure on Sunflower Stalks as Shown by Rumen Digestion	155
8.8.5	Effect of Pressure on Sunflower Stalks as Shown by Rumen Digestion	156
8.8.6	Summary of Effect of Pressure on Sunflower Stalks as Shown by Rumen Digestion	157
8.9.2	Oak and Basswood at 550 psig as Shown by Enzymatic Digestion	158
8.9.4	Effect of Pressure on Basswood and Oak as Shown by Enzymatic Digestion	159
8.10.2	Effect of Pressure on Sugar Cane Rinds as Shown by Enzymatic Digestion	160
8.10.5	Effect of Pressure on Sugar Cane Rinds as Shown by Rumen Digestion	161
8.11.1	Effect of Pressure on Peat as Shown by Rumen Digestion	162
8.12.1	Peak Yields for Glucose with Different Substrates	163
8.12.2	Peak Values for IVCD with Different Substrates	164
8.12.3	Peak Yields for Glucose Plus Xylose with Different Substrates	165
8.13	Variation in Cooking times at 550 psig for for Different Substrates	166

List of Figures

<u>Number</u>	<u>Title</u>	<u>Page</u>
<u>Section 9:</u>		
9.1.1	Fractionation Procedure I	213
9.1.2	Distribution of Lignin When Extracted by Fractionation Procedure I. Aspen at 650 psig for 55 seconds	214
9.1.3	Distribution of Lignin When Extracted by Fractionation Procedure I. Straw	215
9.2.1	Fractionation Procedure II	216
9.2.2	Distribution of Lignin When Extracted by Fractionation Procedure II. Aspen at 560 psig	217
9.2.3	Cumulative Totals of Fractions of Exploded Wood When Extracted by Fractionation Procedure II. Aspen at 560 psig	218
<u>Section 12:</u>		
12.1	Consumption of Formaldehyde by Iotech Lignin	253
12.2	Cutting Pattern	254
12.3	Relationship of Modulus of Elasticity to Density	255
<u>Section 13:</u>		
13.1	Effect of Cook Time on Weight Loss at 560 psig	274
13.2	Lignin Content of Exploded Wood	275
13.3	Methoxyl Content of Exploded Wood	276
13.4	Corrected Lignin Content	277
13.5	Pseudolignin Formation with Increased Cook Time	278
13.6	Pentosan Conversion with Increasing Cook Time	279

List of Figures

<u>Number</u>	<u>Title</u>	<u>Page</u>
13.7	Effect of Cook Time on Pentosan, Pseudolignin and Weight Loss	280
13.8	Summary of Material Balance	281
13.9	Pentosan Content of Exploded Wood Based on Weight of Original Wood	282

Section 14:

14.2	Variation of Steam Cost per Oven- dried Ton with Operating Pressures	295
14.3	Variation of Operating Cost per Oven-dried Ton with Cooking Time	296

Appendix B:

B.1	Schematic Design of Iotech's Pilot Plant	312
-----	---	-----

Appendix C:

C.1	Flow Chart of Enzyme Hydrolysis	319
C.2	Effect of Various Exploded Wood Consistencies as Shown by Enzymatic Digestion	322
C.3	Effect of Consistency on Aspen Exploded at 560 psig as Shown by Enzymatic Digestion	323
C.4	Effect of Increasing Enzyme on Sugar Production. Aspen at 560 psig	324
C.5	Effect of Various Enzyme Concentrations on Sugar Production	325

Appendix E:

E.1	HPLC Runs. Standard Curve with 10 mg/ml of Xylose and Glucose	340
-----	--	-----

List of Figures

<u>Number</u>	<u>Title</u>	<u>Page</u>
E.2	Typical Experimental Curve	341

Appendix F:

F.1	Effect of Cook Time on Toxicity of Exploded Aspen Solubles: Different Extraction Temperatures	348
F.2	Effect of Cook Time on Toxicity of Exploded Aspen Solubles: Different Incubation Times	349
F.3	Effect of Cook Time on Toxicity of Exploded Aspen Solubles: 430 psig (235°C/455°F)	350

Appendix H:

H.1	Weight Loss Correction Chart - to Correct Exploded Biomass to Original Biomass	358
-----	--	-----

LIST OF TABLES

<u>Number</u>	<u>Title</u>	<u>Page</u>
<u>Section 3:</u>		
3.1	Effect of Various Pressures at Different Times on Aspen Chips	26
3.2	Peak Yields at Various Pressures with Corresponding Cooking Times	29
3.3	Glucose ("Alphacellulose") Yield: Net Economic Gain (or Loss) at Various Pressures	30
3.4	Xylose ("Hemicellulose") Yield: Net Economic Gain (or Loss) at Various Pressures	31
3.5	Xylose + Glucose ("Holocellulose") Yield: Net Economic Gain (or Loss) at Various Pressures	32
3.6	Value of Extra 1% Sugar	33
<u>Section 4:</u>		
4.1	Effect of Size of Input Chips at 550 psig	46
4.2	Effect of Size of Input Chips at 640 psig	49
4.3	Effect of Varying Pressure on Big Aspen Chips	50
<u>Section 5:</u>		
5.1	Effect of Moisture Content of Chips at 650 psig	73

List of Tables

<u>Number</u>	<u>Title</u>	<u>Page</u>
5.2	Effect of Moisture Content of Chips at 560 psig	76
5.3	Effect of Moisture Content of Chips at 470 psig	79
5.4	Effect of Moisture Content of Chips at 360 psig	81
5.5	Effect of Moisture Content of Chips at 250 psig	84

Section 6:

6.1	Effect of Presteaming with 150 psig Steam for 12 seconds before Cooking at 650 psig	94
6.2	Effect of Presteaming Barley Straw for 20 seconds	95
6.3	Presteaming Green, Air-dried and Frozen Aspen at 150 psig for 20, 40 and 60 seconds then Cook Time for 55 seconds at 650 psig	96

Section 7:

7.1	Effect of Different Dies on Aspen at 650 psig	105
-----	--	-----

Section 8:

8.1	Effect of Various Pressures on Birch Chips	167
8.2	Effect of Various Pressures on Maple Chips	170
8.3	Effect of Various Pressures on Barley Straw	173

List of Tables

<u>Number</u>	<u>Title</u>	<u>Page</u>
8.4	Effect of Various Pressures on Oat Straw	176
8.5	Effect of Various Pressures on Wheat Straw	179
8.6	Effect of Various Pressures on Bagasse	182
8.7	Effect of Various Pressures on Corn Stover	186
8.8	Effect of Various Pressures on Sunflower Stalks	189
8.9	Effect of Various Pressures on Basswood	192
8.10	Effect of Various Pressures on Sugar Cane Rinds	195
8.11	Effect of Various Pressures on Peat Moss	197
8.12	Comparison of Substrates by Sugar Yield	199

Section 9:

9.1.1	Methoxyl Content of Fractions of Exploded Aspen Wood	219
9.1.2	Lignin Analysis of Fraction of Exploded Wood and Methoxyl Analysis of the Apparent Klason Lignin	220
9.1.3	Amount of Methoxyl in the Lignin of Exploded Wood	221
9.1.4	Lignin Analysis Corrected for Pseudolignin Content	222
9.1.5	Lignin Analysis of Fractions of Exploded Straw	223

List of Tables

<u>Number</u>	<u>Title</u>	<u>Page</u>
9.2.1	Weights of Fractions Obtained from Fractionation Procedure II	224
9.2.2	Composition of Water Solubles (Fraction 1)	225
9.2.3	Composition of Precipitate from Alkali (Fraction 2)	226
9.2.4	Composition of Pulp (Fraction 3)	229
9.2.5	Summary of Amounts and Composition of Fractions of Exploded Wood	232
9.2.6	Lignin Balance	233
 <u>Section 10:</u>		
10.1	Fractionation of Iotech Lignin by Vapour Pressure Osmometry	237
 <u>Section 11:</u>		
11.1	Pepper's Table	240
11.2	Lignin by Extraction Procedure II	240
11.3	Syringaldehyde and Vanillin Yields	241
11.4	Wayman and Chua Results	243
 <u>Section 12:</u>		
12.1	Properties of Waterboard Panels bonded with Modified Iotech Lignin	256

List of Tables

<u>Number</u>	<u>Title</u>	<u>Page</u>
<u>Section 13:</u>		
13.1	Lignin Analysis of Exploded Wood and Methoxyl Content of the Lignin at 560 psig	283
13.2	Lignin Analysis of Exploded Wood, Corrected for Pseudolignin, and Comparison of Calculated Weight Loss with Empirical Actual Observed Loss	285
13.3	Furfural and Pentosan Production	286
13.4	Lignin Yield	287
13.5	Weight Loss Calculation	288
13.6	Difference between Oven-dry and Vacuum-dry Wood	272
13.7	Corrected Empirical Recovery	273
<u>Section 14:</u>		
14.1	Estimated Capital and Operating Cost of Steam Explosion Pretreatment	297
14.2	Variation of Steam Cost with Operating Pressure	298
14.3	Variation of Operation Cost/Ton with Cooking Time	299
<u>Appendix C:</u>		
C.2	Effect of Various Exploded Wood Consistency on Enzymatic Digestion	326

List of Tables

<u>Number</u>	<u>Title</u>	<u>Page</u>
C.3	Effect of Consistence on Aspen Exploded at 560 psig as shown by Enzymatic Digestion	327
C.4	Effect of Increasing Enzyme Concentrations on Enzymatic Digestion	328
C.5	Effect of Various Enzyme Concentrations on Enzyme Digestion	329

Appendix D:

D.1	Composition of <u>in vitro</u> Basal Medium and Inoculum	336
-----	---	-----

Appendix F:

F.1	Effect of Cook Time on Toxicity of Exploded Aspen Solubles	351
F.2	Effect of Cook Time on Toxicity of Exploded Aspen Solubles	352
F.3	Effect of Cook Time on Toxicity of Exploded Aspen Solubles	353

Appendix G:

G.1	Summary of Components of Aspen Wood (Solvent Extracted), and the Products Produced Theoretically on Hydrolysis	355
-----	--	-----

LIST OF PHOTOGRAPHS

<u>Title</u>		<u>Page</u>
Control Aspen (#2459)	200x	303
560 psig 30 sec (#2424	200x	303
560 psig 70 sec (#2415)	200x	303
560 psig 80 sec (#2394)	200x	303
560 psig 90 sec (#2465)	200x	304
560 psig 100 sec (#2483)	200x	304
560 psig 220 sec (#2498)	2000x	304
560 psig 300 sec (#2505)	400x	304
560 psig 30 sec (#2425)	2000x	305
560 psig 80 sec (#2401)	2000x	305
560 psig 90 sec (#2466)	4000x	305
560 psig 100 sec (#2477)	400x	305
Samples of wood chips and exploded wood		360
Partial view of the Iotech Pilot Plant		362

SECTION 2

EXECUTIVE SUMMARY

2.0 EXECUTIVE SUMMARY

Effective pretreatment at low cost is a key factor in achieving cheap ethanol from biomass. Most ligno-cellulosic substrates available as future energy resources give only low yields of sugars without some form of pretreatment.

An ideal pretreatment process can be viewed as a refining operation. It is desirable to achieve a clean fractionation of the various components in the raw material, and the fractions obtained should give high yields in downstream chemical and biological processes.

In addition, when biological methods are employed the pretreatment should provide complete sterilization of the raw material to avoid subsequent contamination. Also, when size reduction is required to achieve high substrate concentrations, the pretreated material should be delivered in a finely-divided form.

Under this contract, we have been working to optimize the Iotech steam explosion pretreatment process to meet the major pretreatment goals outlined above. We have examined many parameters such as pressure, cooking time, particle size, moisture content and die configuration and we have measured the yields of glucose and xylose produced using a standard enzyme hydrolysis assay. At the same time, we tested the pretreated material for cellulose digestibility by rumen microflora. Over a dozen different substrates have been studied. The yield of lignin has been determined under various operating conditions, and the nature of the lignin isolated has been examined with a view to future end uses. An assessment of the operating cost of pretreatment

by steam explosion has been made. Many samples of pretreated biomass and lignin have been sent out to other DOE contractors for evaluation.

Fractionation

When biomass is cooked by steam the yield from each fraction is maximized at a different specific cooking time. In general, xylose recovery peaks at short cooking times followed by glucose and IVCD which peak at an intermediate time. The best lignin yields are obtained at longer cooking times.

This technique of "fractionation by cooking time" is indicated in Figure 2.1 for a typical aspen hardwood substrate.

FIGURE 2.1

EFFECT OF COOKING TIME ON VARIOUS YIELDS

The process parameters can be adjusted to optimize pretreatment in the direction of the fraction with greatest value. In the case shown,

<u>Product</u>	<u>Optimum Cooking time</u>
Xylose	20 sec.
Glucose	50
Glucose + Xylose	40
Rumen Digestibility	60
Lignin	120

For ethanol production, the summation of the glucose and xylose yield is usually of the greatest interest, and the optimum represents a compromise position between peak xylose and peak glucose. As high-value applications for lignin are developed it is conceivable that a long cooking time could become the optimum.

Yields

High yields obtained by the steam explosion process show that the hemicellulose and cellulose are made accessible to enzymes and rumen as the lignin is stripped away from the carbohydrates.

Based on the data obtained from the testing of a dozen substrates, we report the peak yields obtained for the various products, expressed as a percentage of theoretical yield from the original biomass.

<u>Product</u>	<u>Peak yield</u>	
	<u>Average</u>	<u>Range</u>
Glucose	68	46-87
Xylose	64	30-86
Glucose + Xylose	60	39-75
IVCD	82	41-90
Lignin	80	

The standard enzyme assay used to generate the sugar data is believed to be representative of practical conditions in that it uses a NOVO enzyme dosage of 8 IU/g of substrate and a residence time of 24 hours. In tests where we doubled the enzyme dosage we found that the xylose and glucose yields increased by as much as 60%. This indicates that the yields are limited by enzyme activity, not substrate accessibility. We expect that more powerful enzyme complexes, such as the C30 strain, will significantly increase the yield on existing pretreated materials.

Optimum Process Conditions

Our most extensive research has been done on aspen hardwood. As explained earlier, the process conditions can be tuned to give the optimum yield of any desired fraction. Given that the total sugars yield is the most relevant, and after consideration of both technical and economic aspects, we recommend an operating pressure of 500-550 psig for a cooking time of 40-50 seconds. Best performance is obtained with a starting moisture content of 35%. The particle size of the raw material is not a significant factor and ordinary commercial wood chips may be used with success. Additional treatments, such as presteaming, do not appear to improve the yield, nor does the use of constricting die in the outlet nozzle of the gun.

Various Substrates

The steam explosion worked well in general for a wide range of substrates which included hardwoods, straws and agricultural by-products. Birch, maple and aspen

were the best performers in the hardwood tests, while oak gave relatively low yields. All the straws tested gave similar results. In the agricultural by-products, sunflower stalks lagged significantly behind bagasse and corn stover. Peat, (tested by IVCD) gave disappointing results.

We believe that difficulties encountered with some substrates, such as sunflower stalks and peat, relate to the fact that the wetness and lumping of the material do not permit steam penetration into the pores.

Rumen Microflora Behaviour

There are noticeable differences in the performance of rumen microflora and cellulase enzymes. As the cooking time is lengthened, the conversion of cellulose into sugars by both rumen and cellulase increases steadily up to a specific peak value. In the case of aspen hardwood, shot at 560 psig, the peak occurs at about 60 seconds. As the cooking time is extended beyond the peak, however, the rumen yield falls sharply whereas the cellulase yield remains more or less on a plateau.

Our research indicates that the difference in behavior is caused by alterations in the crystalline structure of the cellulose at about the peak value, rather than the generation of toxic compounds. For instance, photomicrographs, included in this report, show steady transformation of the fibres into an amorphous structure. We hypothesize that the pore sizes are reduced, making the cellulose inaccessible to attack by the larger rumen enzymes.

Lignin

A unique feature of steam explosion pretreatment is the quality of the lignin produced. In traditional processes for separation of lignin, such as wood pulping, the resultant lignin is heavily polymerized, difficult to solubilize, unreactive and tarry. It is typically burnt as a low-grade fuel or used as a road filler. The Iotech process, however, produces a lignin which is very soluble in a simple solvent such as ethanol or acetone. Extraction with 0.4% NaOH followed by re-precipitation can recover 80% of the true lignin.

The lignin product has an exceptionally low molecular weight in comparison to traditional pulp lignin. For example, the average molecular weight of commercially extracted Iotech lignin has been measured at 946, whereas pulp lignins typically measure 8,000 to 10,000.

The chemical reactivity of the lignin can be determined by estimating the number of active sites (uncondensed sites) on the benzene ring structure. Iotech lignin has been found to have a reactivity equivalent to the best lignins reported in the literature, which were extracted under mild and carefully controlled conditions. In comparison to lignins extracted after low pressure "autohydrolysis", the Iotech product is significantly superior.

The high quality lignin is made possible by the instantaneous explosion at the end of the Iotech pretreatment. The cooked biomass is decompressed instantly from an operating pressure of 500-600 psig to atmospheric pressure. This explosive step not only pulverizes the biomass, but it also "freezes" the chemical reactions occurring in the cooking vessel. At normal operating temperatures the lignin is partly hydrolyzed and in the

form of molten droplets. When it is cooled slowly, the lignin repolymerizes and cross-links attach to the active sites on the benzene ring (i.e. condensation). With extremely rapid cooling, as in the Iotech system, the degree of repolymerization and condensation is reduced and better quality lignin is produced.

The basic phenol structure of the lignin molecule makes it a prime candidate for replacement of oil as a chemical feedstock in the future. A lignin characterized by high solubility and reactivity makes the economic utilization of lignin feasible today.

Lignin Applications

In a biomass refining plant the tonnage of lignin produced will be similar to the tonnage of ethanol produced when substrates such as hardwoods are used. In most projected flowsheets the lignin byproduct is burned as a fuel and a credit of 3-5¢/lb is taken.

If, however, a high value application can be developed for the lignin, the economics of ethanol liquid fuel production are turned around; that is, the ethanol becomes essentially a by-product of lignin manufacture. It should be noted that utilization of lignin as a chemical feedstock replacement for oil would achieve national energy savings equivalent to replacement of gasoline by ethanol.

We report initial success in some preliminary research to develop an immediate application for lignin. We have synthesized a "lignin formaldehyde" resin which can be substituted for the petroleum-based phenol formaldehyde resins currently used as binders for waferboard,

particle board and plywood. Preliminary board tests indicate the lignin resin exceeds Standards Association requirements and is equivalent to currently available resins. The Iotech resin has several properties which make it superior to similar formulations developed using pulp lignin.

As a feedstock for resin, the lignin can be assigned a value at this time of 20¢/lb and the final resin can be marketed at half the existing prices. With ever-increasing oil prices, the value of the lignin credit can be expected to increase steadily.

High value applications like this for lignin have enormous impact on the cost per gallon of ethanol.

Process Economics

We have determined the operating cost of a pretreatment unit which would be part of an ethanol plant processing 85,000 OD tons/year (250 OD tons/day) of biomass. Based on data scaled up from the Iotech pilot plant and current market prices supplied by equipment manufacturers, our engineering staff has completed a detailed design of pretreatment which incorporates Iotech guns with capacity of 75 OD tons/day operating on a semi-continuous cycle of 3 minutes. The calculations include boiler requirements, feed and discharge equipment, instrumentation, operating cost at the optimum automatic control, buildings and installation process conditions has been estimated at \$7.50/OD ton.

The operating cost is sensitive to cooking time, but our analysis shows that short cooking times at high pressure are favoured when the economics are considered.

It seems clear that \$7.50/OD ton is a low cost for pretreatment which, in a single step, can make the cellulose accessible, can separate high quality lignin, can sterilize the biomass, and can pulverize the material into a powder.

Future Research

Cheap ethanol from biomass will no doubt come as a result of technological advances in all the various processing steps, but it is apparent that pretreatment and lignin development have an important bearing on the final cost per gallon.

Several avenues for future research are apparent in these two fields.

For instance, in the current research we have noted that under certain isolated conditions sugar yields of over 80% have been obtained, and we believe it is possible to systematically optimize steam explosion to routinely achieve the higher yields. One difficulty is that the hemicellulose and cellulose peaks do not occur at the same cooking time, and a compromise is necessary between the two for the maximum total yield. At the compromise point part of the hemicellulose has been converted into furfural and furfural derivatives.

At higher pressures, kinetic theory suggests that the hemicellulose and cellulose peaks should occur closer together. Also, if the cooking time is kept very short, followed by the "freezing" action of the explosion we expect that the hemicellulose fraction does not have time to undergo the degradation reactions and higher yields are possible.

Continued systematic optimization, combined with some basic research on the kinetics, and investigation of potential catalysts is warranted for development of pretreatment.

For lignin, systematic work is required to measure the yields, reactivities and other properties over a whole range of operating conditions. Some basic research is necessary to understand the reactions and kinetics involved in the gun, with a view to improving the quality of the product lignin.

We believe that significant development efforts should be directed towards high value lignin applications such as resins, plastics, surfactants and basic lignin derivatives such as phenol and benzene.

Finally, we urge that research be undertaken to develop hardwoods as a major source of biomass. Results from hybrid poplar plantation studies indicate that wood biomass will achieve much higher biomass tonnages per acre at lower costs than other large scale sources of biomass. Development of microflora specifically selected for high performance in wood solutions is required today.

SECTION 3

EFFECT OF PRESSURE

3.0 EFFECT OF PRESSURE

(Section C in contract)

The effect of pressure on the enzyme and rumen yields was evaluated by shooting aspen chips at five different pressures. At each pressure, the cooking time was varied over a wide range.

The pretreatment performance was monitored with respect to two different assay methods. One is digestion by the NOVO enzyme of a suspension of the exploded wood, followed by measurement by HPLC of the products glucose and xylose; the other an in vitro digestion by rumen microflora. Experimental procedures and assay methods are described fully in Appendices A through E.

In the case of enzyme digestion the experimental results are expressed as % sugars as a percentage of the exploded wood. For rumen digestion, the results are expressed as a percentage of the cellulose in the exploded wood. Experimental data appear in Table 3.1 and are plotted in Figures 3.1 to 3.6.

Discussion

At each pressure, the glucose, xylose and IVCD results exhibit similar patterns. As the cooking time is increased the yield increases until a peak value is obtained. The peak value occurs at different times for the three assays. The xylose usually peaks first, whereas the IVCD and glucose peak later at approximately the same time. For example, at 560 psig, the xylose peak occurs at 13 seconds, the IVCD at 43 seconds and the glucose at 56 seconds. Figure 3.7 is a plot of the pressure against that of the log time at which the glucose yield and the IVCD peak. There is an exponential relationship between the times of the peaks at the various

pressures even though the curves were still increasing at 250 psig after 24 minutes.

After the peak, the xylose yield is seen to decline steadily. Studies of the reaction mechanism in the gun (Section 13) indicate that the hemicellulose is being converted to furfural and furfural derivatives.

In the case of the IVCD, the general pattern is that it declines sharply after the peak cooking time is passed, even down to zero. This is in contrast to the general behavior of the glucose, which usually shows very little decline. It is curious that two different enzyme complexes would show such divergent behaviour, and two hypotheses have been advanced to explain this pattern. First, it was thought that some compound formed as a byproduct in the gun reactions is toxic to the rumen system but not to the NOVO enzymes. Second, it was thought that there may be a transformation of the crystal structure of the cellulose which would inhibit the ability of the rumen to attack the cellulose.

To test the toxicity hypothesis, a sample of undercooked wood was contaminated with the same weight of overcooked wood. If a toxic material was present, it would presumably inhibit rumen activity even on the undercooked portion. It was found that the resultant IVCD was an arithmetic average of the two samples, suggesting the absence of any toxic compounds.

We now believe that the different behavior with NOVO enzymes and rumen is linked to changes in the crystal structure of the cellulose substrate. It can be seen in photomicrographs (Section 15) that the original fibres are transformed into an amorphous mass as the cooking time is increased. The peak yield corresponds approximately to the complete disappearance of crystal

fibres. It is likely that the pore size of the cellulose shrinks, and we believe that the larger rumen enzymes cannot reach the substrate as effectively as the smaller *Trichoderma* enzymes.

The peak yields at each pressure for the rumen, hemicellulose (xylose), alphacellulose (glucose) and holocellulose (xylose + glucose) are tabulated in Table 3.2 and are plotted in Figure 3.8. All the data have been corrected for the weight loss which occurs in the pretreatment process using the chart in Appendix H, so that all data is expressed as a % of original wood entering the gun. It should be noted that the maximum theoretical values for the aspen are as follows (on an extractive-included basis):

IVCD	100%
Glucose	52%
Xylose	20%

It can be seen that the peak rumen digestibility is increased at lower pressures, whereas the hemicellulose (xylose) achieves more yield at higher pressures.

In the case of the alphacellulose (glucose) yield, there is a slight decline with increasing pressure, whereas the holocellulose (xylose + glucose) has an optimum at an intermediate pressure.

It is not simple, therefore, to ascertain the optimum operating pressure. Since the cost of pretreatment is very sensitive to cooking time (Section 14) the relative costs of operating at a certain pressure must be taken into account.

An optimization analysis has been performed incorporating the technical data (Section 3) and the economic data (Section 14). In particular, incremental cost of operating at a pressure below 650 psig is balanced against the incremental value of any extra sugar yield obtained at the lower pressure. The calculations are shown in Tables 3.3 through 3.6. The final net gain (or loss) has been plotted in Figure 3.9.

Conclusion

It can be seen that the optimum for hemicellulose (xylose) yield occurs at 600 psig whereas for maximum alphacellulose (glucose), the process may be economically operated over a wide range from 450-550 psig.

Since maximum yield on holocellulose is most desirable for cheapest ethanol cost, we recommend that the steam explosion pretreatment be operated at pressure of 500-550 psig.

FIGURE 3.1

EFFECT OF PRESSURE ON ASPEN AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 3.1

FIGURE 3.2

EFFECT OF PRESSURE ON ASPEN AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 3.2

FIGURE 3.3

SUMMARY OF EFFECT OF PRESSURE ON ASPEN
AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 3.3

FIGURE 3.4

EFFECT OF PRESSURE ON ASPEN AS SHOWN BY RUMEN DIGESTION

21119528
30717528
41018528

FIGURE 3.4

50716528
60716528

FIGURE 3.5

EFFECT OF PRESSURE ON ASPEN AS SHOWN BY RUMEN DIGESTION

FIGURE 3.5

FIGURE 3.6

SUMMARY OF EFFECT OF PRESSURE ON ASPEN AS SHOWN BY
RUMEN DIGESTION

21119528
30717528
41018528
50716528
60717528

FIGURE 3.6

FIGURE 3.7

RELATIONSHIP BETWEEN ASSAY PEAKS AND PRESSURE

FIGURE 3.7

FIGURE 3.8

EFFECT OF PRESSURE ON YIELDS

FIGURE 3.9

ECONOMIC GAIN (OR LOSS) FROM OPERATION AT
VARIOUS PRESSURES

TABLE 3.1

EFFECT OF VARIOUS PRESSURES AT DIFFERENT TIMES ON ASPEN CHIPS

250 psig					
Iotach #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
21119528	240	58.8	50.0	11.4	14.8
	480	52.4	65.6	9.5	19.6
	720	53.0	75.8	9.1	23.4
	840	53.8	81.4	5.3	20.9
	960	54.8	86.1	4.9	27.6
	1080	55.8	88.2	4.6	29.5
	1200	57.4	87.5	3.0	34.8
	1440	57.0	90.2	3.0	36.7
360 psig					
30717528	74	49.2	27.6	10.6	19.3
	130	49.3	61.0	10.1	24.9
	190	50.8	78.3	7.6	31.9
	260	51.8	83.0	5.3	30.4
	400	52.3	67.7	2.3	28.9

TABLE 3.1

TABLE 3.1 continued

EFFECT OF VARIOUS PRESSURES AT DIFFERENT TIMES ON ASPEN CHIPS

450 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
41018528	100	50.8	68.9	10.2	30.0
	120	50.0	78.8	9.1	31.0
	160	52.0	80.8	6.2	30.5
	180	49.4	79.8	7.7	32.5
	200	52.2	69.7	3.7	31.0
	220	51.8	69.1	5.7	33.0
	240	53.6	71.6	5.7	33.0
	260	53.0	58.5	3.4	32.5
	300	51.2	37.1	2.8	31.3

TABLE 3.1 continued.

TABLE 3.1 continued

EFFECT OF VARIOUS PRESSURES AT DIFFERENT TIMES ON ASPEN CHIPS

560 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
50716528	13	47.8	29.7	13.0	14.3
	25	47.8	50.6	10.5	18.1
	43	47.6	74.4	10.1	25.7
	56	68.3	48.6	9.2	28.6
	86	48.9	4.7	2.9	25.7
	120	45.7	0	1.5	25.7
650 psig					
60716528	5	46.4	49.6	11.4	15.2
	10	41.0	-	11.4	15.2
	20	47.4	65.4	11.0	21.9
	40	48.6	43.2	6.7	26.7
	60	48.1	6.3	2.9	23.8

TABLE 3.1 continued

TABLE 3.2

PEAK YIELDS AT VARIOUS PRESSURES
WITH CORRESPONDING COOKING TIMES

PRESSURE psig	IVCD		XYLOSE ^a		GLUCOSE ^a		XYLOSE + GLUCOSE	
	Peak Yield	Cook Time sec.	Peak Yield	Cook Time sec.	Peak Yield	Cook Time sec.	Peak Yield	Cook Time sec.
250	88.2	1080	11.2	240	30.0	1200	33.6	1200
360	83.0	260	10.2	74	29.0	190	35.9	190
450	80.8	160	9.4	100	27.6	180	37.0	100
560	74.4	43	12.9	13	26.6	56	35.2	56
650	65.4	20	11.3	5	24.8	40	30.6	20

^a % sugars, expressed as % of original wood.

TABLE 3.2

TABLE 3.3

GLUCOSE ("ALPHACELLULOSE") YIELD
NET ECONOMIC GAIN (OR LOSS) AT VARIOUS PRESSURES

1	2	3	4	5	6	7	8
OPERATING PRESSURE	COOK TIME FOR PEAK GLUCOSE YIELD (a)	PEAK GLUCOSE YIELD (a)	INCREMENTAL GLUCOSE YIELD	INCREMENTAL VALUE OF EXTRA GLUCOSE (b)	ESTIMATED OPERATING COST (c)	INCREMENTAL OPERATING COST	NET GAIN (LOSS) (d)
psig	YIELD (a)	% sugars	% sugars	\$/OD ton	\$/OD ton	\$/OD ton	\$/OD ton
650	20 sec	23.5	---	---	6.40	---	---
560	56 sec	26.6	3.1	5.02	7.20	.80	4.22
450	2 min	27.9	4.4	7.13	9.30	2.90	4.23
360	4.3 min	29.0	5.5	8.91	12.05	5.65	3.26
250	20 min	31.0	7.5	12.15	27.83	21.43	(9.28)

TABLE 3.3

(a) Table 3.2

(b) At \$1.62 per 1% sugar (see Table 3.6)

(c) Table 14.1

(d) Column 5 minus Column 7

TABLE 3.4

XYLOSE ("HEMICELLULOSE") YIELD
NET ECONOMIC GAIN (OR LOSS) AT VARIOUS PRESSURES

1	2	3	4	5	6	7	8
OPERATING PRESSURE	COOK TIME FOR PEAK XYLOSE YIELD	PEAK XYLOSE YIELD % sugars	INCREMENTAL XYLOSE YIELD % sugars	INCREMENTAL VALUE OF EXTRA XYLOSE % sugars	ESTIMATED OPERATING COST \$/ OD ton	INCREMENTAL OPERATING COST \$/OD ton	NET GAIN (LOSS) \$/OD ton
psig							
650	5	11.3	---	---	6.12	---	---
560	13	12.9	1.6	2.60	-6.20	.08	2.52
450	100	9.4	-1.9	-3.04	8.60	2.48	(5.52)
360	74	10.2	-1.1	-1.78	7.90	1.78	(3.56)
250	240	11.2	-0.1	-0.16	11.80	5.68	(5.84)

TABLE 3.4

TABLE 3.5

XYLOSE + GLUCOSE ("HOLOCELLULOSE") YIELD
NET ECONOMIC GAIN (OR LOSS) AT VARIOUS PRESSURES

1	2	3	4	5	6	7	8
OPERATING PRESSURE	COOK TIME FOR PEAK SUGAR YIELD	PEAK SUGAR YIELD % sugars	INCREMENTAL SUGAR YIELD % sugars	INCREMENTAL VALUE OF EXTRA SUGAR \$/OD ton	ESTIMATED OPERATING COST \$/OD ton	INCREMENTAL OPERATING COST \$/OD ton	NET GAIN (LOSS) \$/OD ton
psig	YIELD						
650	20 sec	30.6	---	---	6.40	---	---
560	56 sec	35.2	4.6	7.45	7.20	.80	6.65
450	100 sec	37.0	6.4	10.37	8.60	2.20	8.17
360	4.3 min	32.5	1.5	2.43	12.05	5.65	(3.22)
250	20 min	33.6	3.0	4.86	27.83	21.43	(16.57)

TABLE 3.5

VALUE OF EXTRA 1% SUGAR

Basis 1 OD ton (2000 lb) aspen
65% holocellulose

1. Theoretical yield of ethanol from 1 OD ton
= $\frac{2000 \text{ lb} \times 65\% \times 0.568 \text{ lb alcohol/lb cellulose}}{6.29 \text{ lb alcohol/gallon}}$
= 117 gallons
2. Value of alcohol produced at \$1.00/gallon
= \$117
3. % sugars at 100% yield
= $65\% \times 1.111$
= 72% sugars
4. Value of alcohol produced
= $\frac{117}{72}$
= \$1.62/1% sugar

SECTION 4

EFFECT OF PARTICLE SIZE OF STARTING MATERIAL

4.0 EFFECT OF PARTICLE SIZE OF STARTING MATERIAL

The chips for this test were prepared as follows:

large chips - these were taken from a commercial chipper.

medium chips - they were the above chips hammer milled through $1\frac{1}{4}$ " holes and then sieved to remove the fines.

small chips - the fines above were collected by sieving through an $1/8$ " screen.

Fifty individual chips of each kind were picked at random and the chips dimensions were measured. The results are given in the following table.

Dimensions	Big Chips	Medium Chips	Small Chips
average thickness	4.3 mm	2.764 mm	1.72 mm
standard deviation	= 1.555	= 1.011	= 0.536
average width	19.2 mm	6.38 mm	3.18 mm
standard deviation	= 4.907	= 2.193	= 0.747
average length (along the grain)	23.4 mm	23.4 mm	23.4 mm
	= 6.409		

The assay results for these three sizes of chips are shown in Table 4.1 and then plotted on Figures 4.1.1 and 4.1.2. In Table and Figure 4.2 another sample of chips has been used, one inch and $1/8$ inch hammermill and chipper pieces. The general trends are quite similar, but the medium size chips show the smoothest curves. The large chips are not necessarily cooked through in the time they are at pressure, resulting in assays with quite a

wide variation. On the other hand, the dust and fibres, which are broken off the chips in the chipping process, have been included in the small chip samples. When the steam comes into contact with this material it condenses on the fines and forms balls which are uncooked in the center. A lack of activity from the same cause can be seen more dramatically in Figure 4.2, where samples of 1/8 inch diameter material from the hammermill show relatively low results.

Table 4.3 and Figures 4.3.1 to 4.3.6 show the results of cooking large chips at various pressures. These results have the same general trends as have been discussed in Section 3.0.

The size of the particles of the starting material, provided it is of a median size, does not appear to have very much effect on the assay activity.

31004293
21009293
41009293

FIGURE 4.1.1...

EFFECT OF SIZE OF INPUT CHIPS AT 550 psig AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 4.1.1

FIGURE 4.1.2

EFFECT OF SIZE OF INPUT CHIPS AT 550 psig AS SHOWN BY
RUMEN DIGESTION

31004293

21009293

41009293

FIGURE 4.1.2

FIGURE 4.2
EFFECT OF SIZE OF INPUT CHIPS AT 640 psig AS SHOWN BY
RUMEN DIGESTION

31022321
21119321

FIGURE 4.3.1

EFFECT OF PRESSURE ON LARGE ASPEN CHIPS AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 4.3.1

61120321
51009321

FIGURE 4.3.2

EFFECT OF PRESSURE ON LARGE ASPEN CHIPS AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 4.3.2

FIGURE 4.3.3

SUMMARY OF EFFECT OF PRESSURE ON LARGE ASPEN CHIPS

AS SHOWN BY ENZYMATIC DIGESTION

51009321
31018321
21119321
61120321

FIGURE 4.3.3

FIGURE 4.3.4

EFFECT OF PRESSURE ON LARGE ASPEN CHIPS AS SHOWN BY
RUMEN DIGESTION

41018321
31022321
21114321

FIGURE 4.3.4

FIGURE 4.3.5

EFFECT OF PRESSURE ON LARGE ASPEN CHIPS AS SHOWN
BY RUMEN DIGESTION

51009321
41018321
61120321

FIGURE 4.3.5

FIGURE 4.3.6

SUMMARY OF EFFECT OF PRESSURE ON LARGE ASPEN CHIPS AS SHOWN
BY RUMEN DIGESTION

51009321
41018321
31022321
21119321
61120321

FIGURE 4.3.6

TABLE 4.1

EFFECT OF SIZE OF INPUT CHIPS AT 550 psig

<u>Aspen</u>		<u>Small chips</u>			
Iotech #	Time	% cellulose	IVCD	% xylose	% glucose
	(sec)				
21009293	20	50.8	40.9	11.5	17.2
	40	48.6	54.7	13.0	25.7
	60	49.0	55.5	10.1	29.5
	70	57.8	61.4	8.2	25.7
	80	48.6	70.0	8.6	27.6
	85	50.2	50.6	8.2	25.7
	90	51.0	60.4	8.2	26.7
	95	50.6	68.4	7.6	27.6
	100	51.4	56.8	7.6	29.5
	120	52.0	21.5	5.7	28.6

TABLE 4.1

TABLE 4.1 continued

EFFECT OF SIZE OF INPUT CHIPS AT 550 psig

<u>Aspen</u>		<u>Medium chips</u>			
Iotech #	Time	% cellulose	IVCD	% xylose	% glucose
	(sec)				
31004293	20	48.8	39.7	13.4	21.0
	40	49.0	55.9	12.4	25.7
	60	50.4	65.5	11.0	30.4
	70	51.4	74.3	10.1	29.6
	80	52.6	73.4	8.6	29.5
	85	52.4	68.3	6.7	30.4
	90	50.4	60.7	6.7	32.4
	95	51.4	60.9	7.6	31.4
	100	49.0	40.8	8.2	32.3
	105	50.2	45.8	7.6	32.4
	120	51.0	21.2	5.7	39.9

TABLE 4.1 continued

TABLE 4.1 continued

EFFECT OF SIZE OF INPUT CHIPS AT 550 psig

<u>Aspen</u>		<u>Large chips</u>			
Iotech #	Time	% cellulose	IVCD	% xylose	% glucose
	(sec)				
41009293	20	49.6	37.5	11.4	19.0
	40	51.2	54.3	10.5	25.7
	60	49.4	73.7	10.1	25.7
	70	50.0	78.4	9.6	24.9
	80	49.2	74.4	10.5	30.4
	85	53.0	81.1	9.1	28.5
	90	51.2	81.6	8.6	28.5
	95	51.2	77.7	8.2	25.7
	100	50.6	77.1	10.1	33.3
	120	49.4	73.7	7.2	30.4

TABLE 4.1 continued

TABLE 4.2

EFFECT OF SIZE OF INPUT CHIPS AT 640 psig

<u>Aspen</u>		<u>Chips from chipper</u>		<u>1/8 inch hammermill</u>		<u>1 inch hammermill</u>	
Iotech #	Time (sec)	% cellulose	IVCD	% cellulose	IVCD	% cellulose	IVCD
90411597	22	48.5	24.5	51.3	59.4	47.6	47.1
	40	48.2	71.4	47.1	47.8	47.5	73.9
	55	47.9	78.0	47.6	32.4	47.8	76.5
	70	48.2	75.9	46.7	28.5	48.2	61.0
	90	49.1	60.1	44.9	1.3	48.7	36.8
	120	48.3	44.9	44.9	3.9		
	170	46.7	3.2				

TABLE 4.2

TABLE 4.3

EFFECT OF VARYING PRESSURE ON BIG ASPEN CHIPS

250 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
21119321	240	58.8	50.0	11.4	14.8
	480	42.4	65.6	9.5	19.6
	720	53.0	75.8	9.1	23.4
	840	53.8	81.4	5.3	20.9
	960	54.8	86.1	4.9	27.6
	1080	55.2	88.2	4.6	29.5
	1200	57.4	87.5	3.0	34.8
	1440	57.0	90.2	3.0	36.7

TABLE 4.3

TABLE 4.3 continued

EFFECT OF VARYING PRESSURE ON BIG ASPEN CHIPS

350 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
31022321	120	50.4	50.4	18.1	23.8
	180	51.6	65.5	14.8	29.5
	240	51.0	72.5	14.8	31.4
	300	51.4	79.0	11.4	42.8
	360	52.2	80.5	7.2	31.4
	420	54.4	82.0	8.2	34.2
	480	57.2	83.9	4.4	40.9
	600	54.2	71.6	3.8	40.0
	720	65.0	62.8	1.5	38.0

TABLE 4.3 continued

TABLE 4.3 continued

EFFECT OF VARYING PRESSURE ON BIG ASPEN CHIPS

450 psig			
Iotech #	Time (sec)	% cellulose	IVCD
41018321	100	50.8	68.9
	120	50.0	78.8
	160	52.0	80.8
	180	49.4	79.8
	200	52.2	69.7
	220	57.8	69.1
	240	53.6	71.6
	260	53.0	58.5
	300	51.2	37.1

TABLE 4.3 continued

TABLE 4.3 continued

EFFECT OF VARYING PRESSURE ON BIG ASPEN CHIPS

550 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51C09321	20	49.6	37.5	11.4	19.0
	40	51.2	54.3	10.5	25.7
	60	49.4	73.7	10.1	25.7
	70	50.0	78.4	9.6	24.9
	80	49.2	74.4	10.5	30.4
	85	53.0	81.1	9.1	28.5
	90	51.2	81.6	8.6	28.5
	95	51.2	77.7	8.2	25.7
	100	50.6	77.1	10.1	33.3
	120	49.4	73.7	7.2	30.4

TABLE 4.3 continued

Table 4.3 continued

EFFECT OF VARYING PRESSURES ON BIG ASPEN CHIPS

650 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
61120321	10	50.2	38.6		
	20	48.6	55.2	8.2	11.0
	40	48.8	72.1	6.8	18.2
	50	48.0	79.2	6.7	19.0
	55	48.4	73.1	5.9	19.0
	60	47.8	71.1	5.3	19.6
	65	49.8	75.5	4.9	20.9
	70	49.0	40.8	4.9	20.9
	90	50.6	24.5	3.2	21.5
	120	49.0	1.2	2.9	20.0

TABLE 4.3 continued

SECTION 5

EFFECT OF STARTING MOISTURE CONTENT

5.0 EFFECT OF STARTING MOISTURE CONTENT

The effect of moisture content in wood has been tested at five different cook temperatures with four different moisture contents. The results are shown in Tables 5.1 to 5.5 and plotted on Figures 5.1 to 5.5. These figures show the effect of differing moisture contents at each pressure. The same results were re-plotted in Figure 5.6.1 to 5.6.4 showing the rumen digestion results at each average moisture content. Finally, a summary plot was drawn in Figure 5.7 for 650, 550 and 360 psig pressures showing how the moisture content affected the peak glucose, xylose, holocellulose and IVCD production; these were then plotted as a percentage of the original dry weight.

The best results were obtained with 35% moisture at 550 psig. 25% and 50% average moisture was inferior to this. Below 25% moisture the values increased for glucose, xylose and holocellulose but not for the IVCD. The only 63% moisture tested showed that it was worse than 50% moisture. However, even at 650 psig there was still over 60% IVCD activity after 120 seconds, when with lower moisture there is generally little activity left at this point. The higher the moisture content the broader is the peak for the rumen digestion.

FIGURE 5.1.1

EFFECT OF MOISTURE CONTENT AT 650 psig ON THE GLUCOSE PRODUCTION

ASPEN

60613536
50417536
30417536
20613536
00417536

FIGURE 5.1.1

FIGURE 5.1.2...

EFFECT OF MOISTURE CONTENT AT 650 psig ON THE XYLOSE PRODUCTION

ASPEN

60613536
50417536
30417536
20613536
00417536

FIGURE 5.1.2

FIGURE 5.1.3

EFFECT OF MOISTURE CONTENT AT 650 psig AS SHOWN BY

RUMEN DIGESTION

ASPEN

60613536
50417536
30417536
20613536
60417536

FIGURE 5.1.3

FIGURE 5.2.1.1

EFFECT OF MOISTURE CONTENT AT 560 psig ON THE GLUCOSE PRODUCTION

ASPEN

30712256
20716256
10712256
51004256

FIGURE 5.2.1.1

30712256
20716256
10712256
51004256

FIGURE 5.2.2

EFFECT OF MOISTURE CONTENT AT 560 psig ON THE XYLOSE PRODUCTION

ASPEN

FIGURE 5.2.2

51004256
 30712256
 20716256
 10712256

FIGURE 5.2.3
 EFFECT OF MOISTURE CONTENT AT 560 psig AS SHOWN BY
 RUMEN DIGESTION
 ASPEN

FIGURE 5.2.3

FIGURE 5.3.3

EFFECT OF MOISTURE CONTENT AT 470 psig AS SHOWN BY
RUMEN DIGESTION
ASPEN

50710476
30712476
20716476
00718476

FIGURE 5.3.3

30713396
20717396
00718396

FIGURE 5.4.1

EFFECT OF MOISTURE CONTENT AT 360 psig AS SHOWN BY

GLUCOSE PRODUCTION

ASPEN

FIGURE 5.4.1

30713396
20717396
00718396

FIGURE 5.4.2

EFFECT OF MOISTURE CONTENT AT 360 psig ON
XYLOSE PRODUCTION
ASPEN

FIGURE 5.4.2

FIGURE 5.4.3

EFFECT OF MOISTURE CONTENT AT 360 psig AS SHOWN BY

RUMEN DIGESTION

ASPEN

30713396

20717396

00718396

FIGURE 5.4.3

50503236
30713236
00718236

FIGURE 5.5.3.0

EFFECT OF MOISTURE CONTENT AT 200 psig AS SHOWN BY RUMEN DIGESTION

FIGURE 5.5.3

FIGURE 5.6.1

EFFECT OF VARYING PRESSURES ON ASPEN CHIPS

WITH 50% AVERAGE MOISTURE

50417536
51004396
50710476
41022396

- 200 psig
- △ 360 psig
- 470 psig
- 500 psig
- ▲ 650 psig

FIGURE 5.6.1

FIGURE 5.6.2

EFFECT OF PRESSURE ON ASPEN CHIPS WITH 35%
AVERAGE MOISTURE

30417536
30712256
30712476
30713396
30713236

FIGURE 5.6.2

FIGURE 5.6.3

EFFECT OF PRESSURE ON ASPEN CHIPS WITH 22%
AVERAGE MOISTURE

20613536
20716256
20716476
20717396

FIGURE 5.6.3

00417536
00718476
00718396
00718236

FIGURE 5.6.4

EFFECT OF PRESSURE ON ASPEN CHIPS WITH 7% AVERAGE MOISTURE

FIGURE 5.6.4

FIGURE 5.7

SUMMARY OF PEAK ACTIVITIES OF GLUCOSE, XYLOSE, HOLOCELLULOSE
AND IVCD WITH DIFFERENT MOISTURE CONTENTS

TABLE 5.1

EFFECT OF MOISTURE CONTENT OF CHIPS AT 650 psig

Aspen

63% moisture					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
60613536	15	48.4	37.2	12.0	17.2
	30	50.2	62.9	10.5	21.9
	45	49.0	77.6	12.0	27.6
	60	48.8	80.7	12.9	32.3
	80	51.2	76.2	9.1	30.4
	120	52.4	61.4	4.8	26.6
50% moisture					
50417536	5	48.3	30.0	14.9	13.3
	20	47.8	56.9	18.6	30.4
	40	48.4	79.4	16.2	31.4
	55	40.4	82.6	11.0	36.1
	70	51.8	48.3	7.6	35.2

TABLE 5.1

TABLE 5.1 continued

EFFECT OF MOISTURE CONTENT OF CHIPS AT 650 psig

Aspen

36% moisture					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
30417536	5	47.9	70.4	17.1	15.2
	20	47.8	67.3	18.0	32.4
	40	47.5	79.8	14.8	40.0
	55	48.3	48.2	5.7	27.6
	70	48.0	14.2	3.8	32.3
21% moisture					
20613536	15	46.4	65.5	10.5	19.0
	30	47.8	65.7	8.2	23.8
	45	49.0	20.0	4.8	21.9
	60	49.6	11.3	4.4	23.8
	80	50.6	5.9	2.5	34.2
	120	51.0	0	0	28.5

TABLE 5.1 continued

TABLE 5.1 continued

EFFECT OF MOISTURE CONTENT OF CHIPS AT 650 psig

Aspen

6% moisture					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
00417536	5	47.4	17.3	15.8	9.5
	20	47.8	62.8	15.2	29.5
	40	48.4	43.5	7.6	32.4
	55	48.6	24.7	2.9	27.6
	70	47.7	0	0.6	31.4
	100	41.0	0	1.0	22.8

TABLE 5.1 continued

TABLE 5.2

EFFECT OF MOISTURE CONTENT OF CHIPS at 560 psig

Aspen

50% moisture					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51004396	20	48.8	39.7	13.4	21.0
	40	49.0	55.9	12.4	25.7
	60	50.4	65.5	11.0	30.4
	70	51.4	74.3	10.1	29.6
	80	52.6	73.4	8.6	29.5
	85	52.4	68.3	6.7	30.4
	90	50.4	60.7	6.7	32.4
	95	51.4	60.9	7.6	31.4
	100	49.0	40.8	8.2	32.3
	105	50.2	45.8	7.6	32.4
	120	51.0	21.2	5.7	39.9

TABLE 5.2

TABLE 5.2 continued

EFFECT OF MOISTURE CONTENT OF CHIPS AT 560 psig

Aspen

35% moisture					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
30712256	13	46.7	34.0	18.6	22.8
	25	48.2	50.2	21.9	40.0
	43	47.7	73.2	15.2	40.0
	56	48.3	83.4	13.9	43.8
	86	48.4	52.5	8.2	43.7
	120	46.8	14.7	6.7	43.7
	400	47.0	0	5.7	43.8
25% moisture					
20716256	13	47.8	29.7	13.0	14.3
	25	47.8	50.6	10.5	18.1
	43	47.6	74.4	10.1	25.7
	56	48.6	68.3	9.2	28.6
	86	48.9	4.7	2.9	25.7
	120	45.7	0	1.5	25.7

TABLE 5.2 continued

TABLE 5.2 continued

EFFECT OF MOISTURE CONTENT OF CHIPS AT 560 psig
Aspen

12% moisture					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
10712256	13	47.7	26.6	14.9	18.2
	25	47.3	41.2	14.8	22.8
	43	48.3	71.0	10.5	26.6
	56	49.1	58.5	9.6	36.3
	36	49.3	30.9	4.8	38.0
	120	47.1	4.5	2.5	31.4
	180	46.9	5.8	1.9	34.2

TABLE 5.2 continued

TABLE 5.3

EFFECT OF MOISTURE CONTENT OF CHIPS AT 470 psigAspen

52% moisture			
Iotech #	Time (sec)	% cellulose	IVCD
50710476	30	48.0	42.5
	60	47.0	45.5
	120	49.2	66.6
	240	49.7	50.9
	300	49.8	24.5
	360	47.9	8.1
	480	45.6	7.5
35% moisture			
30712476	33	48.1	44.3
	55	47.8	63.6
	90	49.4	77.7
	130	50.6	86.8
	200	50.9	32.4
	320	49.5	17.2

TABLE 5.3

TABLE 5.3 continued

EFFECT OF MOISTURE CONTENT OF CHIPS AT 470 psigAspen

25% moisture			
Iotech #	Time (sec)	% cellulose	IVCD
20716476	55	48.4	47.9
	90	49.6	53.6
	130	50.4	40.1
	200	50.6	20.2
	320	48.7	1.4
	480	48.4	4.5
8% moisture			
00718476	33	47.8	28.9
	55	48.5	52.2
	90	49.2	67.9
	130	50.5	47.3
	200	49.5	13.1
	320	50.0	8.0
	480	49.2	16.7

TABLE 5.3 continued

TABLE 5.4

EFFECT OF MOISTURE CONTENT OF CHIPS AT 360 psig

Aspen

46% moisture					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
41022396	120	50.4	50.4	18.1	23.8
	180	51.6	65.5	14.8	29.5
	240	51.0	72.5	14.8	31.4
	300	51.4	79.0	11.4	42.8
	360	52.2	80.5	7.2	31.4
	420	54.4	82.0	8.2	34.2
	480	57.2	83.9	4.4	40.9
	600	54.2	71.6	3.8	40.0
	720	65.0	62.8	1.5	38.0

TABLE 5.4

TABLE 5.4 continued

EFFECT OF MOISTURE CONTENT OF CHIPS AT 360 psigAspen

35% moisture					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
30713396	74	50.7	39.4	13.7	20.3
	130	50.1	62.9	12.7	29.5
	190	44.4	78.1	9.5	34.6
	260	50.9	86.2	5.5	31.2
	400	51.7	88.0	5.1	35.3
	760	50.7	40.0	1.7	28.9
	960	50.9	31.2	0.9	33.3

TABLE 5.4 continued

TABLE 5.4 continued

EFFECT OF MOISTURE CONTENT OF CHIPS AT 360 psig
Aspen

20% moisture					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
20717396	74	49.2	27.6	10.6	19.3
	130	49.3	61.0	10.1	24.9
	190	50.8	78.3	7.6	31.9
	260	51.8	83.0	5.3	30.4
	400	52.5	67.7	2.3	28.9
6% moisture					
00718396	74	49.9	21.4	9.2	8.6
	130	50.2	52.9	17.0	38.6
	190	52.9	42.5	3.9	20.7
	260	53.3	39.2	3.6	15.6
	400	52.2	32.2	0	17.6
	600	52.2	32.2	0	33.3
	760	52.0	34.0	0	29.1

TABLE 5.4 continued

TABLE 5.5

EFFECT OF MOISTURE CONTENT OF CHIPS AT 250 psigAspen

52% moisture			
Iotech #	Time (sec)	% cellulose	IVCD
50503236	120	48.9	33.7
	240	51.3	35.7
	480	49.3	40.4
	900	54.0	56.7
	1800	57.9	71.0
35% moisture			
30713236	240	50.0	40.8
	480	49.3	59.0
	1800	56.5	80.5
	3600	56.3	89.7

TABLE 5.5

TABLE 5.5 continued

EFFECT OF MOISTURE CONTENT OF CHIPS AT 250 psig

Aspen

6% moisture			
Iotech #	Time (sec)	% cellulose	IVCD
00718236	240	52.6	30.4
	480	53.2	23.7
	960	53.5	20.0
	3600	64.7	86.7

TABLE 5.5 continued

SECTION 6

EFFECT OF PRE-CONDITIONING

6.0 EFFECT OF PRE-CONDITIONING

Aspen and barley straw were pre-conditioned by treating with steam at 100 to 150 psig for 12 to 60 seconds. The results are tabulated in Tables 6.1 to 6.3, and are plotted in Figures 6.1.1 to 6.3.2. Both the aspen and the straw show an improvement in the xylose yield, but there is not much difference in the glucose yield. The pre-steaming appears to cause the IVCD to peak and decline sooner. When the green air-dried and frozen chips were pre-steamed, the glucose yield showed little difference. The xylose yield was down noticeably in the air-dried chips whether pre-steamed or not. Rumen digestibility of the green and frozen chips was reduced by the pre-steaming. It appears to cause the same result as a longer cook time in spite of being at a much lower pressure. There is a dramatic decline in rumen digestibility of air-dried chips, with moisture content of 6%, which is only marginally increased with the pre-steaming. It appears that the loss of moisture when the chips are dried is sufficient to reduce the pore size of the cellulose so the rumen microflora are unable to digest it; steaming for this length of time is not sufficient for the cellulose to reabsorb moisture and return to a normal configuration.

The conditions at which the pre-conditioning experiments have been done do not noticeably increase the yields. The only improvement appears to be in the xylose yield, when the pre-steaming reduces the decomposition of xylose to furfural.

FIGURE 6.1.1

EFFECT OF PRESTEAMING AT 150 psig FOR 12 SECONDS ON ENZYMATIC DIGESTION

ASPEN AT 650 psig

FIGURE 6.1.1

FIGURE 6.1.2

EFFECT OF PRESTEAMING AT 150 psig FOR 12 SECONDS ON RUMEN DIGESTION

ASPEN AT 650 psig

FIGURE 6.1.2

51123329

51211329

FIGURE 6.2.1

EFFECT OF PRESTEAMING BARLEY STRAW AT 100 psig FOR 20 SECONDS
AS SHOWN BY ENZYMATIC DIGESTION

550 psig

FIGURE 6.2.1

51123328
51211328

FIGURE 6.2.2

EFFECT OF PRESTEAMING BARLEY STRAW AT 550 psig FOR
20 SECONDS AS SHOWN BY RUMEN DIGESTION

FIGURE 6.2.2

FIGURE 6.3.1

EFFECT OF PRESTEAMING WITH 150 psig STEAM ON GREEN, AIR-DRIED AND FROZEN CHIPS

ASPEN AT 650 psig FOR 55 SECONDS

FIGURE 6.3.1

FIGURE 6.3.2

EFFECT OF PRESTEAMING WITH 150 psig STEAM ON GREEN, AIR-DRIED
AND FROZEN CHIPS

ASPEN AT 650 psig FOR 55 SECONDS

FIGURE 6.3.2

TABLE 6.1

EFFECT OF PRESTEAMING WITH 150 psig STEAM
FOR 12 SECONDS BEFORE COOKING AT 650 psig

presteaming					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
60613479	15	48.4	57.8	13.3	20.9
	30	47.8	76.2	10.5	22.8
	45	48.8	81.6	16.0	30.4
	60	49.8	65.9	15.0	28.6
	80	50.6	45.8	15.0	28.6
	120	51.4	26.8	13.0	24.7
untreated					
60613479	15	48.4	37.2	12.0	17.2
	30	50.2	62.9	10.5	21.9
	45	49.9	77.6	12.0	27.6
	60	48.8	80.7	12.9	32.3
	80	51.2	76.2	9.1	30.4
	120	52.4	61.4	4.8	26.6

TABLE 6.1

TABLE 6.2

EFFECT OF PRESTEAMING BARLEY STRAW FOR 20 SECONDSuntreated

550 psig

Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51123328	20	41.6	35.1	9.8	22.4
	30	43.2	44.4	10.5	26.8
	40	46.4	52.6	8.2	35.2
	50	45.6	60.1	5.3	30.2
	60	43.6	49.1	2.7	30.8
	70	45.0	60.0	2.7	32.1
	80	45.2	69.9	3.0	33.5
	90	45.4	70.5	2.0	29.6
	100	43.6	73.4	2.0	30.8
	120	43.4	71.0	3.3	30.5

presteamed

51211328	20	43.4	38.2	13.5	20.1
	30	42.6	63.8	14.4	26.4
	40	44.6	52.9	10.6	28.7
	50	42.8	44.4	11.0	31.0
	60	44.6	61.4	6.8	30.6
	70	45.0	68.9	7.6	31.0
	90	47.6	73.9	6.8	33.4
	110	45.0	62.2	4.8	34.8

TABLE 6.2

TABLE 6.3

PRESTEAMING GREEN, AIR DRIED AND FROZEN
ASPEN AT 150 psig FOR 20, 40 & 60 SECONDS,
THEN COOK TIME FOR 55 SECONDS AT 650 psig

Aspen

green					
Iotech #	Time	Presteam Time	IVCD	% xylose	% glucose
	(sec)	(sec)			
60430625	55	0	78.0	9.1	23.8
	55	20	74.4	9.1	25.7
	55	40	52.0	6.3	23.8
	55	60	44.7	6.7	24.7
air dried					
60430625	55	0	1.4	2.5	22.8
	55	20	6.9	4.8	30.4
	55	40	4.9	2.5	22.8
	55	60	6.1	2.5	25.7

TABLE 6.3

TABLE 6.3 continued

PRESTEAMING GREEN, AIR DRIED AND FROZEN
ASPEN AT 150 psig FOR 20, 40 & 60 SECONDS,
THEN COOK TIME FOR 55 SECONDS AT 650 psig

Aspen

frozen					
Iotech #	Time (sec)	Presteam Time (sec)	IVCD	% xylose	% glucose
60430625	55	0	76.2	9.1	22.8
	55	20	57.4	9.1	27.6
	55	40	57.4	8.2	26.6
	55	60	61.1	6.7	25.7

TABLE 6.3 continued

SECTION 7

EFFECT OF DIE CONFIGURATIONS

7.0 EFFECT OF DIE CONFIGURATIONS

An initial experiment was run using three die types: sieve, star and circular. Diagrams of these are shown in Figures 7.1 to 7.3. The results are tabulated in Table 7.1 and have been plotted in Figures 7.4 and 7.5. Very little difference in activity can be found and it was concluded that as good results could be obtained using no die.

FIGURE 7.1

STAR DIE 316 S/S

FIGURE 7.2

SIEVE DIE 316 S/S

FIGURE 7.3

CIRCULAR DIE 316 S/S

FIGURE 7.4

EFFECT OF USING DIFFERENT DIES AS SHOWN BY ENZYMATIC DIGESTION

ASPEN AT 650 psig

FIGURE 7.4

FIGURE 7.5

EFFECT OF USING DIFFERENT DIES AS SHOWN BY RUMEN DIGESTION
ASPEN AT 650 psig

FIGURE 7.5

TABLE 7.1

EFFECT OF DIFFERENT DIES ON ASPEN AT 650 psig

none					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
60417743	5	48.3	30.0	14.9	13.3
	16	47.3	42.1	14.9	18.2
	20	47.8	56.9	18.6	30.4
	40	48.4	79.4	10.3	31.4
	55	47.8	82.6	11.0	32.3
	70	51.8	48.3	7.6	35.2
star					
60417743	16	47.5	45.7	13.9	19.0
	40	47.2	80.0	15.2	27.6
	55	47.5	83.6	10.5	29.6
	70	48.4	71.1	10.1	32.3
	80	47.4	65.0	5.7	30.4
	90	47.4	14.8	5.7	31.4

TABLE 7.1

TABLE 7.1 continued

EFFECT OF DIFFERENT DIES ON ASPEN AT 650 psig

sieve					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
60417748	16	48.0	56.7	15.2	20.9
	40	48.1	80.5	12.4	28.3
	55	47.5	84.4	9.1	28.3
	70	47.5	51.6	7.6	31.4
	90	46.6	14.8	4.4	27.6
circular					
60417748	16	47.9	49.1	16.2	24.7
	40	47.6	82.4	12.0	32.3
	55	48.0	74.2	10.1	30.4
	70	47.7	45.1	5.3	30.4
	90	48.0	14.6	3.8	28.6

TABLE 7.1 continued

SECTION 8

INVESTIGATION OF DIFFERENT SUBSTRATES

8.0 INVESTIGATION OF DIFFERENT SUBSTRATES

Three groups of substrates were investigated: (1) wood, comprising aspen, birch, maple, basswood and oak; (2) straws, comprising barley straw, oat straw and wheat straw; and (3) agricultural by-products, comprising corn stover, bagasse, sugar cane rind, sunflower stalks and peat. Experimental results are shown in Tables 8.1 to 8.11, and plotted in Figures 8.1.1 to 8.11.1.

The general pattern for all substrates was similar to aspen as reported in Section 3. At a given pressure, as the cooking time is increased, the yields of the various products increase to a peak, and then decline. The cooking time for the peak yield of xylose is shorter than for the peak yield of glucose, and a compromise cooking time will usually give the highest yield of total sugars. As the pressure is increased, the cooking time necessary for the peak yield becomes shorter.

The theoretical yields of glucose and xylose have been calculated from literature values of cellulose and pentose content and are shown in Table 8.12. The peak yield for each substrate has been tabulated, and the percent of theoretical yield calculated. For glucose, it can be seen from Table 8.12 that the average yield over all the substrates is 68%, with a range from 46% to 87%. The data is charted in Figure 8.12.1. In general, the woods give higher yields than the other substrates, centering around 72%, though oak is disappointing at only 47%. The general level for straws and the agricultural by-products is about 65%, with barley straw and corn stover above the average, and sunflower stalks below.

Generally similar results pertain to the IVCD and total sugar yield as shown in Figures 8.12.2 and 8.12.3. Some IVCD tests were done on peat, but a low result of 42% IVCD was obtained.

It is known that the hemicellulose fraction of ligno-cellulosics begins to degrade if the biomass is exposed to adverse conditions for long time periods. In particular, the sunflower stalks tested were wet and badly-weathered, which may account for the low result. Materials such as peat, which clump together on exposure to steam, are difficult to treat satisfactorily in the Iotech process.

The cooking times for peak glucose yield have been compared at 550 psig for the different substrates. Results are shown in Table 8.13 and plotted in Figure 8.13. We can observe that, in general, the woods require less time for maximum recovery than the straws and agricultural by-products.

Conclusion

Combining the results from all substrates, we have achieved average peak yields as follows:

	Peak Yield	Range
Glucose	68	46-87
Xylose	64	30-86
Glucose + Xylose	60	41-75
IVCD	82	42-90

Yields from wood substrates are generally slightly better than those from straws and agricultural products. Aspen, birch, maple, barley straw and corn stover achieved good yields, whereas oak and sunflower stalks were below average.

FIGURE 8.1.1

21109758

EFFECT OF PRESSURE ON BIRCH CHIPS AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 8.1.1

FIGURE 8.1.2

51108758
41127758

EFFECT OF PRESSURE ON BIRCH CHIPS AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 8.1.2

FIGURE 8.1.3

SUMMARY OF EFFECT OF PRESSURE ON BIRCH CHIPS AS SHOWN BY
ENZYMATIC DIGESTION

51108758

21109758

41121758

FIGURE 8.1.3

FIGURE 8.1.4

21120758

EFFECT OF PRESSURE ON BIRCH CHIPS AS SHOWN BY RUMEN DIGESTION

FIGURE 8.1.4

FIGURE 8.1.5

51108758

41121758

EFFECT OF PRESSURE ON BIRCH CHIPS AS SHOWN BY RUMEN DIGESTION

FIGURE 8.1.5

FIGURE 8.1.6

SUMMARY OF EFFECT OF PRESSURE ON BIRCH CHIPS AS SHOWN BY
RUMEN DIGESTION

51108758
21109758
41121758

FIGURE 8.1.6

FIGURE 8.2.1

21109931

EFFECT OF PRESSURE ON MAPLE CHIPS AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 8.2.1

FIGURE 8.2.2

51102931

41122931

EFFECT OF PRESSURE ON MAPLE CHIPS AS SHOWN BY ENZYMATIC
DIGESTION

FIGURE 8.2.3

SUMMARY OF EFFECT OF PRESSURE ON MAPLE CHIPS AS SHOWN
BY ENZYMATIC DIGESTION

51102931
21109931
41122931

△ 250 psig
□ 450 psig
○ 550 psig
— glucose
--- xylose

FIGURE 8.2.4

EFFECT OF PRESSURE ON MAPLE CHIPS AS SHOWN BY
RUMEN DIGESTION

FIGURE 8.2.4

FIGURE 8.2.5

51102931

41122931

EFFECT OF PRESSURE ON MAPLE CHIPS AS SHOWN BY RUMEN DIGESTION

FIGURE 8.2.5

FIGURE 8.2.6

SUMMARY OF EFFECT OF PRESSURE ON MAPLE CHIPS AS SHOWN
BY RUMEN DIGESTION

51102931
41122931
21109931

FIGURE 8.2.6

21207328

FIGURE 8.3.1

EFFECT OF PRESSURE ON BARLEY STRAW AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 8.3.1

FIGURE 8.3.2

51116328
41206328

EFFECT OF PRESSURE ON BARLEY STRAW AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 8.3.2

FIGURE 8.3.3

SUMMARY OF EFFECT OF PRESSURE ON BARLEY STRAW AS SHOWN BY
ENZYMATIC DIGESTION

51116328
41205328
21207328

FIGURE 8.3.3

21207328

FIGURE 8.3.4

EFFECT OF PRESSURE ON BARLEY STRAW AS SHOWN BY
RUMEN DIGESTION

FIGURE 8.3.4

51146328
41206328

FIGURE 8.3.5

EFFECT OF PRESSURE ON BARLEY STRAW AS SHOWN BY RUMEN
DIGESTION

FIGURE 8.3.5

FIGURE 8.3.6

SUMMARY OF EFFECT OF PRESSURE ON BARLEY STRAW AS SHOWN BY
RUMEN DIGESTION

21207328
41206328
51116328

FIGURE 8.4.1

EFFECT OF PRESSURE ON OAT STRAW AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 8.4.2

51128720
41128720

EFFECT OF PRESSURE ON OAT STRAW AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 8.4.3,

SUMMARY OF EFFECT OF PRESSURE ON OAT STRAW

AS SHOWN BY ENZYMATIC DIGESTION

51128720

41128720

21129720

△ 250 psig

□ 450 psig

○ 550 psig

— glucose

--- xylose

FIGURE 8.4.3

FIGURE 8.4.4.

21129720

EFFECT OF PRESSURE ON OAT STRAW AS SHOWN BY RUMEN DIGESTION

FIGURE 8.4.4

51128720
41128720

FIGURE 8.4.5

EFFECT OF PRESSURE ON OAT STRAW AS SHOWN BY RUMEN DIGESTION

FIGURE 8.4.5

21129720
41128720
51128720

FIGURE 8.4.6

SUMMARY OF EFFECT OF PRESSURE ON OAT STRAW AS SHOWN BY
RUMEN DIGESTION

FIGURE 8.4.6

FIGURE 8.5.1

EFFECT OF PRESSURE ON WHEAT STRAW AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 8.5.1

FIGURE 8.5.2

51203525
41204525

EFFECT OF PRESSURE ON WHEAT STRAW AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 8.5.2

FIGURE 8.5.3

SUMMARY OF EFFECT OF PRESSURE ON WHEAT STRAW AS SHOWN BY
ENZYMATIC DIGESTION

51203525
41204525
31202525

FIGURE 8.5.3

FIGURE 8.5.4

21205525

EFFECT OF PRESSURE ON WHEAT STRAW AS SHOWN BY
RUMEN DIGESTION

FIGURE 8.5.4

FIGURE 8.5.5

EFFECT OF PRESSURE ON WHEAT STRAW AS SHOWN BY
RUMEN DIGESTION

41204525
51203525

FIGURE 8.5.6

SUMMARY OF EFFECT OF PRESSURE ON WHEAT STRAW AS SHOWN
BY RUMEN DIGESTION

51203525
41204525
21205525

FIGURE 8.5.6

FIGURE 8.6.1

EFFECT OF PRESSURE ON BAGASSE AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 8.6.1

FIGURE 8.6.2

40507482
50507482

EFFECT OF PRESSURE ON BAGASSE AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 8.6.2

FIGURE 8.6.3

SUMMARY OF EFFECT OF PRESSURE ON BAGASSE AS SHOWN BY
ENZYMATIC DIGESTION

40507482
50507482
20507482

FIGURE 8.6.3

FIGURE 8.6.4

EFFECT OF PRESSURE ON BAGASSE AS SHOWN BY RUMEN DIGESTION

FIGURE 8.6.4

FIGURE 8.6.5

EFFECT OF PRESSURE ON BAGASSE AS SHOWN BY RUMEN DIGESTION

50507482

40507482

60507482

FIGURE 8.6.5

FIGURE 8.6.6.

SUMMARY OF EFFECT OF PRESSURE ON BAGASSE AS SHOWN BY
RUMEN DIGESTION

40507482
50507482
60507482
20509482
50606482

FIGURE 8.6.6

FIGURE 8.7.1

21126371
21204371

EFFECT OF PRESSURE ON CORN STOVER AS SHOWN BY ENZYMATIC DIGESTION

FIGURE 8.7.1

FIGURE 8.7.2

51114371
41126371

EFFECT OF PRESSURE ON CORN STOVER AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 8.7.2

FIGURE 8.7.3

SUMMARY OF EFFECT OF PRESSURE ON CORN STOVER AS SHOWN BY
ENZYMATIC DIGESTION

51114375
41126375
21126375
21204375

FIGURE 8.7.3

FIGURE 8.7.4

EFFECT OF PRESSURE ON CORN STOVER AS SHOWN BY
RUMEN DIGESTION

21126375
21204375

FIGURE 8.7.4

FIGURE 8.7.5

51114375
41126375

EFFECT OF PRESSURE ON CORN STOVER AS SHOWN BY RUMEN DIGESTION

FIGURE 8.7.5

FIGURE 8.7.6

SUMMARY OF EFFECT OF PRESSURE ON CORN STOVER AS SHOWN
BY RUMEN DIGESTION

51114375
41126375
21204375
21126375

FIGURE 8.7.6

FIGURE 8.8.1

21212574

EFFECT OF PRESSURE ON SUNFLOWER STALKS AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 8.8.1

FIGURE 8.8.2

EFFECT OF PRESSURE ON SUNFLOWER STALKS AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 8.8.2

FIGURE 8.8.3

SUMMARY OF EFFECT OF PRESSURE ON SUNFLOWER STALKS AS SHOWN

BY ENZYMATIC DIGESTION

41130571

51122571

21212571

FIGURE 8.8.3

FIGURE 8.8.4

EFFECT OF PRESSURE ON SUNFLOWER STALKS AS SHOWN BY
RUMEN DIGESTION

FIGURE 8.8.4

FIGURE 8.8.5

EFFECT OF PRESSURE ON SUNFLOWER STALKS AS SHOWN BY
RUMEN DIGESTION

41130574
51122574

FIGURE 8.8.5

FIGURE 8.8.6

SUMMARY OF EFFECT OF PRESSURE ON SUNFLOWER STALKS
AS SHOWN BY RUMEN DIGESTION

51122574
41130574
21212574

FIGURE 8.8.6

FIGURE 8.9.2

CAK AND BASSWOOD AT 550 psig AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 8.9.2

FIGURE 8.9.4

EFFECT OF PRESSURE ON BASSWOOD AND OAK AS SHOWN BY
RUMEN DIGESTION

40524592

50523592

60524592

51102592

FIGURE 8.9.4

40524592
50523592
60522592

FIGURE 8.10.2

EFFECT OF PRESSURE ON SUGAR CANE RINDS AS SHOWN BY
ENZYMATIC DIGESTION

FIGURE 8.10.2

FIGURE 8.10.5

EFFECT OF PRESSURE ON SUGARCANE RINDS AS SHOWN BY
RUMEN DIGESTION

40524592
50523592
60522592

FIGURE 8.10.5

FIGURE 8.11.1

EFFECT OF PRESSURE ON PEAT AS SHOWN BY RUMEN DIGESTION

50525592

40524592

30524592

FIGURE 8.11.1

FIGURE 8.12.1

PEAK YIELDS FOR GLUCOSE WITH DIFFERENT SUBSTRATES

FIGURE 8.12.1

FIGURE 8.12.2

PEAK VALUES FOR IVCD WITH DIFFERENT SUBSTRATES

FIGURE 8.12.2

FIGURE 8.12.3

PEAK YIELDS FOR GLUCOSE PLUS XYLOSE WITH DIFFERENT SUBSTRATES

FIGURE 8.12.3

FIGURE 8.13

VARIATION IN COOKING TIMES AT 550 psig FOR DIFFERENT SUBSTRATES
AT PEAK GLUCOSE YIELD

FIGURE 8.13

TABLE 8.1

EFFECT OF VARIOUS PRESSURES ON BIRCH CHIPS

250 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
21109758	120	47.8	18.4	11.0	10.3
	360	46.6	48.1	12.2	20.0
	600	50.6	71.0	9.2	26.2
	720	51.0	75.3	6.7	32.2
	840	53.6	79.5	8.7	34.2
	960			8.4	34.2
	1080			7.4	35.8

TABLE 8.1

TABLE 8.1 continued

EFFECT OF VARIOUS PRESSURES ON BIRCH CHIPS

450 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
41121758	40	45.2	30.1	17.3	27.4
	60	44.2	53.8	15.7	31.4
	80	48.8	80.3	15.4	37.1
	90	45.2	82.7	15.4	37.1
	100	45.0	88.0	12.4	38.6
	110	45.8	84.7	13.3	38.6
	120	45.0	91.0	14.1	38.0
	140	46.4	90.1	10.6	38.0
	160	46.0	87.0	8.6	31.0
	200	47.8	92.0	6.7	41.4

TABLE 8.1 continued

TABLE 8.1 continued

EFFECT OF VARIOUS PRESSURES ON BIRCH CHIPS

550 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51108758	20	43.0	21.3	14.3	20.9
	40	44.0	37.7	14.3	25.3
	60	44.4	54.9	14.3	29.5
	70	42.2	42.1	11.4	29.1
	80	44.2	38.9	8.4	20.0
	90	42.8	27.1	10.5	28.1
	100	44.0	19.0	5.9	24.3
	120	45.0	21.3	6.7	24.3

TABLE 8.1 continued

TABLE 8.2

EFFECT OF VARIOUS PRESSURES ON MAPLE CHIPS

250 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
21109931	120	44.8	16.5	9.1	10.1
	600	46.6	55.2	7.8	24.7
	720	29.8	41.6	4.8	32.3
	840	47.6	65.5	3.8	33.3
	960	48.2	70.1	3.4	37.6
	1080	49.8	82.7	3.4	36.7

TABLE 8.2

TABLE 8.2 continued

EFFECT OF VARIOUS PRESSURES ON MAPLE CHIPS

450 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
41122931	40	45.8	14.8	10.6	18.1
	60	44.4	28.8	10.1	24.3
	80	45.2	35.4	9.5	29.5
	100	46.8	55.1	10.6	40.9
	120	45.0	61.8	9.1	41.4
	140	45.4	70.0	7.6	39.0
	160	45.8	71.6	4.4	35.2
	180	46.8	77.4	3.8	37.6
	200	48.2	88.7	3.4	36.1
	240	45.4	86.3	3.8	34.8

TABLE 8.2 continued

TABLE 8.2 continued

EFFECT OF VARIOUS PRESSURES ON MAPLE CHIPS

550 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51102931	20	43.2	6.0	5.9	11.4
	40	49.8	40.2	5.3	16.2
	60	45.0	52.0	4.9	21.9
	70	40.6	56.2	4.9	21.5
	80	42.2	49.8	4.6	25.7
	90	43.2	53.4	3.0	22.8
	100	44.0	42.3	2.5	24.3
	120	44.6	30.9	2.5	26.2

TABLE 8.2 continued

TABLE 8.3

EFFECT OF VARIOUS PRESSURES ON BARLEY STRAW

250 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
21207328	240	44.2	48.4	13.5	19.6
	360	44.0	51.4	15.0	30.0
	480	44.0	59.1	13.5	32.9
	500	45.4	61.2	11.4	34.2
	720	46.6	63.9	10.3	34.2
	840	47.8	67.4	6.8	38.6
	960	48.2	65.1	7.2	38.6
	1200	49.6	69.8	4.8	51.3

TABLE 8.3

TABLE 8.3 continued

EFFECT OF VARIOUS PRESSURES ON BARLEY STRAW

450 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
41206328	20	44.6	35.4	13.9	21.5
	40	42.8	45.8	19.6	25.7
	50	41.4	45.4	15.0	23.8
	60	40.6	43.8	13.5	25.7
	70	41.8	54.5	13.5	28.5
	80	43.6	54.6	13.3	32.3
	90	44.2	59.3	12.5	32.9
	110	43.4	65.4	9.1	34.2
	140	44.6	73.1	6.5	34.2
	180	46.4	78.4	5.7	37.1

TABLE 8.3 continued

TABLE 8.3 continued

EFFECT OF VARIOUS PRESSURES ON BARLEY STRAW

550 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51116328	6	45.2	34.1	6.7	12.9
	20	42.4	52.8	14.3	17.7
	30	42.6	47.9	10.6	20.9
	40	43.2	61.6	10.3	25.7
	50	42.8	66.8	10.3	31.4
	60	43.6	70.6	5.8	27.7
	70	43.2	75.0	5.8	29.1
	80	44.6	73.1	5.3	32.9
	100	44.2	78.3	6.3	34.8
	120	45.2	57.5	1.7	25.2

TABLE 8.3 continued

TABLE 8.4

EFFECT OF VARIOUS PRESSURES ON OAT STRAW

250 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
21129720	120	38.8	52.1	9.9	20.0
	240	36.6	51.9	10.6	19.0
	300	36.0	67.2	10.8	25.8
	350	38.4	69.8	11.4	31.2
	420	36.8	73.9	10.6	34.6
	480	36.8	70.1	8.4	36.2
	600	37.8	76.7	6.1	40.3
	720	38.8	74.2	5.3	37.1
	840	39.4	80.2	4.0	37.9
	960	41.2	81.6	2.3	38.0

TABLE 8.4

TABLE 8.4 continued

EFFECT OF VARIOUS PRESSURES ON OAT STRAW

450 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
41128720	20	36.2	55.8	10.6	20.3
	40	34.4	56.4	14.3	29.6
	50	34.8	60.9	13.5	27.6
	60	34.6	62.4	10.6	23.8
	70	45.6	73.2	10.3	26.0
	80	36.6	69.9	10.6	32.1
	90	35.8	70.4	10.8	40.5
	110	37.6	75.5	8.7	35.2
	140	38.6	85.5	4.6	31.7
	160	37.0	80.5	2.3	29.8

TABLE 8.4 continued

TABLE 8.4 continued

EFFECT OF VARIOUS PRESSURES ON OAT STRAW

550 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51128720	10	34.6	56.1	13.5	23.6
	20	36.4	58.2	14.1	24.5
	30	34.6	64.2	13.1	32.1
	40	34.4	67.8	10.8	32.7
	50	34.6	71.7	12.4	34.0
	60	35.2	74.4	10.5	32.1
	70	36.8	77.7	9.3	34.0
	80	37.2	82.8	10.3	37.8
	100	36.4	67.0	5.9	35.0

TABLE 8.4 continued

TABLE 8.5

EFFECT OF VARIOUS PRESSURES ON WHEAT STRAW

250 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
21205525	240	39.8	64.3	18.2	28.5
	360	39.4	64.0	13.5	31.0
	480	41.4	69.1	11.2	35.2
	540	40.0	74.5	8.7	38.0
	600	45.0	72.9	10.6	45.6
	720	43.8	74.0	9.1	43.7
	840	43.4	77.8	5.9	46.6
	1200	45.8	77.7	3.4	40.9

TABLE 8.5

TABLE 8.5 continued

EFFECT OF VARIOUS PRESSURES ON WHEAT STRAW

450 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
412C4525	20	43.8	53.4	20.5	21.1
	40	40.0	58.0	17.9	19.2
	50	40.8	49.0	15.0	23.8
	60	40.6	63.5	12.5	24.5
	70	40.8	66.2	16.5	33.3
	80	41.8	69.4	8.2	20.3
	90	41.2	72.8	10.5	26.8
	110	42.0	67.1	8.2	30.2
	140	42.4	67.4	4.4	30.0
	180	42.8	66.8	2.9	39.7

TABLE 8.5 continued

TABLE 8.5 continued

EFFECT OF VARIOUS PRESSURES ON WHEAT STRAW

550 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51203525	10	37.8	55.0	14.3	21.5
	20	38.0	68.4	15.0	22.4
	30	36.6	66.7	11.2	23.8
	40	35.4	66.1	13.5	27.6
	50	37.6	66.0	13.5	29.1
	60	36.6	68.9	9.3	30.4
	70	40.8	69.1	9.1	28.5
	80	39.2	75.5	6.5	32.9
	90	38.8	78.4	4.8	32.9

TABLE 8.5 continued

TABLE 8.6

EFFECT OF VARIOUS PRESSURES ON BAGASSE

200 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
20509482	60	44.3	36.3		
	120	46.9	42.2		
	240	44.5	45.2		
	480	44.4	52.7		
	960	46.1	62.2		
	1800		69.5		
280 psig					
20509482	30		44.5	2.1	14.1
	60		49.7	5.1	15.4
	120		47.2	7.6	19.4
	240		58.6	10.6	26.2
	480		69.8	13.1	34.8

TABLE 8.6

TABLE 8.6 continued

EFFECT OF VARIOUS PRESSURES ON BAGASSE

400 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
40507482	10	42.5	40.2	1.7	7.8
	30	41.4	43.0	3.8	16.5
	40	44.9	47.5	5.1	20.7
	50	51.0	49.8	6.3	22.2
	60	42.3	51.3	6.8	21.1
	80	40.3	51.9	10.5	26.2
	120	41.6	55.5	10.5	24.5
	180	42.4	69.1	11.4	32.9
	240	42.2	70.6	11.0	34.4

TABLE 8.6 continued

TABLE 8.6 continued

EFFECT OF VARIOUS PRESSURES ON BAGASSE

550 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
50507482	10	41.2	45.2	15.2	22.6
	30	40.6	56.1	17.7	24.5
	45	40.6	62.1	15.2	27.7
	50		63.6	13.9	29.3
	60		69.6	13.9	29.3
	90		72.4	5.9	28.1
	120		76.2	10.5	30.6
	160		59.2	8.6	28.3
	240		20.2	4.2	28.1

TABLE 8.6 continued

TABLE 8.6 continued

EFFECT OF VARIOUS PRESSURES ON BAGASSE

650 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
60507482	10	62.0	61.4		
	30	63.0	65.5		
	40	62.5	73.0		
	50	65.0	70.4		
	60	64.5	66.3		
	80	64.0	58.7		
	120	22.1	40.7		
	180	13.4	41.1		
	240	8.4	37.9		

TABLE 8.6 continued

TABLE 8.7

EFFECT OF VARIOUS PRESSURES ON CORN STOVER

250 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
21126375	120	38.2	56.0	7.6	16.2
21204375	240	38.0	62.6	10.0	23.4
	360	37.2	67.7	10.5	25.7
	480	38.2	70.7	10.3	31.9
	600	38.6	76.0	9.2	32.7
	720	39.4	77.7	13.3	40.5
	840	41.4	79.2	10.8	45.0
	960	43.0	79.5	4.9	34.6
	1080	49.4	76.1	4.9	35.2
	1200	41.8	78.0	3.6	42.4

TABLE 8.7

TABLE 8.7 continued

EFFECT OF VARIOUS PRESSURES ON CORN STOVER

450 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
41126375	20	37.4	53.5	13.1	21.5
	40	37.4	62.6	11.6	22.8
	60	37.4	61.0	10.3	25.7
	80	37.2	67.2	8.2	29.1
	90	36.8	70.7	6.8	38.6
	100	36.6	73.8	7.6	38.6
	120	38.8	74.7	7.2	41.8
	140	39.4	78.2	4.6	33.8
	180	38.4	79.2	2.1	30.0
	240	47.6	83.6	2.1	27.2

TABLE 8.7 continued

TABLE 8.7 continued

EFFECT OF VARIOUS PRESSURES ON CORN STOVER

550 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51114375	10	38.6	60.1	9.5	18.6
	20	37.4	63.6	10.6	20.0
	30	38.0	69.5	10.6	26.6
	40	37.6	64.9	9.5	24.7
	50	38.8	73.2	9.1	28.5
	60	39.6	73.7	7.6	32.9
	70	41.2	73.8	6.3	33.8
	80	39.2	76.0	5.3	32.9
	90	40.2	79.6	4.6	34.2
	120	42.0	71.4	1.9	32.3
	180	39.4	50.8	2.1	32.9

TABLE 8.7 continued

TABLE 8.8

EFFECT OF PRESSURE ON SUNFLOWER STALKS

250 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
21212574	240	36.8	25.0	14.1	13.3
	360	36.2	23.2	10.1	5.1
	480	35.4	23.2	8.6	12.0
	600	35.8	21.2	8.2	12.0
	840	37.0	23.7	7.6	16.7
	1080	34.6	11.6	5.7	18.2
	1440	35.6	21.9	6.1	22.6
	1800	40.4	33.2	4.4	23.2
	2160	37.2	41.5	4.8	25.8

TABLE 8.8

TABLE 8.8 continued

EFFECT OF PRESSURE ON SUNFLOWER STALKS

450 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
41130574	40	35.2	26.7	10.1	13.9
	60	34.6	24.3	11.4	15.6
	80	33.0	23.6	10.1	17.5
	100	33.8	24.3	9.7	22.4
	120	33.6	28.6	6.3	19.8
	140	34.6	32.4	4.9	21.1
	160	35.0	34.3	5.3	22.4
	180	33.8	38.5	6.3	26.2
	220	34.2	49.7	3.6	27.7
	240	33.2	33.1	6.8	21.3

TABLE 8.8 continued

TABLE 8.8 continued

EFFECT OF PRESSURE ON SUNFLOWER STALKS

550 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51122574	20	34.2	22.8	10.5	13.3
	40	33.6	25.0	10.1	15.8
	60	31.4	21.0	8.6	24.3
	80	31.2	27.6	5.5	21.9
	100	30.2	27.8	3.4	24.7
	120	30.4	20.4	5.0	24.3
	140	31.0	34.2	2.1	25.3
	180	30.8	27.9	3.6	25.3

TABLE 8.8 continued

TABLE 8.9

EFFECT OF VARIOUS PRESSURES ON BASSWOOD

400 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
40515592	30	47.4	14.0		
	120	45.6	50.6		
	240	47.8	77.0		
550 psig					
50515592	6	46.2	15.7	6.3	11.4
	15	45.8	12.7	9.5	10.5
	30	44.9	18.5	12.4	20.0
	60	44.9	67.8	11.4	26.6
	80	47.2	80.0	6.7	31.4
	120	49.0	63.6	3.4	31.4
	240	48.4	11.3	1.5	32.4

TABLE 8.9

TABLE 8.9 continued

EFFECT OF VARIOUS PRESSURES ON OAK

550 psig					
Ictech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
51102592	20	42.5	36.8	6.5	13.9
	30	44.0	40.5	6.8	17.7
	60	43.6	66.5	6.7	22.8
	80	43.2	55.1	5.9	22.8
	100	44.2	43.0	4.6	21.9

TABLE 8.9 continued

TABLE 8.9 continued

EFFECT OF VARIOUS PRESSURES ON BASSWOOD

650 psig			
Iotech #	Time (sec)	% cellulose	IVCD
60515592	6	43.2	12.5
	15	43.0	16.7
	30	43.2	54.2
	60	44.7	82.6
	80	45.7	30.9
	120	47.1	11.3
	240	44.4	4.1

TABLE 8.9 continued

TABLE 8.10

EFFECT OF VARIOUS PRESSURES ON SUGAR CANE RINDS

400 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
40524592	15	41.3	20.9	6.3	7.9
	30	38.6	31.1	10.8	16.8
	48	40.5	37.8	16.9	21.7
	75	38.1	51.2	12.8	30.4
	90	39.6	70.2	7.4	30.7
	120	39.7	71.8	7.0	35.0
	164	41.6	77.9	10.7	36.1
550 psig					
50523592	5	40.5	23.9	10.8	13.6
	15	37.1	39.9	14.1	20.1
	25	37.5	63.2	14.8	25.5
	35	39.0	67.2	10.8	26.6
	45	39.1	71.9	9.9	26.3
	55	33.6	67.4	5.8	24.2
	75	39.4	24.5	3.8	28.2

TABLE 8.10

TABLE 8.10 continued

EFFECT OF VARIOUS PRESSURES ON SUGAR CANE RINDS

650 psig					
Iotech #	Time (sec)	% cellulose	IVCD	% xylose	% glucose
60522592	5	37.9	62.0	5.8	12.8
	15	38.1	64.5	10.7	19.3
	25	37.7	37.4	7.0	19.0
	35	39.3	64.9	7.9	19.0
	45	38.9	0	4.3	21.5
	57	35.9	0	3.1	25.5

TABLE 8.10 continued

TABLE 8.11

EFFECT OF VARIOUS PRESSURES ON PEAT MOSS

350 psig			
Iotech #	Time (sec)	% cellulose	IVCD
30524592	15	18.1	29.3
	30	18.4	35.9
	45	16.7	37.7
	60	17.1	42.7
	120	15.9	34.6
	180	15.8	38.1
400 psig			
40524592	5	18.4	25.4
	15	15.6	25.9
	30	17.6	22.2
	45	15.7	35.0
	60	16.7	24.6
	120	15.8	29.1
	180	16.1	40.6

TABLE 8.11

TABLE 8.11 continued

EFFECT OF VARIOUS PRESSURES ON PEAT MOSS

560 psig			
Iotech #	Time (sec)	% cellulose	IVCD
50525592	5	16.3	26.9
	15	15.6	30.8
	30	17.4	21.4
	45	16.4	22.6
	60	15.9	24.5
	90	15.2	21.0
	120	15.0	20.0
	180	16.8	25.0

TABLE 8.11 continued

TABLE 8.12
COMPARISON OF SUBSTRATES BY SUGAR YIELD

SUBSTRATE	COMPOSITION ^a OF SUBSTRATE			LITERATURE REFERENCE	THEORETICAL HYDROLYSIS YIELD			PEAK YIELDS ^b			% OF THEORETICAL		
	Cellulose	Pentosan	Lignin		% Glucose c	% Xylose d	% Glucose & Xylose	% Glucose	% Xylose	% Glucose & Xylose	% Glucose	% Xylose	% Glucose & Xylose
BARLEY STRAW	48	28	15	1	54	31	85	47.2	18.0	51.6	87	58	61
OAT STRAW	53	15	16	1	60	17	77	37.3	13.2	47.2	62	78	61
WHEAT STRAW	58	20	15	1	65	22	87	42.9	18.9	51.7	66	86	59
ASPEN	46	19	24	2	52	21	73	37.6	17.1	54.3	72	81	74
BIRCH	39	23	22	3	44	26	70	35.5	15.9	48.3	81	61	69
MAPLE	39	17	23	3	44	19	63	38.0	9.8	47.4	86	52	75
OAK	40	19	24	3	45	21	66	20.9	6.3	27.1	46	30	41
BASSWOOD	40	17	20	3	45	19	64	29.8	11.4	35.0	66	60	55
CORN STOVER	45	20	16	1	51	22	73	41.4	12.1	51.3	81	55	70
BAGASSE				} estimate same as corn stover				32.0	16.3	44.1	63	74	60
SUGAR CANE RIND								33.2	15.3	43.0	65	70	59
SUNFLOWER STALKS								25.5	13.0	28.8	50	59	39
AVERAGE											68	64	60

FIGURE 8.12

LITERATURE REFERENCE

1. Hansen, R.G. et al. University of Illinois
Agriculture Experiment Station, Bulletin 634 Nov. 1958
2. Iotech experimental data.
3. Rydholm, S.A. Pulping Processes Interscience
Publishers 1965.

- a Unextracted basis
- b An 8% weight loss has been assumed.
- c % Cellulose x 1.111 + 1%(from glucomannan)
- d % Pentosan x 1.111

SECTION 9

EXTRACTION OF LIGNIN

9.0 EXTRACTION OF LIGNIN

Introduction

Two different methods were tried to separate the individual components of cooked wood into separate fractions. The first separation, Fractionation Procedure I, was basically the method outlined under Section G of the contract. While this yielded a reasonable separation it was not nearly as satisfactory as a second method, Fractionation Procedure II, which resolved the three main components, cellulose, lignin and water solubles, more completely. Figures 9.1.2, 9.1.3 and 9.2.2 show the amount of lignins in each fraction.

Fractionation Procedure I with Aspen and Straw

Aspen wood was subjected to steam at 650 psig for 55 seconds and was then explosively decompressed. The resulting wet exploded wood, which was approximately 2/3 water by weight, was extracted three times with ethanol which removed much of the lignin and left behind a cellulose-rich pulp (Fraction 1). The alcohol extract was further divided by evaporation to approximate dryness and dissolution again of the acetone-soluble portion. After removal of the undissolved portion (Fraction 2), the acetone filtrate was concentrated by evaporation and a lignin-rich fraction (Fraction 3) was obtained by precipitation into water. The water filtrate, containing mainly free sugars, oligosaccharids and other material from the hemicellulose, was evaporated to dryness (Fraction 4). This process is shown diagrammatically in Figure 9.1.1.

Methoxyl Balance in Fractions

The weight and methoxyl content of each fraction is listed in Table 9.1.1.¹ This table also shows that the total amount of methoxyl in all fractions amounted to 4.63% of the weight of original wood put into the gun.

The methoxyl content of typical unextracted aspen wood is 5.11%, of which 0.40% is located in the hemicellulose, and the remaining 4.71% is in the lignin. The methoxyl attached to the hemicellulose is known to be labile under hydrolytic conditions, whereas that attached to the lignin is much more stable. The good agreement, between the above 4.63% and the 4.71%, is interpreted as meaning that the hemicellulose methoxyl has been lost and that virtually all of the lignin methoxyl has survived the steam treatment.

Each fraction in Table 9.1.1 was subjected to a Klason lignin analysis, using the standard TAPPI Method.² The acid-soluble lignin in the Klason filtrate was determined by UV absorption. The results are listed in Table 9.1.2. The "apparent" Klason lignin obtained from each fraction was also analyzed for methoxyl.

The low methoxyl content of the "apparent" Klason lignin from each fraction does not result from loss of methoxyl by the lignin, but reflects the fact that the true Klason lignin is accompanied by a "pseudolignin" as a diluent. The "pseudolignin" is formed from the hemicellulose during drastic steaming treatments and during subsequent standard Klason lignin analysis. This "pseudolignin" also, of course, causes the "apparent" Klason lignin analyses, reported in Table 9.1.2, to be high. Another factor which raises this apparent Klason lignin analysis is the weight loss in the gun caused by this change in the hemicellulose.

In Table 9.1.3 are listed the amounts of methoxyl in the apparent Klason lignin of each fraction of the exploded wood. Although the methoxyl analysis of the acid soluble lignin in each Klason filtrate is not known, an estimation of the amount of methoxyl in this lignin has been made by assuming its methoxyl analysis to be 15%: a value for aspen which has been reported in the literature. Its use is justified because of the relatively small amount present (0.40%) and the resulting small effect that even a large error in this assumption could make on the overall methoxyl balance.

Table 9.1.3 shows that the total methoxyl in the apparent Klason lignin and in the acid soluble lignin is 4.56% of the weight of original wood put into the gun. This value is in excellent agreement with the value of 4.63% reported in Table 9.1.1 as the total methoxyl in all the complete fractions of the exploded wood including the carbohydrate. This agreement, therefore, shows that within the accuracy of the data all the methoxyl of this exploded wood was in the lignin (or possibly pseudolignin). It also confirms the interpretation, above, that the hemicellulose methoxyl has been lost and that virtually all of the lignin methoxyl has survived the steam treatment. Figure 9.1.2 has been plotted using the data from Table 9.1.3 and 9.1.4 to show the make-up of the varying extraction fractions.

This latter conclusion is also supported by the analysis of our typical original starting aspen wood. The unextracted wood contained 20.60% Klason lignin and 3.02% acid-soluble lignin (unextracted basis). The methoxyl in the Klason lignin was 19.84%. If, as above, the methoxyl content of acid-soluble lignin is taken as 15%, the total methoxyl component in the lignin of the original wood was 4.54% which is almost identical with the 4.56% calculated in Table 9.1.3 from the exploded wood analyses. This shows, convincingly, that little or no methoxyl was lost from the lignin in the gun.

Lignin Balance in Fractions

Knowing that the methoxyl content of "true" Klason lignin of typical starting aspenwood before steaming was 19.84% (as shown above), and that methoxyl is not lost from the lignin on steam treatment under these conditions, it is possible to calculate the amount of true Klason lignin in "apparent" Klason lignin, by simply multiplying the methoxyl content of the latter by $\frac{100}{19.84}$.

Although this procedure is valid when the soluble exploded wood mixture is analyzed as one, it is less certain when applied to the individual fractions because of possible enrichment of some fractions in either high- or low-methoxyl lignin.

Assuming that no such major enrichment had occurred, the data of Table 9.1.4 was obtained. The pseudolignin content was calculated simply by difference of the "apparent" and "true" Klason lignin.

Recovery from Gun

Table 9.1.4 shows that the total true Klason lignin and acid soluble lignin amounted to 26.80% of the exploded wood. Therefore, because the total lignin content of the typical starting material was 23.62%, the theoretical recovery was

$$\frac{23.62}{26.80} \times 100 = 88.14\%$$

This recovery of 88.14% was the value used in the above calculations.

An empirical recovery was not obtained for this particular material. However, the above value agrees closely with the empirical recovery of 88.5% obtained (based on vacuum dried material over P_2O_5) for a 560 psig shot at 100 seconds. This latter condition was calculated to be equivalent to 650 psig at 55 seconds, by assuming the Arrhenius relation and a doubling of reaction rate for a $10^\circ C$ increase in temperature.

A similar fractionation of exploded barley straw was done. In this case, the water-soluble straw material remaining in solution in the water, after precipitation of Fraction 3 from acetone into water, was subdivided into material remaining in the water (Fraction 4₁), and material extractable by ethyl acetate in a separatory funnel (Fraction 5). Table 9.1.5 shows the production of a large amount of pseudolignin, as reflected in the total of 44.57% of apparent Klason lignin and acid-soluble lignin. These results are plotted in Figure 9.1.3.

Fractionation Procedure II with Aspen

A second fractionation procedure was evolved which was much more superior to the first scheme in that it produced a more complete extraction of the lignin, with a sharper separation of the three main components into three fractions only. The difficulty with the pseudolignin is also largely overcome. Compare Figures 9.1.2 and 9.1.3 with 9.2.2

This sample of aspen wood was cooked at 560 psig for times ranging from 20 to 120 seconds and was then explosively decompressed. The exploded wood was first extracted with water, at room temperature, to remove the water solubles, mainly partially hydrolyzed hemicellulose (Fraction 1). The residue was then treated with 0.4% sodium hydroxide, at room temperature, to remove much of the lignin. The residual pulp after this extraction (Fraction 3) was cellulose-rich. Acidification of the alkaline solution caused the lignin to precipitate giving Fraction 2.

Arrangement of Attached Tables

The first Table, 9.2.1, shows the weights of the three fractions: water solubles Fraction 1, precipitate from alkali Fraction 2, and residual pulp Fraction 3. The weights are expressed both as % of exploded wood, and as % of wood put into gun.

The next Table, 9.2.2, shows the amounts of lignin and of xylose in Fraction 1, i.e. in the water solubles. These amounts are expressed in three ways: as % of the water solubles, as % of exploded wood, and as % of original wood put into gun.

The next Table, 9.2.3, shows the amounts of lignin and pseudolignin (and indicates the calculation method) in Fraction 2, i.e. in the precipitate from alkali. These amounts are expressed also in three analogous ways: as % of precipitate, as % of exploded wood, and as % of original wood put into gun.

Table 9.2.4 similarly shows the lignin and pseudolignin in Fraction 3, i.e. the residual pulp. These are also expressed in three analogous ways. From this Figure 9.2.2 was plotted.

Table 9.2.5 is a summary of the weights and composition of all three fractions. A summary graph has been plotted, Figure 9.2.3, showing the varying compositions after increasing cooking times.

Table 9.2.6 shows the total true lignin accounted for, and provides a lignin balance.

Results

Analysis of Original Wood put into Gun

Klason lignin	20.60%	all on unextracted wood basis
Acid-soluble lignin	3.02%	
	<hr/>	
Total lignin	23.62%	
Methoxyl in Klason lignin:	19.84%	

A number of trends are evident in Table 9.2.5.

1. From Column 2 it can be seen that, by 20 seconds, the maximum water solubility has almost been reached. Water solubility then very slowly falls off after about 60 seconds.
2. The soluble lignin in the water soluble fraction, however, remains constant (Column 3) from 20 to 120 seconds, showing that some part of the lignin (apparently the ester bond portion) is quickly and completely hydrolyzed by the steam within the first 20 seconds. Whether or not it is substantially altered, the amount in solution at least remains constant in weight.
3. Column 4 shows that the amount of free xylose monomer is quite small at 20 seconds, representing at this time only about 1% of the potential maximum amount possible. The concentration of xylose rises till about 80 seconds, after which it is apparently destroyed faster than it is produced. At this time (80 seconds) only about one half of the original pentosan (from which the xylose is liberated) remains. The relatively low maximum amounts of free xylose, and of soluble lignin, together account for only about 30% of the water solubles at 80 seconds, suggesting that much of the water solubles consists of oligomers from the hemicellulose. This suggestion is

supported by pentosan analysis on the water solubles,³ which show that at 80 seconds, a little less than one half of the water solubles is pentosan.

4. Columns 5, 8 and 9 show a steady increase in the amount of lignin extractable with dilute alkali and recoverable by acidification. The amount is still increasing at 120 seconds, at which point it has reached 78.8% of the total true lignin of the original wood. This 78.8%, together with another 11.2% (2.65% of 23.62% total lignin) already removed into the water solubles, represents 90% of the total true lignin.

Column 14 shows that only 1.36% of the original weight of wood remains as true lignin in the pulp. This represents only 5.76% of the original lignin of the wood, and represents a very high degree of delignification. Furthermore, there is no indication that maximum delignification has been reached at 120 seconds.

5. Columns 10 and 15 show that pseudolignin in both the precipitate (Fraction 2) and in the pulp (Fraction 3) increase slowly with time.

Table 9.2.6 is very significant as it shows that the total true lignin accounted for is remarkably constant and almost complete regardless of time of steam treatment. Part, at least, of the small unaccounted-for lignin was undoubtedly lost in the supernatant solution remaining after the lignin-rich precipitate was filtered off. The remainder represents accumulated small errors. There is no indication of any slow demethylation of the lignin occurring with time. Most (19.4% 19.3%, Columns 9 + 14) of the total true lignin accounted for (22.2% to 22.9%, Columns 3 + 9 + 14) was in the true Klason lignin, which was calculated by assuming a constant methoxyl of 19.84% (as in the Klason lignin of the original wood). In fact,

after the first 40 seconds, the total true Klason lignin varied insignificantly between 18.96% and 19.34% and showed no particular trend. There was, therefore, no gradual loss of methoxyl from the lignin with increasing time of cooking.

Conclusion

Using Fractionation Procedure II, increasing amounts of lignin are released for recovery. At 560 psig and 120 seconds, a high yield of 79% of the original lignin can be obtained after extraction into a solution of 0.4% NaOH and re-precipitation.

REFERENCES

1. TAPPI Standard T2m-43 (modified)
2. TAPPI Standard T222 os-74.
3. TAPPI Standard T223 os-71.

FIGURE 9.1.1

FRACTIONATION PROCEDURE I

FIGURE 9.1.2

DISTRIBUTION OF LIGNIN WHEN EXTRACTED BY FRACTIONATION PROCEDURE I
ASPEN AT 650 psig FOR 55 SECONDS

FIGURE 9.1.2

FIGURE 9.1.3

DISTRIBUTION OF LIGNIN WHEN EXTRACTED BY FRACTIONATION PROCEDURE I
STRAW

FIGURE 9.1.3

FIGURE 9.2.1

FRACTIONATION PROCEDURE II

FIGURE 9.2.2

DISTRIBUTION OF LIGNIN WHEN EXTRACTED BY FRACTIONATION PROCEDURE II

ASPEN AT 560 psig

FIGURE 9.2.2

FIGURE 9.2.3

CUMULATIVE TOTALS OF FRACTIONS OF EXPLODED WOOD WHEN EXTRACTED
BY FRACTIONATION PROCEDURE II

ASPEN AT 560 psig

TABLE 9.1.1

METHOXYL CONTENT OF FRACTIONS OF EXPLODED ASPEN WOOD

FRACTION AS IN FIG. G-1.1	FRACTION WEIGHT (% OF EXPLODED WOOD)	METHOXYL ANALYSIS OF FRACTION (% OF FRACTION)	METHOXYL IN FRACTION AS % OF EXPLODED WOOD	METHOXYL IN FRACTION AS % OF ORIGINAL WOOD PUT INTO GUN*
(1)	60.42	2.89	1.75	1.54
(2)	6.46	2.06	0.13	0.12
(3)	18.63	15.95	2.97	2.62
(4)	13.44	3.04	0.41	0.36
TOTAL	98.94		5.26	4.63

*obtained by multiplying preceeding column
by the calculated recovery
from the gun, i.e. 0.8814

TABLE 9.1.1

TABLE 9.1.2

LIGNIN ANALYSIS OF FRACTIONS OF EXPLODED WOOD
AND METHOXYL ANALYSIS OF THE APPARENT KLASON LIGNIN

FRACTION AS IN FIG. G-1.1	APPARENT KLASON LIGNIN ANALYSIS (% OF FRACTION)	ACID-SOLUBLE LIGNIN BY UV (% OF FRACTION)	METHOXYL ANALYSIS OF APPARENT KLASON LIGNIN (% OF APPARENT KLASON LIGNIN)
(1)	20.89	0.65	13.59
(2)	16.01	6.30	9.87
(3)	88.16	4.92	15.53
(4)	13.13	9.39	11.03

TABLE 9.1.2

TABLE 9.1.3

AMOUNT OF METHOXYL IN THE LIGNIN OF EXPLODED WOOD

FRACTIONS AS IN FIG. G-1.1	METHOXYL IN APPARENT KLASON LIGNIN OF FRACTION, AS % OF EXPLODED WOOD	ESTIMATED* METHOXYL IN ACID-SOLUBLE LIGNIN IN KLASON LIGNIN FILTRATES AS % OF EXPLODED WOOD	ESTIMATED**
			TOTAL METHOXYL IN APPARENT KLASON LIGNIN ACID IN ACID-SOLUBLE LIGNIN AS % OF ORIGINAL WOOD PUT INTO GUN
(1)	1.72	0.059	1.56
(2)	0.10	0.061	0.14
(3)	2.71	0.137	2.51
(4)	0.20	0.189	0.34
TOTAL	4.73	0.447	4.56

*assuming methoxyl content of acid-soluble
lignin to be 15%

**sum of values of preceeding two columns
multiplied by 0.8814

TABLE 9.1.3

TABLE 9. 1.4

LIGNIN ANALYSIS CORRECTED FOR PSEUDOLIGNIN CONTENT

	COMPOSITION OF FRACTION AS % OF FRACTION WEIGHT				COMPOSITION OF FRACTION AS % OF EXPLODED WOOD			
FRACTIONS AS IN FIG. G-1.1	TRUE KLASON LIGNIN	ACID- SOLUBLE LIGNIN	SUM OF TRUE KLASON & ACID- SOLUBLE LIGNIN	PSEUDO- LIGNIN	TRUE KLASON LIGNIN	ACID- SOLUBLE LIGNIN	SUM OF TRUE KLASON & ACID- SOLUBLE LIGNIN	PSEUDO- LIGNIN
(1)	14.31	0.65	14.96	6.58	8.65	0.39	9.04	3.98
(2)	7.96	6.30	14.26	8.05	0.51	0.41	0.92	0.52
(3)	73.45	4.92	78.37	14.71	13.68	0.92	14.60	2.74
(4)	7.30	9.39	16.69	5.83	0.98	1.26	2.24	0.78
TOTAL					23.82	2.98	26.80	8.02

TABLE 9. 1.4

TABLE 9.1.5

LIGNIN ANALYSIS OF FRACTIONS OF EXPLODED STRAW

FRACTIONS AS IN FIG. G-1.1	FRACTION WEIGHT (% OF EXPLODED STRAW)	APPARENT KLASON LIGNIN ANALYSIS (% OF FRACTION)	ACID-SOLUBLE LIGNIN ANALYSIS (% OF FRACTION)	SUM OF APPARENT KLASON LIGNIN & ACID-SOLUBLE LIGNIN (% OF FRACTION)	SUM OF APPARENT KLASON LIGNIN & ACID-SOLUBLE LIGNIN (% OF EXPLODED STRAW)
(1)	65.91	34.38	1.70	36.08	23.78
(2)	9.07	30.11	8.36	38.47	3.49
(3)	13.63	85.39	7.53	92.92	12.66
(4) ₁	6.29	10.26	13.48	23.74	1.49
(5)	5.10	37.22	24.49	61.71	3.15
TOTAL					44.57

TABLE 9.1.5

TABLE 9.2.1

<u>WEIGHTS OF THREE FRACTIONS OBTAINED,</u> <u>AS % OF VACUUM DRY EXPLODED WOOD</u>				<u>WEIGHTS OF THREE FRACTIONS OBTAINED,</u> <u>AS % OF WOOD PUT INTO GUN</u>			
TIME OF SHOT (SEC)	WATER SOLUBLES (VACUUM DRY)	PRECIPITATE (FROM ALKALI)	PULP	WATER SOLUBLES (VACUUM DRY)	PRECIPITATE (FROM ALKALI)	PULP	TOTALS
20	23.67	12.19	57.86	23.32	12.01	57.02	92.35
40	24.71	14.55	54.18	23.88	14.06	52.35	90.29
60	25.49	17.55	53.34	24.01	16.53	50.23	90.77
80	24.65	20.21	53.05	22.59	18.52	48.63	89.74
100	22.15	22.50	52.17	19.55	19.86	46.03	85.44
120	19.41	24.58	51.75	16.60	21.02	44.26	81.88

TABLE 9.2.1

TABLE 9.2.2

COMPOSITION OF WATER SOLUBLES
(FRACTION 1)

TIME OF SHOT (SEC)	AS % OF VACUUM DRY WATER SOLUBLES		AS % OF VACUUM DRY EXPLODED WOOD		AS % OF ORIGINAL WOOD PUT INTO GUN	
	LIGNIN (BY UV)	XYLOSE (BY HPLC) *	LIGNIN (BY UV)	XYLOSE (BY HPLC) *	LIGNIN (BY UV)	XYLOSE (BY HPLC) *
20	11.73	1.0	2.78	0.23	2.74	0.22
40	11.52	3.1	2.85	0.77	2.75	0.75
60	11.41	10.7	2.91	2.7	2.74	2.6
80	12.64	17.8	3.12	4.4	2.86	4.0
100	13.87	17.6	3.07	3.9	2.71	3.4
120	15.88	10.4	3.08	2.0	2.64	1.7

*concentration determined by Iotech

TABLE 9.2.2

TABLE 9.2.3

COMPOSITION OF PRECIPITATE FROM ALKALI
(FRACTION 2)
AS A % OF PRECIPITATE

PRIMARY DATA				CALCULATED COMPOSITION			
TIME (SEC)	APPARENT KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	METHOXYL IN APPARENT KLASON LIGNIN AS % OF APPARENT KLASON LIGNIN	TRUE KLASON LIGNIN*	ACID- SOLUBLE LIGNIN (BY UV)	TOTAL TRUE LIGNIN (KLASON + ACID- SOLUBLE)	PSEUDOLIGNIN BY DIFFERENCE (i.e. APPARENT KLASON LESS TRUE KLASON)
20	86.69	3.36	19.85	89.74	3.36	93.10	-0.05
40	91.15	3.51	19.72	90.60	3.51	94.11	0.55
60	92.58	2.67	19.34	90.25	2.67	92.92	2.33
80	93.08	2.60	19.12	89.70	2.60	92.30	3.38
100	92.97	3.01	18.62	87.25	3.01	90.26	5.72
120	94.18	2.41	18.15	86.16	2.41	88.57	8.02

*calculated from "apparent Klason lignin" and methoxyl content, by assuming "true Klason lignin" to contain 19.84% methoxyl, as did the Klason lignin of the original wood.

TABLE 9.2.3

TABLE 9.2.3 continued

COMPOSITION OF PRECIPITATE FROM ALKALI
(FRACTION 2)
AS A % OF VACUUM DRY EXPLODED WOOD

PRIMARY DATA				CALCULATED COMPOSITION			
TIME (SEC)	APPARENT KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	METHOXYL IN APPARENT KLASON LIGNIN AS % OF APPARENT KLASON LIGNIN	TRUE KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	TOTAL TRUE LIGNIN (KLASON + ACID- SOLUBLE)	PSEUDOLIGNIN BY DIFFERENCE (i.e. APPARENT KLASON LESS TRUE KLASON)
20	89.69	3.36	19.85	10.94	0.41	11.35	0.006
40	91.15	3.51	19.72	13.18	0.51	13.69	0.080
50	92.58	2.67	19.34	15.84	0.47	16.31	0.41
80	93.08	2.60	19.12	18.13	0.53	18.65	0.68
100	92.97	3.01	18.62	19.64	0.68	20.31	1.29
120	94.18	2.41	18.15	21.18	0.59	21.77	1.97

TABLE 9.2.3 continued

TABLE 9.2.3 continued

COMPOSITION OF PRECIPITATE FROM ALKALI
(FRACTION 2)
AS A % OF ORIGINAL WOOD PUT INTO GUN

PRIMARY DATA				CALCULATED COMPOSITION			
TIME (SEC)	APPARENT KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	METHOXYL IN APPARENT KLASON LIGNIN AS % OF APPARENT KLASON LIGNIN	TRUE KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	TOTAL TRUE LIGNIN (KLASON + ACID- SOLUBLE)	PSEUDOLIGNIN BY DIFFERENCE (i.e. APPARENT KLASON LESS TRUE KLASON)
20	89.69	3.36	19.85	10.78	0.40	11.19	-0.006
40	91.15	3.51	19.72	12.74	0.49	13.23	0.077
60	92.58	2.67	19.34	14.92	0.44	15.36	0.39
80	93.08	2.60	19.12	16.62	0.48	17.10	0.63
100	92.97	3.01	18.62	17.33	0.60	17.92	1.14
120	94.18	2.41	18.15	18.12	0.51	18.62	1.69

TABLE 9.2.3 continued

TABLE 9.2.4

COMPOSITION OF PULP
(FRACTION 3)
AS A % OF PULP

PRIMARY DATA				CALCULATED COMPOSITION			
TIME (SEC)	APPARENT KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	METHOXYL IN APPARENT KLASON LIGNIN AS % OF APPARENT KLASON LIGNIN	TRUE KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	TOTAL TRUE LIGNIN (KLASON + ACID- SOLUBLE)	PSEUDOLIGNIN BY DIFFERENCE (i.e. APPARENT KLASON LESS TRUE KLASON)
20	13.38	1.16	19.78	13.34	1.16	14.50	0.04
40	11.50	0.92	19.17	11.11	0.92	12.03	0.39
60	8.73	0.64	18.30	8.05	0.64	8.69	0.68
80	6.62	0.55	16.83	5.62	0.55	6.17	1.00
100	4.92	0.41	15.25	3.78	0.41	4.19	1.14
120	5.10	0.36	10.58	2.72	0.36	3.08	2.38

TABLE 9.2.4

TABLE 9.2.4 continued

COMPOSITION OF PULP
(FRACTION 3)
AS A % OF VACUUM DRY EXPLODED WOOD

PRIMARY DATA				CALCULATED COMPOSITION			
TIME (SEC)	APPARENT KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	METHOXYL IN APPARENT KLASON LIGNIN AS % OF APPARENT KLASON LIGNIN	TRUE KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	TOTAL TRUE LIGNIN (KLASON + ACID- SOLUBLE)	PSEUDOLIGNIN BY DIFFERENCE (i.e. APPARENT KLASON LESS TRUE KLASON)
20	13.38	1.16	19.78	7.72	0.67	8.39	0.02
40	11.50	0.92	19.17	6.02	0.50	6.52	0.21
60	8.73	0.64	18.30	4.29	0.34	4.64	0.36
80	6.62	0.55	16.83	2.98	0.29	3.27	0.53
100	4.92	0.41	15.25	1.97	0.21	2.19	0.59
120	5.10	0.36	10.58	1.41	0.19	1.59	1.23

TABLE 9.2.4 continued

TABLE 9.2.4 continued

COMPOSITION OF PULP
(FRACTION 3)
AS A % OF ORIGINAL WOOD PUT INTO GUN

PRIMARY DATA				CALCULATED COMPOSITION			
TIME (SEC)	APPARENT KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	METHOXYL IN APPARENT KLASON LIGNIN AS % OF APPARENT KLASON LIGNIN	TRUE KLASON LIGNIN	ACID- SOLUBLE LIGNIN (BY UV)	TOTAL TRUE LIGNIN (KLASON + ACID- SOLUBLE)	PSEUDOLIGNIN BY DIFFERENCE (i.e. APPARENT KLASON LESS TRUE KLASON)
20	13.38	1.16	19.78	7.61	0.66	8.27	0.02
40	11.50	0.92	19.17	5.82	0.48	6.30	0.20
60	8.73	0.64	18.30	4.04	0.32	4.27	0.34
80	6.62	0.55	16.83	2.73	0.27	3.00	0.49
100	4.92	0.41	15.25	1.74	0.19	1.93	0.52
120	5.10	0.36	10.58	1.20	0.16	1.36	1.05

TABLE 9.2.4 continued

TABLE 9. 2.5

SUMMARY OF AMOUNTS AND COMPOSITION OF FRACTIONS OF EXPLODED WOOD,
ALL* BASED ON WEIGHT OF ORIGINAL WOOD PUT INTO GUN

COLUMN 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
WATER SOLUBLES FRACTION 1				PRECIPITATE FROM ALKALI FRACTION 2						PULP FRACTION 3				
				TOTAL TRUE LIGNIN AS % OF TOTAL TRUE LIGNIN										
TIME (SEC)	WEIGHT OF WATER SOLS.	SOL. LIGNIN BY UV	XYLOSE BY HPLC	WEIGHT BY PPTE	TRUE KLASON LIGNIN	ACID- SOL. LIGNIN (BY UV)	TOTAL TRUE LIGNIN	IN ORIG. WOOD	PSEUDO LIGNIN	WEIGHT OF PULP	TRUE KLASON LIGNIN	ACID- SOL. LIGNIN (BY UV)	TOTAL TRUE LIGNIN	PSEUDO LIGNIN
20	23.32	2.74	0.22	12.01	10.78	0.40	11.19	(47.33)	-0.006	57.02	7.61	0.66	8.27	0.02
40	23.88	2.75	0.75	14.06	12.74	0.49	13.23	(56.01)	0.077	52.35	5.82	0.48	6.30	0.20
60	24.01	2.74	2.6	16.53	14.92	0.44	15.36	(65.03)	0.39	50.23	4.04	0.32	4.37	0.34
80	22.59	2.86	4.0	18.52	16.62	0.48	17.10	(72.35)	0.63	48.63	2.73	0.27	3.00	0.49
100	19.55	2.71	3.4	19.36	17.33	0.60	17.92	(75.87)	1.14	46.03	1.74	0.19	1.93	0.52
120	16.60	2.64	1.7	21.32	18.12	0.51	18.62	(78.83)	1.69	44.26	1.20	0.16	1.36	1.05

*Data in column 9 (in parentheses) are based on the total true lignin of the original wood.

TABLE 9.2.6

LIGNIN BALANCE*

TIME (sec)	SUM OF TRUE KLASON LIGNIN & SOLUBLE LIGNIN ACCOUNTED FOR IN ALL FRACTIONS, (i.e. IN WATER SOLUBLES, ALKALI EXTRACTED AND PRECIPITATED, AND PULP), AS A % OF WEIGHT OF WOOD PUT INTO GUN	SUM OF PSEUDOLIGNIN IN ALL FRACTIONS	SUM OF TRUE KLASON LIGNIN AND SOLUBLE LIGNIN NOT ACCOUNTED FOR** (i.e. ORIGINAL LIGNIN OF WOOD LESS LIGNIN ACCOUNTED FOR)
20	22.2	0.01	1.4
40	22.3	0.28	1.3
60	22.5	0.73	1.2
80	22.9	1.12	0.7
100	22.6	1.66	1.1
120	22.6	2.74	1.0

* All values listed are expressed as % of original wood put into gun; original wood contained 23.62% of true Klason lignin + acid-soluble lignin.

**Most of lignin unaccounted for was in the supernatant solution remaining after precipitation of the lignin-rich fraction from the alkali extract by acidification.

TABLE 9.2.6

SECTION 10

MOLECULAR WEIGHT OF IOTECH LIGNIN

10.0 MOLECULAR WEIGHT OF IOTECH LIGNIN

The molecular weight of lignin was assayed in a sample of Iotech lignin which was extracted commercially by another company, using ethanol as the extracting solvent. Gun conditions were 650 psig for 55 seconds (equivalent to 258.6°C), a fairly severe treatment. This extracted lignin contained only 52% of the total true lignin content of the exploded wood.

Iotech's commercially extracted lignin (average):

Apparent Klason lignin	87.52%
Acid-soluble lignin	5.51%
	<hr/>
Methoxyl content of above	16.97%

The calculation of the molecular weight of various fractions of the lignin are shown in Table 10.1. The number average molecular weight is given by:

$$\begin{aligned}
 M_n &= \frac{\sum n_i M_i}{\sum n_i} \\
 &= \frac{100}{0.1057149} \\
 &= 945.94
 \end{aligned}$$

The number average molecular weight for the whole fraction before fractionation is therefore 946.

It is useful to compare the Iotech lignin with Milled Wood Lignin (MWL) which is commonly used as a standard in the wood industry. MWL is extracted under mild conditions in a wet ball mill, and typically about 30-35% of the total lignin is extracted. By comparison the yield of Iotech lignin in this commercially extracted sample is 52%, and even higher yields are attainable with careful procedures (Section 9). The average molecular weight of MWL is considerably higher than Iotech lignin, in the range of 5,000 to 10,000.

TABLE 10.1

FRACTIONATION OF IOTECH LIGNIN BY VAPOUR PRESSURE OSMOMETRY

FRACTION #	FRACTION WEIGHT	NUMBER AVERAGE MOLECULAR WEIGHT	FRACTION WEIGHT % TOTAL	CUMULATIVE WEIGHT % TOTAL	FRACTION WGT. NO.AVE. MOL.WGT.
14	7.5622	463	13.78	13.78	0.297624
22	2.9427	453	5.36	19.14	0.0118322
23 & 24	8.0673	637	14.70	33.84	0.0230769
30	1.3507	664	2.46	36.30	0.0037048
34	1.3752	616	2.51	38.81	0.0040747
35	11.9030	1300	21.69	60.50	0.0166846
33	14.7929	2186	26.96	87.46	0.0123330
27	3.4255	2644	6.24	93.70	0.0023601
21	3.4590	3340	6.30	100.00	0.0018862
TOTALS	54.8795		$\sum n_i M_i = 100.00$		$\sum n_i = 0.1057149$

TABLE 10.1

SECTION 11

REACTIVITY OF IOTECH LIGNIN

11.0 REACTIVITY OF IOTECH LIGNIN

Principle of Method

Aspen and other hardwood species have substantial amounts of syringyl and of guaiacyl groups in their lignins.

syringyl

guaiacyl

When these woods are oxidized with alkaline nitrobenzene, as much as 30% of the weight of the lignin is converted to syringaldehyde, and an additional 12% is converted to vanillin in the case of aspen.

syringaldehyde

vanillin

If, however, the lignin has been subjected to conditions which cause cross-links to form between guaiacyl or syringyl groups, such cross-linked groups can no longer form vanillin or syringaldehyde. Consequently, by measuring the amount of syringaldehyde and vanillin formed by a lignin sample on oxidation, it is possible to determine whether or not the sample has been subjected to drastic conditions. Drastic treatment leads to cross-linking and therefore low yields. Cross-linked lignin is less reactive chemically and is therefore of less interest chemically. Even short treatments with hot water have been found to reduce the yields substantially.

Pepper, Manopoulo and Burton¹ in describing a gas-liquid chromatographic procedure for determining the yields of syringaldehyde and vanillin list the following yields:

TABLE 11.1

Sample	Yield (% of lignin)	
	Syringaldehyde	Vanillin
Pepper's Aspen Wood Meal	30.8	11.7
Pepper's Isolated Lignin from Aspen Wood	13.6	5.5
Aspen Wood Meal (literature value quoted by Pepper)	29.1	11.5
Isolated Lignin from Aspen Wood (literature value quoted by Pepper)	19.3	6.7

Pepper's chromatographic procedure was used in the present work, with three samples cooked at 560 psig for 20, 60 and 100 seconds and extracted by extraction procedure II.

TABLE 11.2

Time (sec)	Yield* of lignin rich precipitate	Weight of True Klason Lignin + acid soluble lignin in the precipitate, as % of weight of precipitate	Weight of True Klason Lignin + acid soluble lignin in the precipitate, as % of total original weight of lignin in wood
20	12.01	93.10	47.33
60	16.53	92.92	65.03
100	19.86	90.26	75.87

* yield of precipitate as a % of weight of original wood put into gun

Yields of syringaldehyde and vanillin, listed below, were obtained after the method was checked out using a standard sample of aspen wood meal.

TABLE 11.3

Sample	Yield (%)	
	Syringaldehyde	Vanillin
ForinTek standard aspen meal	29.30 ^a	12.39 ^a
Precipitate, 20 sec shot	18.33 ^a	7.09 ^a
Precipitate, 60 sec shot	15.63 ^a	6.45 ^a
Precipitate, 100 sec shot	14.66 ^a	5.77 ^a
Precipitate, 20 sec shot	17.05 ^b	6.60 ^b
Precipitate, 60 sec shot	14.52 ^b	5.99 ^b
Precipitate, 100 sec shot	13.23 ^b	5.20 ^b

a) yield based on lignin content of precipitate or of wood (i.e. true Klason lignin content + acid soluble lignin content)

b) yield based on precipitate weight

Discussion:

The results in Table 11.3 show that the method of analysis was working satisfactorily. The values obtained for the lignin in standard aspen wood are in good agreement with the values reported, or quoted, by Pepper (Table 11.1). The syringaldehyde yield fell between the two values reported in Table 11.1 and the vanillin yield lay only slightly above the values in Table 11.1. This difference is well within the variability of the wood.

The syringaldehyde yields from the three precipitates, when based on the lignin contents of the precipitates, also all fell between the two comparable yields listed for isolated lignin in Table 11.1. The corresponding vanillin yields lay about the higher vanillin yield in Table 11.1.

It can be seen, therefore, that although the Iotech lignin in the precipitates is certainly more condensed (i.e. with more cross-linkage) than that in the original wood, it is not significantly different in this respect from the relatively mildly isolated lignins reported by Pepper.

The progress of condensation is reflected in the decreasing yields of both aldehydes, as the time of steaming is increased from 20, through 60, to 100 seconds.

Wayman and Chua² have reported the results of alkaline nitrobenzene oxidations of lignin extracted with 90% dioxane at 70°C from wood heated in an autoclave with water. Their results are listed below in Table 11.4.

When Tables 11.4 and 11.3 are compared, it can be seen that all of Wayman's extracted lignins are much more condensed than are the three Iotech lignins. All of the extracted lignins, even that with the shortest steaming, 300 seconds, gave significantly lower yields of both aldehydes than did even the longest, 100 seconds, of Table 11.3. Even Wayman's milled wood lignin, which is generally believed to be mildly isolated, gave lower yields of syringaldehyde than did all three Iotech lignins, and gave a yield of vanillin comparable to that of an Iotech shot between 60 and 100 seconds.

It appears, therefore, that as both Wayman and Falkehag predicted at the Battelle Columbus meeting in 1979, Iotech lignin is less condensed, at least for the times and pressures studied here, than is wayman's "autohydrolysis lignin".

TABLE 11.4

Sample	Yield (%)	
	Syringaldehyde	Vanillin
Aspen milled wood lignin	9.74	6.15
Extracted Lignin, 5 min steam.	10.22	4.14
" " 10 " "	7.83	3.96
" " 20 " "	5.13	3.08
" " 30 " "	4.29	2.67
" " 40 " "	2.13	1.54
" " 60 " "	1.99	1.53
" " 90 " "	2.46	1.57
" " 120 " "	2.33	1.58

REFERENCES

1. Pepper, Manopoulo and Burton: Canadian Journal of Chemistry 40 1976-1980 (1962).
2. Wayman and Chua: Canadian Journal of Chemistry 57 2599 (1979).

SECTION 12

LIGNIN RESIN INVESTIGATION

12.0 PRELIMINARY EXAMINATION OF MODIFIED
IOTECH LIGNIN AS A WAFERBOARD ADHESIVE

246

BY

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DECEMBER 1979

OBJECTIVES

The objectives of this study were to modify extracted lignin by methylation and to combine the product with small amounts of phenol-formaldehyde; the product was then to be evaluated as a waferboard adhesive.

MATERIALS

Lignin - Iotech Commercially Extracted Lignin

Aspen chips were exploded after 55 sec. of steam treatment at 650 psig. Lignin was then extracted with 95 percent ethanol and the solvent removed by evaporation.

Phenol Formaldehyde (PF)

A fresh batch of resole-type phenol-formaldehyde was prepared in the laboratory following standard resin preparation methods. The resin produced had a mole ratio of 1 to 1.8 for phenol and formaldehyde respectively. It had a solid content of 55.1 percent and a water dilutability of 1 to 1.3. The final PH of the resin was adjusted to 6.7 with para-toluene sulfonic acid.

Furnish

A quantity of commercial wafers was obtained from a waferboard plant in Longlac, Ontario which employs a CAE disc type waferizer. The wafers were found to be 0.028 to 0.032 inches in thickness, 1.5 inches in length and random width.

The wafers were sieved on a 4 mesh screen to remove undersize particles then dried to 5.0 percent moisture and sealed in plastic bags until the blending operation. The wafers were 85 to 90 percent poplar (Populus tremuloides Michx.) with small percentages of white and yellow birch (Betula papyrifera Marsh) and (Betula alleghaniensis Britton).

Wax

Slack wax - 778 Imperial Oil.

EXPERIMENTAL

Lignin Methylation

1134 gms of lignin was dissolved in 3400 gms of 1.0N NaOH to which 680 mls of 30 percent solution of formaldehyde was added. The methylation reaction was allowed to proceed for 50 hours. The consumption of formaldehyde by the lignin was monitored by the determination of free formaldehyde content following a sodium sulphite method (1). The free formaldehyde content of the lignin solution is shown in Figure 12.1.

Lignin - Phenol Formaldehyde Combination

1172 gms of methylolated lignin was dissolved in a dilute solution of ammonia. The resulting solution was found to be 22.7 percent with respect to lignin solids. 912 gms of resole at 55.1 percent resin solids was added to the vigorously stirred lignin solution. This produced a 70/30 ratio of lignin solids to resole solids.

The Lignin - pF solution was then spray dried in a laboratory scale spray drier with an inlet temperature of 135°C and an outlet temperature of 80°C. The solution was atomized by a spray nozzle at 40 psi air pressure. The dry powder was collected by means of a cyclone attached to the apparatus.

The yield of powder was determined and its melting and softening characteristics were examined on a cure plate. The moisture content of the powder was also determined.

Composit Panel Preparation

Sufficient furnish for 3 panels 18 x 18 x 0.4375 inches was prepared at one time in a batch type blender. Tumbling furnish at the desired moisture content was sprayed with 2 percent hot slack wax. The adhesive powder, 2.5 percent with respect to the oven-dry weight of furnish, was then added to the blender and mixing continued for 5 minutes.

The blended furnish was spread out by hand into an 18" x 18" wooden forming box placed over a metal caul plate. When the forming box was removed, the free standing mat was covered by a second metal caul plate and the assembly was placed in a steam heated press at 410°F (210°C). As the press contacted the top caul plate, a timer was started. Time to reach nominal thickness of 0.4375 inches was set at 50 ± 5 seconds.

At the end of the prescribed pressing times (5 and 7.5 mins) the press was opened and the panel measured at all four corners to determine "hot thickness". The rough edges of the pressed panel were trimmed to remove loose flakes and low density areas. Specimens for the various tests were then cut according to the pattern shown in Figure 12.2. The specimens were stored in a conditioning chamber at 65 percent relative humidity and 21°C (70°F) for several days prior to testing.

Panel Evaluation

The conditioned dimensions and weight of each specimen were recorded prior to testing by heating in a forced air oven at 105°C for 24 hours. Since all samples were conditioned in an identical manner the moisture content of the static bending specimen was considered as representative for the whole study.

Modulus of rupture (static bending), accelerated aging, modulus of elasticity and internal bond tests were carried out on specimens in accordance with the test requirements of CSA Waferboard Standard CAN 3-0188.2M78(2).

RESULTS AND OBSERVATIONS

Observation on the Preparation of the Lignin Adhesive Powder

The methylation and subsequent acidification and filtrations steps of the lignin were carried out in a manner similar to those steps used in the production of kraft adhesive (Ref. 3,4). No significant difference in handling techniques were required. The modified lignin also mixed easily with the resole and remained in solution during the spray drying operation.

The solution spray dried well with only minor sticking observed. The powder collected was very fine and uniform. The "clean-up" of the spray drier was difficult because the hot water used in the "clean-up" operation softened the particles adhering to the walls which required more than normal scrubbing to remove.

The powder was found to soften readily when placed on a cure plate at 150°C. Its softening and curing characteristics were found to be quite similar to commercial phenol formaldehyde adhesives.

Kraft lignin adhesive powders have always had the disadvantage of poor softening and flow qualities which necessitated slightly longer press times for composit products than commercially available phenolic resins. The excellent softening observed for this 70/30 lignin/pF tested may reduce the press time and more importantly may permit the reduction in the amount of phenolic resin required to meet composit strength requirements.

Panel Preparation

The adhesive powder was found to contain 4.95 percent moisture. The powder was free flowing and adhered well to the prepared wood flakes.

No sticking of the hot mat to the caul plates was observed during the board pressing operation. Release agent was not used in this study.

Variability in density exists between board samples and between test specimens within a board sample. All board properties are affected by these density variations. To reduce the effect of this variable in this study, the density of each test specimen has been determined and plotted against the strength property being measured.

Fig. 12.3 is an example of a typical density plot. From these graphs the regression equation has been found using the least square method. The measured strength value of each specimen was subsequently adjusted to density 42 pcf using the regression equation. The adjusted strength values were then averaged and reported.

The results should be considered in the light of several limiting factors. The number of sample panels tested was very small in comparison to normal composite evaluations. The regression plots made also had a very small population. Further the samples were tested at a moisture content of 3.5 percent instead of the normal 7 percent. This higher moisture content requires several weeks of controlled conditioning which was not possible in the short time frame allotted to the study.

As a result one should only consider general trends based on results shown in table 12.1.

1. Panels were found to meet minimum standards at a 5 minute press time.
2. Longer press time improves the core cure as shown by a higher internal bond and a reduction in swelling during a two hour boil.
3. The wet MOR is unusually high in comparison to the dry MOR.

CONCLUSIONS

1. The Iotech lignin behaves similar to the kraft lignin during the steps of methylation and blending with phenol formaldehyde.
2. The Lignin/pF mixture spray dries at normal temperatures and pressures.
3. The 70/30 Lignin/pF powder adhesive appears to have excellent melting and curing characteristics.
4. The strength properties of the waferboard panels produced meet the minimum CSA standards for exterior waferboard.

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FIGURE 12.1 Consumption of Formaldehyde by Iotech Lignin

FIGURE 12.2 Cutting Pattern

FIGURE 12.3 Relationship of Modulus of Elasticity to Density

TABLE 12.1 Properties of Waferboard Panels Bonded with Modified
Iotech Lignin

Properties	5 minute Press time	7.5 minute Press time	CSA ¹ Minimum Standard
Wet MOR (psi)	1941	1964	1000
Dry MOR (psi)	3334	3194	2000
MOE (psi x 10 ³)	560	600	400
IB (psi)	44.6	74.5 ²	40
Thickness Swelling During 2 hr Boil	74.7%	58.0%	-

¹ National Standard of Canada CAN 3-0188.2M78

² Insufficient data to adjust for density variability

SECTION 13

PRELIMINARY MATERIAL BALANCE

13.0 PRELIMINARY MATERIAL BALANCE OF IOTECH STEAM EXPLOSION PROCESS

Introduction

When wood is heated with steam, part of the pentosan (which consists almost entirely of xylose sub-units in the case of aspen wood) is hydrolyzed to pentose sugars (xylose). Some of the xylose molecules split out three molecules of water to form furfural, some of which is believed to condense with the lignin, i.e. become attached chemically to the lignin. Another molecule of water is probably split out, either during condensation with lignin or at least during the Klason lignin analysis.

The weight of the "true" lignin is thus increased by the amount of the "pseudolignin" or furfural-derived material attached to it.

The standard Klason lignin analysis, (which consists of treating the woody sample with sulfuric acid to dissolve the cellulose and hemicellulose, leaving the lignin behind to be weighed) does not dissolve the furfural-derived material and thus gives an erroneously high result. Table 13.1 and the middle plot of Figure 13.2 show the increasing content of apparent Klason lignin (i.e. true Klason lignin + pseudolignin) with increasing time of heating at 560 psig.

Approximately 10% to 15% of the lignin of hardwoods is soluble in sulfuric acid treatment and appears in the filtrate where it is determined by UV absorption. The upper plot in Figure 13.2 shows the total apparent lignin (apparent Klason lignin + acid soluble lignin by UV).

It has been found in the present work that there are two causes for the increasing content of apparent lignin:

- 1) formation of pseudolignin, discussed above; and,
- 2) reduction in weight of steam-treated wood by the splitting-out of water and other volatiles, largely from the hemicellulose (i.e. hydrogen and hydroxyl groups are removed as water).

The base weight for percentages of components of exploded wood expressed as percentages of the exploded wood is thus reduced. The percentage of these components, and the yields of products made from them, are thus significantly lower if expressed in terms of weight of original wood introduced into the gun.

In the present work it has been found that the total amount of "true" Klason lignin present in aspen wood, before heating in steam, is virtually unchanged by the steam treatment and explosion. Furthermore, the methoxyl content of the true Klason lignin itself is virtually unchanged. This is not true of the methoxyl content of the hemicellulose. However, of the 5.32% methoxyl in solvent-extracted aspen wood, only 0.41% is attached to the hemicellulose, the remainder being part of the lignin.

Thus, 100 grams of original unextracted aspen wood, whether subsequently steam-treated or not, continued to contain 23.62 grams of true lignin (20.60 grams of Klason lignin + 3.02 grams of acid soluble UV lignin).

Also, the methoxyl content of the true Klason lignin of aspen wood subjected to mild acid prehydrolysis continues to be 20.50%. This stability of the true Klason lignin, and of its methoxyl, is not surprising because both the steam treatment and Klason lignin treatments are acid hydrolysis treatments, and the Klason lignin treatment is the more severe of the two.

Exploded wood is, therefore, analyzed by first doing a standard Klason lignin analysis and then determining the methoxyl content of the apparent Klason lignin produced. If the methoxyl content is less than 20.50% as shown in Table 13.1, it can be assumed that true Klason lignin of methoxyl content 20.50% is diluted with pseudolignin of zero methoxyl content.

$$\text{True Klason Lignin} = \% \text{ apparent Klason lignin} \times \frac{\% \text{ methoxyl}}{20.50\%}$$

The decrease in methoxyl content of apparent Klason lignin, obtained from exploded wood, with increase in heating times at 560 psig is shown in Table 13.1 and in Figure 13.3. Corrected, or true Klason lignin content, is shown in Table 13.2 and in Figure 13.4. The pseudolignin content (also included in Table 13.2) is obtained simply by subtracting the true Klason lignin from the apparent Klason lignin.

The lower plot in Figure 13.4 shows the true Klason lignin content of exploded wood as a function of time of heating at 560 psig. The upper plot also includes the acid soluble lignin determined by UV. The increase in true lignin content is caused only by the decrease in base weight.

$$\text{Weight of exploded wood} = \frac{23.62 \times 100}{\text{true lignin as a \% of exploded wood}}$$

(The weight of wood is expressed as a percentage of the original weight of wood before heating, i.e. the theoretical maximum recovery of exploded wood and weight loss, obtained by subtracting the above percentage from 100, is shown in Table 13.2 and Figure 13.1 as a function of time of heating).

Knowing the theoretical percentage recovery of exploded wood is important because it permits calculations of mass balance and enables an analysis based on weight of exploded wood to be converted to one based on weight of original wood put into gun.

$$\% \text{ of original wood} = \% \text{ of exploded wood} \times \frac{\text{theoretical maximum \% recovery}}{100}$$

Analysis for pentosan and free furfural

In brief, the conventional pentosan determination involves heating of the sample of wood material with strong hydrochloric acid which converts the pentosan to furfural. The furfural is removed by distillation and is determined colorimetrically in the distillate. When applied to exploded wood, this analysis includes any free furfural present in the sample. In order to determine the pentosan content, and not to include the furfural, the sample is first air-dried and extracted with ether to remove the furfural. The residue is then subjected to the standard pentosan determination, the base weight being corrected to include the non-volatile ether extractives. Free furfural was determined using wet samples as received without air drying. The free furfural was recovered by distillation with water (no hydrochloric acid), and then determined colorimetrically, the required acid being added directly to the distillate.

The results of a number of pentosan and free furfural analyses of exploded aspen wood samples, provided by Iotech, are shown in Table 13.3. All analyses in this table are based on the oven-dry weight of exploded wood.

The dozen samples analyzed for the series of times at 560 psig show a steady destruction of pentosan from the 19.5% present in original aspen wood to only 5.19% remaining after 300 seconds. The free furfural shows a steady increase from zero in original wood to 1.49% after 300 seconds. The amount of pentosan equivalent to the free furfural is also listed. Much more furfural, however, has been produced. Some has been lost as a vapour (azeotrope with water) on explosion and some has condensed with lignin to form pseudolignin (discussed below).

Fate of the Original Wood Components

The composition of exploded wood, expressed as a percentage of the original weight of wood introduced into the gun, is shown in Table 13.4. In obtaining this data, the empirical weight loss shown in Table 13.2 was used. The total true lignin content of 23.62% has been assumed as explained above.

In Figure 13.7 the weight lost, the pentosan lost, and the pseudolignin formed are all plotted against time of heating at 560 psig, all based on original weight of wood before heating.

In addition, the weight of pentosan equivalent to the pseudolignin is plotted, as well as the pentosan equivalent to the sum of both the pseudolignin and recovered free furfural in Figure 13.8. Details of this calculation are shown later. This last plot thus shows the amount of pentosan that would have had to be consumed if all of the pseudolignin formed had come from the pentosan.

Taking the data on total weight lost on explosion, the data on weight of pseudolignin formed, and the data on the weight of pentosan lost, it is possible to set up and solve three simultaneous equations in three unknowns (X, Y and Z, in Table 13.5). The only additional assumption involved is that all the pentosan lost is converted to furfural (losing two molecules of water), part of the furfural remains free (much of it escaping with the steam), the other part condenses with lignin losing another molecule of water; all of the cellulose lost forms hydroxymethylfurfural (losing two molecules of water), and all of the hydroxymethylfurfural condenses with lignin, losing another molecule of water.

These assumptions are not unreasonable. Hydroxymethylfurfural is much less volatile with steam than is furfural. The pentosan determination depends on this fact. Furthermore, hydroxymethylfurfural is more reactive chemically than furfural.

Agreement of Observed Degradation of Pentosan with Theoretical Expectations

Stable Pentosan

In Figure 13.7, the plot of pentosan lost shows a pronounced "levelling-out" after 120 seconds at 560 psig, when 5.31% of the initial 20.75% pentosan still remains. This relative stability of the remaining pentosan is to be expected from the known composition of aspen wood and the known stability of biuronic acids to acid hydrolysis.

Hardwood hemicellulose consists mainly of an xylan composed of a chain of anhydroxylose units. To an average of about one in every ten there is attached a 4-0-methylglucuronic acid residue. During the pentosan determination this glucuronic acid is decarboxylated and dehydrated to furfural, and thus acts as though it were another anhydroxylose unit. However, under milder conditions, as in the gun, the glycosidic linkage between the glucuronic acid and the anhydroxylose unit to which it is directly attached is stable. Thus, approximately 2 out of every 11 units of the glucuronoxylan are stable and remain undecomposed.

In aspen wood, the uronic acid content is 4.14% (on an unextracted basis) which, in the pentosan, is equivalent to $4.14 \times \frac{132}{176} = 3.1\%$ of anhydroxylose. The anhydroxylose, to which this uronic acid is attached directly, amounts to another 3.1% of anhydroxylose, making a total of 6.2% of stable pentosan. This value of 6.2% is in good agreement with the observed 5.3% of stable pentosan observed above.

Kinetics of Pentosan Degradation

In a first order degradation the rate at which the material is degrading, at any instant, is proportional to the amount of the material still remaining at that instant. Mathematically this leads to a relation which gives a straight line plot when the logarithm of the amount of material still undecomposed is plotted against time.

In Figure 13.9, such a plot has been made, and it shows the expected linear relationship, thus giving more confidence to the experimental data. The plot shows linearity between 32 and 120 seconds. After 120 seconds there is a break in the plot, corresponding to the change in the process when the more easily hydrolyzed pentosan is approaching exhaustion and the more resistant material remains, as discussed above.

The observed decomposition of pentosan is thus much as would be expected.

Pentosan Equivalent to Pseudolignin

The reactions expected are:

A weight loss from 132 to 78 is assumed. It is not certain however whether a third molecule of water is split out on formation of the complex with lignin. However if addition to the carbonyl group of furfural is involved, an hydroxyl group alpha to the aromatic ring would be formed. Such benzyl alcohols tend to form carbonium ions which condense with C-6 sites in other aromatic rings with elimination of a molecule of water. Such an elimination is expected during the drastic Klason lignin treatment.

Loss of Weight when Cellulose is Converted to Pseudolignin

The overall reactions expected are:

A weight loss from 162 to 108 (i.e. of 56) is assumed. As with pentosan, the loss of the third molecule of water is uncertain.

Thus, to account for a 1% loss in weight as water splits out of cellulose, the weight loss of cellulose required would be $1 \times \frac{162}{52} = 2.89\%$

Solution of 3 Simultaneous Equations in 3 Unknowns

Assuming one knows: 1) total weight lost on explosion
 2) weight of pseudolignin formed
 3) weight of pentosan lost
 and if one makes the following assumptions:

- 1) All the cellulose lost loses a net amount of 3 molecules of H_2O in condensing with lignin to form pseudolignin

i.e. net reaction:

- 2) Part of the pentosan lost similarly loses a net amount of 3 molecules of water in condensing with lignin also to form pseudolignin.

i.e. net reaction:

- 3) The rest of the pentosan lost a net amount of 2 molecules of water in forming furfural, none of which condensed with lignin

net reaction:

The following 3 simultaneous equation in X, Y and Z can be set up and solved:

Equation 1: Total weight lost (less acetyl)

= water from cellulose lost +
water from both parts of the pentosan lost +
furfural from the 2nd part of the pentosan lost

$$\text{i.e. } \frac{54}{162}x + \frac{54}{132}y + \frac{36}{132}z + \frac{96}{132}z = \text{total weight lost (less acetyl lost)}$$

$$0.333X + 0.409Y + Z = \text{total weight lost} \dots (1)$$

(less acetyl lost)

Equation 2: Pseudolignin formed

= pseudolignin from cellulose +
pseudolignin from pentosan

$$\frac{108}{162}X + \frac{78}{132}Y \rightleftharpoons \text{pseudolignin formed}$$

$$0.667X + 0.591Y = \text{pseudolignin formed} \dots\dots (2)$$

Equation 3:

$$\text{Total pentosan lost} = \text{pentosan lost (to pseudolignin)} + \text{pentosan lost (to furfural)}$$

$$Y + Z = \text{pentosan lost}$$

Solution:

$$\text{Double equation (1)} \quad 0.667X + 0.818Y + 2Z = 2 \text{ weight lost}$$

$$\text{Subtract equation (2)} \quad 0.667X + 0.519Y = \text{pseudolignin}$$

$$0.227Y + 2Z = 2 \text{ weight lost} - \text{pseudolignin}$$

From equation (3):

$$2Z = 2(\text{pentosan lost} - Y)$$

$$\therefore \text{ in \#4 } 0.227Y + 2 \text{ pentosan lost} - 2Y = 2 \text{ weight lost} - \text{pseudolignin}$$

$$\text{rearrange } 1.773Y + 2 \text{ pentosan lost} + \text{pseudolignin} - 2 \text{ weight lost}$$

$$\therefore Y = \frac{2 \text{ pentosan lost} + \text{pseudolignin formed} - 2 \text{ weight lost}}{1.773}$$

$$Z = \text{pentosan lost} - Y$$

$$X = \frac{\text{pseudolignin formed} - 0.591Y}{0.667}$$

The data used in Table 13.4 were substituted in these general solutions and the values obtained for X, Y and Z were listed in Table 13.5; a summary is plotted in Figure 13.8.

Conclusions.

The weight loss obtained, based on lignin analysis, agree well with the empirically observed results, thus confirming the general accuracy of the material balance calculations.

It can be seen that, in the operating range, only a small percentage loss results from conversion of the cellulose to breakdown products.

Most Recent Correction of Recovery from Gun

Subsequent to this work further investigation was done when a discrepancy was found between the oven-dried weight and phosphorus pentoxid-vacuum dried weight. A very careful investigation has shown that this discrepancy is due to decomposition of some component of the water solubles in the 105°C laboratory oven. This discrepancy amounts to about 2 1/4% of the weight of the exploded wood. Reported recoveries from the gun are therefore low by up to this amount; also calculated amounts of components based on unexploded wood put into the gun are slightly wrong because the base of the percentage is low by up to this amount. This is the case because the decomposition occurring in the water soluble fraction, on oven drying, will also occur in the oven drying of the whole exploded wood which has about a quarter of its weight as water solubles.

TABLE 13.6

1 TIME 560 psig (seconds)	WEIGHT OF DRIED WATER- SOLUBLES AS A % OF WEIGHT OF OVEN-DRY EXPLODED WOOD		4		5
	2 P ₂ O ₅ vac.dried to constant weight	3 Oven-dried 11 hours at 105°C	DIFFERENCE BETWEEN P ₂ O ₅ VAC. DRIED WGT. & OVEN-DRIED WGT.		CONVERSION FACTOR
20	23.70	23.55	0.15		0.99854
40	24.90	24.14	0.76		0.99244
60	25.91	24.27	1.64		0.98390
80	25.17	23.04	2.13		0.97916
100	22.65	20.41	2.24		0.97804
120	19.83	17.64	2.19		0.97855

This discrepancy, as seen from the above table, increases with cooking time, presumably because the amount of free sugars (mainly xylose) increases with time. Xylose is not very stable under conditions in the drying oven.

Correction of Data

If 100 gm of oven dried exploded wood had been dried under vacuum over P_2O_5 instead of in the oven, it would have come to a constant weight of 100 gm plus the amount in Column 4 of Table 13.6, eg. at 100 sec cook, 100 gm of oven dry exploded wood would have been 102.24 gm of P_2O_5 vacuum dry exploded wood. To correct data presently based on oven dry exploded wood, multiply by

$$\frac{100}{102.24} = 0.9780$$

The corrected empirical recovery for each cook time was obtained by adding the amount of difference between the P_2O_5 -vacuum dried weight and the oven-dry weight of the water solubles, corrected to a P_2O_5 -vacuum dried as shown below in Table 13.7.

TABLE 13.7

TIME (sec) 560 psig	RECOVERY OF OVEN-DRY EXPLODED WOOD	COLUMN 4 TABLE 13.6	CORRECTED EMPIRICAL RECOVERY
20	98.40	0.15	98.54
40	95.90	0.76	96.63
60	92.66	1.64	94.18
80	89.75	2.13	91.66
100	86.30	2.24	88.24
120	83.70	2.19	85.53

FIGURE 13.1

EFFECT OF COOK TIME ON WEIGHT LOSS AT 560 psig

FIGURE 13.1

FIGURE 13.2

LIGNIN CONTENT OF EXPLODED WOOD

FIGURE 13.3

METHOXYL CONTENT OF EXPLODED WOOD

FIGURE 13.3

FIGURE 13.4

CORRECTED LIGNIN CONTENT

FIGURE 13.4

FIGURE 13.5

PSEUDOLIGNIN FORMATION WITH INCREASING COOK TIME

FIGURE 13.5

FIGURE 13.6

PENTOSAN CONVERSION WITH INCREASING COOK TIME

FIGURE 13.6

FIGURE 13.7

EFFECT OF COOK TIME ON PENTOSAN, PSEUDOLIGNIN AND WEIGHT LOSS

FIGURE 13.7

FIGURE 13.8

SUMMARY OF MATERIAL BALANCE

FIGURE 13.8

FIGURE 13.9

PENTOSAN CONTENT OF EXPLODED WOOD BASED ON WEIGHT OF

FIGURE 13.9

TABLE 13.1

LIGNIN ANALYSIS OF EXPLODED WOOD AND METHOXYL CONTENT OF THE LIGNIN AT 560 psig

IOTECH SAMPLE NUMBER	TIME AT PRESSURE (seconds)	LIGNIN ANALYSIS ¹			METHOXYL ² ANALYSIS OF APPARENT KLASON LIGNIN (%)
		APPARENT KLASON LIGNIN (%)	ACID- SOLUBLE LIGNIN BY UV (%)	APPARENT TOTAL LIGNIN (%)	
Original Aspen Wood ³	0	20.60 ⁴	3.02 ⁴	23.62	20.50 ⁵
W790914(01)	5	21.38	2.78	24.16	19.83
W790914(02)	15	21.87	2.63	24.50	19.70
W790621(20+21)	32	23.55	2.60	26.15	18.75
W790914(04)	60	35.77	3.00	28.77	18.28
W790621(18+19)	70	26.60	3.08	29.68	17.58
W790621(22+23)	80	27.56	3.06	30.62	17.87
W790611(01)	85	27.80	3.22	31.02	17.23
W790621(24+25)	90	28.20	3.18	31.38	17.34
W790621(26+27)	100	29.22	3.23	32.45	17.04
W790621(28+29)	120	30.50	3.50	34.00	16.38
W790622(05+06)	220	33.10	3.54	36.64	15.52
W790914(05)	240				
W790625(01+02)	300	33.07 ⁶	3.90 ⁶	36.97 ⁶	15.47 ⁶

TABLE 13.1

TABLE 13.1 continued

- 1 Based on oven-dry exploded wood
- 2 Based on oven-dry apparent Klason lignin
- 3 Unextracted aspen wood used by Iotech
- 4 Actual analysis (not a value from the literature), extractives-containing basis
- 5 Obtained by extrapolating plot, of Methoxyl analysis of apparent Klason Lignin vs time of heating in gun, to zero time. Value of 20.50 agrees well with literature value of 20.52 reported by I. Pearl and Beyer (Tappi 40, No1, 45 (1957)) although most literature values are higher. (of the order 21.6).
- 6 These analyses of 300 second shot are corrections of earlier erroneous values (which had resulted from incomplete washing of lignin)

TABLE 13.2

Lignin Analysis of Exploded Wood, Corrected for Pseudolignin, and Comparison of Calculated Weight Loss (Based on Klason Lignin analysis and Methoxy content) with Empirical Actual Observed Loss.

TIME AT 560 PSIG (seconds)	CORRECTED LIGNIN ANALYSIS			PSEUDO- LIGNIN (%)	CALCULATED RECOVERY OF EXPLODED WOOD (%)	CALCULATED WEIGHT LOSS (%)	EMPIRICAL OBSERVED WEIGHT LOSS (%)
	TRUE KLASON LIGNIN (%)	ACID- SOLUBLE LIGNIN BY UV (%)	TOTAL TRUE LIGNIN (%)				
0	20.60	3.02	23.62	0	100.00	0	0
5	20.68	2.73	23.46	0.70	100.68	-0.68	0.40
15	21.02	2.63	23.65	0.85	99.87	0.13	1.15
32	21.54	2.60	24.14	2.01	97.85	2.15	3.05
60	22.98	3.00	25.98	2.79	90.92	9.08	7.34
70	22.81	3.08	25.89	3.79	91.23	8.77	8.84
80	24.02	3.06	27.08	3.54	87.22	12.78	10.25
85	23.37	3.22	26.59	4.43	88.83	11.17	11.07
90	23.85	3.18	27.03	4.35	87.38	12.62	11.85
100	24.29	3.23	27.52	4.93	5.83	14.17	13.65
120	24.37	3.50	27.87	6.13	84.75	15.25	16.30
220	24.06	3.54	28.60	8.04	82.59	17.41	~ 18.
240							
300	24.96	3.90	28.86	8.11	81.84	18.16	

TABLE 13.2

TABLE 13.3

IOTECH SAMPLE NUMBER	MAXIMUM PRESSURE (psig)	TIME AT PRESSURE (seconds)	FREE FURFURAL			TOTAL PENTOSAN & FURFURAL EXPRESSED AS PENTOSAN (%)
			PENTOSAN (NOT INCLUDING FREE FURFURAL) (%)	EXPRESSED AS FURFURAL (%)	EXPRESSED AS EQUIVALENT PENTOSAN (%)	
Standard aspen wood	0	0	19.00	0	0	19.00
W 790914(01)	560	5	18.83	0.11	0.15	18.98
W 790914(02)	560	15	18.37	0.14	0.19	18.56
W 790621(20+21)	560	32	17.92	0.33	0.45	18.37
W 790914(04)	560	60	12.64	0.50	0.69	13.33
W 790621(18+19)	560	70	12.23	0.66	0.91	13.14
W 790621(22+23)	560	80	11.20	0.84	1.16	12.36
W 790611(01)	560	85	9.78	1.02	1.40	11.18
W 790621(24+25)	560	90	9.52	0.94	1.29	10.81
W 790621(26+27)	560	100	8.21	1.09	1.50	9.71
W 790621(28+29)	560	120	6.63	1.37	1.88	8.51
W 790622(05+06)	560	220	6.23	1.39	1.91	8.14
W 790914(05)	560	240				
W 790625(01+02)	560	300	5.19	1.49	2.05	7.24

1) All Analyses expressed as % of oven dry exploded wood

2) Wood slurried at 5% consistency with water at 50°C for 1 hour (same result obtained at 10% consistency & at 1/2 hour)

TABLE 13.4

TIME AT 560 psig (seconds)	LIGNIN			PSEUDO- LIGNIN (%)	METHOXYL IN APPARENT KLASON-LIGNIN (% of orig- inal wood)	PENTOSAN (NOT INCLUDING FREE FURFURAL) (%)	FREE FURFURAL		TOTAL PENTOSAN & FURFURAL (EXPRESSED AS EQUIVALENT PENTOSAN) (%)
	TRUE KLASON LIGNIN (%)	ACID- SOLUBLE LIGNIN BY UV (%)	TOTAL TRUE LIGNIN (%)				EXPRESSED AS FURFURAL) (%)	EXPRESSED AS EQUIVALENT PENTOSAN (%)	
0	20.60	3.02	23.62	0	4.22	19.00 ²	0	0	19.00
5	20.60	2.77	23.37	0.70	4.22	18.75	0.11	0.15	18.90
15	20.78	2.60	23.38	0.84	4.26	18.16	0.14	0.19	18.35
32	20.88	2.52	23.40	1.95	4.28	17.37	0.32	0.44	17.81
60	21.29	2.78	24.07	2.59	4.36	11.71	0.46	0.64	12.35
70	20.79	2.81	23.60	3.45	4.26	11.15	0.60	0.83	11.98
80	21.56	2.75	24.30	3.18	4.42	10.05	0.75	1.04	11.09
85	20.78	2.86	23.65	3.94	4.26	8.70	0.91	1.35	9.94
90	21.02	2.80	23.83	3.83	4.31	9.39	0.83	1.14	9.53
100	20.97	2.79	23.76	4.26	4.30	7.09	0.94	1.30	8.38
120	20.40	2.93	23.33	5.13	4.18	5.55	1.15	1.57	7.12
220	20.55	2.90	23.45	6.59	4.21	5.11	1.14	1.57	6.67
240									
300	20.43	3.19	23.62	6.64	4.19	4.25	1.22	1.68	5.93

1) Calculated from analyses in Tables 1,2 &3, and assuming observed empirical weight loss, except for 300 second shot where no empirical weight loss was available. In this case the weight loss calculated from Klason lignin and Methoxyl analyses was used.

2) Standard Aspen.

TABLE 13.5

TIME AT 560 PSIG (seconds)	TOTAL WEIGHT LOST (FROM TABLE A) (%)	ASSUMED LOSS OF ACETYL (%)	DATA USED IN CALCULATION			SOLUTION OF EQUATIONS		
			LOSS TO BE ACCOUNTED FOR ² (%)	PENTOSAN LOST (%)	PSEUDOLIGNIN FORMED (%)	X CELLULOSE TO PSEUDO- LIGNIN (-3H ₂ O) (%)	Y PENTOSAN TO PSEUDO- LIGNIN (-3H ₂ O) (%)	Z PENTOSAN TO FURFURAL (-2H ₂ O) (%)
0	0	0	0	0	0			
5	0.40	3.56	-3.16	0.25	0.70)	Not all Acetyl has been lost at short times		
15	1.15	3.56	-2.41	0.84	0.85)			
32	3.05	3.56	-0.51	1.63	2.01)			
60	7.34	3.56	3.78	7.29	2.79	-0.72	5.53	1.76
70	8.84	3.56	5.28	7.85	3.79	1.22	5.04	2.81
80	10.25	3.56	6.69	8.95	3.54	1.28	4.55	4.40
85	11.07	3.56	7.57	10.30	4.43	1.64	5.65	4.65
90	11.85	3.56	8.29	10.61	4.35	1.51	4.78	5.83
100	13.65	3.56	10.09	11.91	4.93	3.11	4.83	7.08
120	16.30	3.56	12.74	13.45	6.13	5.42	4.25	9.20
220	17.41	3.56	13.85	13.89	8.04	8.59	3.91	9.98
300	18.20	3.56	14.64	14.75	8.11	7.96	4.74	10.01

1) Details of calculations, and assumptions in text.

2) Total weight lost, less the acetyl lost, i.e. the difference from the preceding two columns.

SECTION 14

PROCESS ECONOMICS

14.0 PROCESS ECONOMICS

Capital Cost

An estimate has been made of the capital cost of an Iotech steam explosion pretreatment plant by scaling-up pilot plant data. The projection has been made based on a plant designed to handle 85,000 OD tons/year of aspen (250 OD tons/day) for conversion into ethanol and lignin. Designed for a pressure of 600 psig and a cooking time of 60 seconds, four 45 cu ft guns would be required.

For the estimate we have completed detailed engineering design for the gun and auxilliary equipment, and we have obtained current prices from machine shops and hardware suppliers. Equipment includes feed and discharge valves, feed and discharge hoppers, screw conveyors, instrumentation and automatic controls. Installation costs have been included.

The cost of buildings has been allocated to the steam explosion process on the basis of area used, and the cost of the steam generating plant has been allocated according to the proportion of steam consumed in the steam explosion operation.

With an item for engineering fees and contingency the total capital cost for this steam explosion installation is estimated at \$1,890,000.00 as shown in Table 14.1. This figure indicates the average cost for a single gun at \$473,000.00, but we consider the marginal cost of a single gun addition, where no significant buildings and boiler capacity need be added, at \$270,000.00.

Operating Cost

The operating cost is built up from the individual costs of capital, maintenance, labour and steam.

The annual capital cost is calculated by write-off of the plant over 10 years at 12% interest.

Maintenance charges are included at 6% of the capital cost of the plant.

Labour charges are estimated as one worker/shift for four guns.

Steam costs are based on empirical pilot plant data. As the operating pressure is raised, steam cost increases, as shown in Table 14.2 and Figure 14.2. The cost may vary from 92¢/OD ton at 800 psig down to 66¢ at 250 psig.

For the particular installation studied the operating cost is \$7.32/OD ton, as shown in Table 14.1.

It can be seen that capital-related costs contribute 72% of the total operating cost and the cost of steam is a relatively unimportant factor in the cost economics.

Value of Pretreatment

An operating cost of \$7.32/OD ton represents a low cost for a pretreatment process which achieves several desirable effects in a single step.

- (a) the sugar yield is increased from about 20% for untreated wood up to 55% for the treated product.
- (b) the lignin is separated in a form which is soluble, reactive and suitable for conversion into useful chemicals.
- (c) the biomass is sterilized by the high temperature reaction, so that risk of contamination in the downstream biological processes is reduced.

- (d) the treated product has been comminuted by the explosion to a size which permits dissolution into water at high concentrations.

It is worth noting that a 20% increase in sugar yield is worth at least \$25 in reference to the value of the alcohol produced.

Comparison with Milling

It is known that various types of milling can break up the crystalline structure of the cellulose and result in enhanced sugar yields. We believe, however, that the steam explosion offers a much better approach, because the cost of milling is considerably higher.

Millett et al.¹ quote energy costs for comminution of dry pine down to various mesh sizes. At a size equivalent to the Iotech exploded wood (50% through 200 mesh) their data indicates an energy cost alone of \$30/ton. This figure does not include the cost of drying and capital-related expenses which would push the cost of milling up to about \$60/ton.

Effect of Cooking Time

At different pressures the optimum cooking time varies greatly. For example, at a pressure of 650 psig the best cooking time is 20-40 seconds, whereas at 250 psig the best time is 15-25 minutes.

These differences in cooking time have a large effect on the cost of pretreatment. At longer cooking times, more guns are necessary to process a given tonnage of biomass. At a cooking time of 1 minute, four would be required whereas 27 guns are necessary for a cooking time of 20 minutes. The capital-related costs increase

sharply with additional cooking time as shown in Table 14.3 and Figure 14.3.

It can be seen that the operating cost estimate increases from \$7.32 at 1 minute cooking time (600 psig) up to \$27.83 at 20 minutes (250 psig).

Under certain conditions longer cooking times can produce higher yields of sugars. Since the cost of production at the longer cooking times is greater, an optimum economic cooking time can be found. Calculations to determine such an optimum have been done in Section 3 (see Figure 3.9) of this report, and the analysis indicates an economic optimum of 500-550 psig at a cooking time of 40-50 seconds.

Conclusion

At the optimum conditions the cost of steam explosion pretreatment is estimated at \$7.50/OD ton.

REFERENCES

1. Millett et al. Biotechnology Symposium U.S. Army
Natick Research Development Command, 1976.

FIGURE 14.2

VARIATION OF STEAM COST PER OVEN-DRIED TON WITH
OPERATING PRESSURE

FIGURE 14.3

FIGURE 14.3

TABLE 14.1

ESTIMATED CAPITAL AND OPERATING COST
OF STEAM EXPLOSION PRETREATMENT

Basis 85,000 OD tons/year (250 OD tons/day)
 4 Iotech gun 45 cu.ft each
 600 psig operating pressure
 Steam cost (ex. wood boiler) \$2.14/million BTU

A. CAPITAL COST

Guns and auxiliary equipment	\$ 900,000
Building (allocation)	250,000
Boiler (allocation)	360,000
Engineering fees/contingency	380,000
	<hr/>
Total capital cost	<u>\$1,890,000</u>
Annual capital cost (write off over 10 years @ 12%)	 <u>\$ 336,000</u>

B. OPERATING COST/TON

Capital cost	\$3.95
Maintenance (6% capital)	1.33
Steam	.86
Labour	1.18
	<hr/>
Total operating cost/ton	<u>\$7.32</u>

TABLE 14.2

VARIATION OF STEAM COST WITH OPERATING PRESSURE

Operating Pressure psig	Operating Temperature		Estimated Steam Cost
	°F	°C	¢/ton
200	388	197	66¢
400	448	231	78
600	489	254	86
800	520	271	92

TABLE 14.3

VARIATION OF OPERATING COST/TON WITH COOKING TIME

BASIS: 85,000 OD tons/year

COOKING TIME	TURN- AROUND TIME	NUMBER OF GUNS REQUIRED	ESTIMATED CAPITAL COST \$	ESTIMATED OPERATING COST \$/ton				
				CAPITAL	MAINTENANCE	LABOUR	STEAM	TOTAL
5 sec	2 min	3	\$1,520,000	3.18	1.07	.95	(a) .92	6.12
1 min	3 min	4	1,890,000	3.95	1.33	1.18	.86	7.32
5 min	7 min	9	3,470,000	7.26	2.45	2.16	.78	12.65
10 min	12 min	15	5,090,000	10.66	3.59	3.18	.74	18.17
20 min	22 min	27	7,920,000	16.59	5.59	4.93	.72	27.83

(a) Assume 5 sec = 800 psig

1 min = 600 psig

5 min = 400 psig

10 min = 330 psig

20 min = 250 psig

from Figure

TABLE 14.3

SECTION 15

MICROSCOPIC EXAMINATION OF EXPLODED ASPEN

15.0 MICROSCOPIC EXAMINATION OF EXPLODED ASPEN

Method

The samples were air dried and stored in a dessicator over phosphorus pentoxide. They were mounted on aluminium stubs with double-adhesive scotch tape, coated with gold, and the preparations were examined and photographed with a JEOL (JSM-35) scanning electron microscope.

Discussion

The series at 560 psig pressure, from 30 seconds to 300 seconds, has been chosen to show the progressive breakdown in the wood with increasing cooking time. Wiley-milled aspen (photo #2459) is used as the control. At 30 sec (#2424) there are still large, intact, fibre bundles, showing ray crossings, very similar to the control, though the bundles are smaller and appear more loosely bonded together. The cell walls are encrusted, with a bubbly appearance (#2425). As the cooking time is increased (70 sec #2415, 80 sec #2394, and 90 sec #2465) the fibre bundles begin to break down. Fibres still remain at 90 sec, but by 100 sec (#2483) the appearance is quite different: a debris of broken fibres which seem to have coalesed. The cell walls at 70 sec are still encrusted but present a wrinkled appearance, showing an increased extraction of the encrusting material. By 80 sec small droplets are appearing (#2401) and the fibre bundles have started to break apart longitudinally. The cell walls become even more wrinkled at 90 sec (#2466), but by 100 sec they appear smoother, with many more liquid-like spheres (#2477). At the longer cooking time of 220 sec these spheres are more numerous (#2498) and the fibres even more fragmented. Finally at 300 sec the droplets are large (#2525) and set in a matrix of cell debris.

The development of wrinkles in the cell wall is believed to be due to excessive shrinkage resulting from the loss of hemicellulose. Associated with this loss is an increase in looseness and porosity of the cell walls which is thought to facilitate the release of larger lignin molecules to form the droplets. After 100 sec it appears the cellulosic material becomes covered by a more or less continuous film of extractives; this may or may not affect the digestibility of the material. It would appear however that extractives, presumably partly lignin, do not react with the cellulose, as the lignin can be additively extracted with increasing cook time by Fractionation Procedure II. Alternatively the reduction in digestibility after 100 sec at 560 psig is the change in the physical form of the cellulose material. This alteration may make it indigestible to much rumen microflora whereas apparently this is not so with the NOVO enzymes.

Conclusion

Based on the anatomy of the cooked wood it appears the digestibility of the material increases up to a limit with the gradual breakdown of the fibre bundles, the removal of extractives from the cell walls (shown by the production of wrinkles) and the breakdown of the cell walls, both by transverse separation and longitudinal shredding. Over-cooking beyond this point results in a rapid decrease of digestibility.

Control Aspen (#2459) 200X

560 psig 30 sec (#2424) 200X

560 psig 70 sec (#2415) 200X

560 psig 80 sec (#2394) 200X

560 psig 90 sec (#2465) 200X

560 psig 100 sec (#2483) 200X

560 psig 220 sec (#2498) 2000X

560 psig 300 sec (#2505) 400X

560 psig 30 sec (#2425) 2000X

560 psig 80 sec (#2401) 2000X

560 psig 90 sec (#2466) 4000X

560 psig 100 sec (#2477) 400X

APPENDIX A

SOURCES AND PREPARATION OF ASPEN AND OTHER SUBSTRATES

APPENDIX A: SOURCES AND PREPARATION OF ASPEN
AND OTHER SUBSTRATES

Description of Aspen Chips used in Iotech's Gun Tests

Unless otherwise described, all aspen chips processed in the period from April 1979 to July 24, 1979 were obtained from Iroquois Mill, Ontario, and aspen chips processed from July 25, 1979 to date were obtained from Papineauville, Quebec.

Iroquois Aspen Chips

Approximately 5 tons of aspen logs from a 30 year old stand were obtained. The logs were debarked then chipped. Only chips selected between 5/8 in. hole screen and 1 1/4 in. hole screen were accepted. Chips were let stand over the weekend and probably absorbed some rain (the chips' moisture content was about 52%). Chips were mixed by a backhoe and shovels then placed in plastic bags and stored at sub-zero temperature in a commercial freezer.

Papineauville Chips

About 5 tons of aspen chips were obtained from a 30-50 year old stand. Method of chip preparation was similar to above. Aspen chips have moisture content of about 48%.

Preparation of Chips at Pilot Plant

Bags of chips were taken out of the cold storage as needed and then hammer milled through a 1 in. hole screen. Hammer milled chips are left in covered wooden boxes to warm up to room temperature before shooting.

Other Hardwood Species

White birch and hard maple 4 ft x 10 in diam. bolts were obtained from the Petawawa Forest Station, Petawawa, Ontario. The logs had been slightly seasoned and have

an average moisture content of about 24% (wet weight). All the bark was removed before chipping. 1/4 inch chips were obtained with a small single knife disc chipper.

Straw

Barley straw, oat straw and corn stover were obtained from local farms. Wheat straw came from Kingston, Ontario. The straws were harvested in the fall; therefore the moisture content was reduced to about 10%. Straw was hammer milled through 1 1/4 in. screen and the dusty particles were removed with a vibratory separator. Earlier tests indicated that the dust was charred when it came in contact with the hot gun wall and this burnt material caused lower in vitro cellulose digestibility. The hammer milled straw was then wetted with water to bring the moisture content to about 40%. The corn stover must have been soaked with some rain when obtained, as it was very wet. It was air dried at room temperature to about 50% moisture content.

Agricultural By-products

The air-dried sunflower stalks were provided by Dominion Experimental Station, Morden, Manitoba. The sunflower stalks were wetted with water to moisture content of about 50%. The wetted material was kept in sealed plastic bags several days prior to testing to facilitate water soaking evenly into the fibers.

APPENDIX B

PROCEDURE FOR GUN EXPERIMENTS

APPENDIX B: PROCEDURE FOR GUN EXPERIMENTS

1. Standard experiments: for process variation, effect of pressure, cooking time, moisture content of starting material, and different substrates.

Four kg of wood chips (or 1.5 kg of wetted straw) was used in each shot. The charge was poured into the gun through a metal funnel fitted to the gun top opening. Each charge occupies about 3/4 of the gun volume. The gun was tightly closed with a locking cap then the air operated steam valve was quickly opened to inject high pressure steam into the gun near the top and bottom. As the pressure inside the gun reached the predetermined value the cooking time started. The pressure was almost constant during the cooking period and was maintained by a pressure regulator. Three seconds before the end of the cook cycle the steam valve was shut off. At the end of the cycle the gun bottom valve was quickly released and the contents of the gun discharged violently into the cyclone where some of the superheated water flashed off. The exploded material was collected in a stainless steel tub placed beneath the cyclone. The inside wall of the cyclone was scraped clean between shots to minimize sample contamination. The exploded material was thoroughly mixed in the tub, then kept in plastic bags. All samples were stored in a freezer for future use. Unless otherwise indicated most of the tests were carried out without any die, i.e. the gun bottom opening is 1 9/16 in. diameter.

2. Experiment with different dies: tests were done with star die, sieve die, circular die and without die to investigate the effect of the gun nozzle shape on digestibility (see Section 7). The die can be lifted out or lowered into the gun nozzle using a T-bar rod and hooks screwed into the die.

3. Presteam experiment: to see whether precooking of frozen or air-dried chips would improve the digestibility. Low pressure steam was introduced into the gun via the low pressure line (see equipment diagram Figure B.1) for a predetermined period then high pressure steam was injected as in standard tests.
4. Varying moisture content of input chips: green wood chips were spread on cement floor to dry at room temperature to desired moisture content.

FIGURE B.1

SCHEMATIC DIAGRAM OF IOTECH'S PILOT PLANT

APPENDIX C

MATERIALS AND METHOD OF ENZYME HYDROLYSIS ASSAY

APPENDIX C: MATERIALS AND METHOD OF ENZYME HYDROLYSIS ASSAY

Enzymes

Obtained from NOVO Laboratories Corp., 59 Denbery Road, Wilton, Kentucky 06897, U.S.A.

Celluclast 100 L (liquid) activity 100 C₁U/g

Cellobiase 250 L (liquid) activity 250 CbU/g

Unit Definition

C₁U/g - unit of celluclast activity is equal to the amount of enzyme required to produce 1 μ mole/min reducing carbohydrate under standard conditions:

pH 4.80

Buffer 0.1M acetate buffer

Substrate C₁ 10 g/l Avicel

Incubation at 50°C for 20 min.

Enzyme dilution of C₁ 0.01 to 0.03 C₁U/ml

CbU/g - unit of cellobiase activity is the amount of enzyme which at pH 5.0 and 50°C will, in 15 min reaction time with cellobiose as substrate. liberate 2 μ moles of glucose per minute.

Substrate

The cellulosic substrates in this research are various exploded wood and agricultural products steamed (cooked) at different pressures and durations, designated as pressure/time with units psig. and sec.

Apparatus

50°C $\pm$ 0.05°C hot air chamber fitted on a platform shaker

Thermostat water bath 50°C $\pm$ 0.05°C

SP6-400 UV Spectrophotometer

Precision Scientific Co., Chicago drying oven Model 18

Analytical Procedures for Sugar Determination

Somoygi Method

This method was originally used but due to the large amounts of reducing sugars present in the hydrolysate, several dilutions were required and this made the procedure tedious and time consuming. The DNS method for reducing sugar determination was used instead.

DNS Method

The DNS sugar assay employed the colorimetric reaction by dinitrosalicylic acid 5,1. The chromophore formed has an absorption maximum at 550 nanometres (nm). The change in absorbance at 550 nm is, to a certain extent, directly proportional to the reducing sugar concentration in the sample. The assay procedure is as follows:

- 1) One ml of hydrolysate is added in a 16 x 150 mm test tube with 1 ml of distilled H₂O. Mix well.
- 2) 2 ml of DNS reagent is added to the diluted hydrolysate and well mixed.
- 3) The test tube is then covered with tin-foil and heated in a boiling water bath for 15 min.
- 4) Cool to room temperature in a cold water bath to stop the reaction.
- 5) Then, add 16 ml distilled H₂O to the mixture and mix by inverting the tube several times.
- 6) Measure absorbance of diluted sample at 550 nm.
- 7) Determine the sugar concentration from glucose standard curve prepared from standard solutions of glucose in the range 0.2 to 2 mg/ml.
- 8) Glucose standards are run with each set of samples at 0.5 mg/ml and/or 1.0 mg/ml.

The DNS method also requires a lot of sample dilution before assay even though the sensitivity is from 0.2 to 2 mg/ml. Both the Somoygi and the DNS methods determine all reducing substances such as glucose, cellobiose, xylose, arabinose, and aldehydes. Since the sugars in the hydrolysate will proceed to ethanol fermentation it is important to know the levels of the individual sugars. High pressure liquid chromatography (HPLC) was finally used for accurate measurement of the sugars; see Appendix E.

Filter Paper Activity (FPA)

During the course of this research, different batches of the enzyme celluclast were introduced due to low supply of the originally used celluclast 250s. Hence, a quick assay to determine the different enzymes' activities is called for in order to maintain the uniformity of enzyme dosage used throughout the research. This crude assay of activity is commonly measured against filter paper to give an overall evaluation of the hydrolytic strength. The method of determining Filter Paper Activity (FPA) as used in this research is as follows:

- 1) 1 ml of celluclast sample (2 mg/ml) is added to 1 ml of 0.05M citrate buffer (pH 5.0).
- 2) To this mixture a 1x6 cm strip (54 mg) of Whatman #1 filter paper that has previously been rolled up is added.
- 3) Incubate in a 50°C water bath for 1 hour.
- 4) Remove from water bath and cool in cold water bath for 5 min.
- 5) Pipette 1 ml of solution from the mixture making sure to avoid removing any of the filter paper residue.
- 6) Determine reducing sugars by modified DNS method (see below)
- 7) The concentration of sugar in mg/ml multiplied by the dilution factor of 2 is the FPA.

Modified DNS Method

For the purpose of this FPA assay, the method outlined below was used:

- 1) To one ml of the sample, 3.0 ml of DNS reagent is added and mixed.
- 2) Heat mixture in a boiling water bath for 5 min.
- 3) Cool in cold water bath for 5 min.
- 4) Dilute with 20.0 ml of water, mixing well by inverting the tube several times.
- 5) Measure absorbance of diluted sample at 600 nm.
- 6) Determine sugar concentration from curve prepared from standard glucose solutions of 0.5 - 2.5 mg/ml.

The variations in this method as opposed to the previous DNS method is necessary to maintain sensitivity over a wider range.

Preparation of Enzyme Hydrolysate

A flow-chart is shown in Figure C.1 with the step-by-step procedure for preparing the enzyme hydrolysate for the standard enzyme test. First moisture is determined by drying the wood in an oven at 105°C for 24 hours. The percentage consistency of the wood is calculated by the weight of the oven-dried wood, i.e. 5 gm oven-dried wood corresponds to 5% consistency. Wet wood is then used to give the equivalent of 5 gm of oven-dried wood. To this is added the enzyme solution made from 2.5 parts of 100 L-cellulast to one part of 250 L-cellobiase. The enzyme solution was used in a concentration of 35% to oven-dried wood, i.e. 1.75 gm is added in 100 gm. The whole is made up to 100 gm with 0.4M sodium acetate buffer pH 4.8. This is incubated at 50°C for 24 hours. The flask is then cooled before filtering to prevent elution of glucose from the filter paper by the enzyme.

FIGURE C .1

FLOW CHART OF ENZYME HYDROLYSIS

Wood Consistency

Figure C.2 shows a range of wood consistencies (i.e. number of grams of oven-dried wood per 100 gm incubation mixture is equivalent to % consistency). The enzyme was added to these samples to give 5% enzyme to wood dosage to all samples. Initially these incubations were only analyzed for reducing sugars by the DNS method and an erroneous conclusion was drawn that there was no effect on enzyme activity for 5% to 15% consistency. Later on, when the HPLC was working well, glucose and xylose were assayed for. It can be seen that there is a gradual decrease in the glucose produced as the wood consistency increases, which is even greater above 15% consistency.

A comparison was done with two enzyme consistencies of 5% and 10%. The results are shown in Figure C.3. The yield of glucose is much greater with 5% consistency while the xylose does not show much difference. All the enzyme assays in this report have been done at 5% wood consistency.

Enzyme Concentration

The effect of the enzyme concentration was investigated, as shown in Figure C.4. After 24 hours incubation there is little effect on the xylose produced but the glucose increases markedly, and the total reducing sugars show the same trend. Incubating further for two and five days showed no increase in the sugar produced. A further experiment was done, this time using ethanol washed wood and incubating for 24 hours at 50°C. These results are shown in Figure C.5 which shows that the glucose yield goes on increasing up to 90% enzyme to

washed wood. Since it is uneconomic to use this concentration of enzyme, 35% enzyme to wood was taken, as this is a point after the slope of the graph has levelled off. All assays in this study have been done with 2.5 parts celluclast 100 L to one part cellobiase 250 L enzymes at 35% to the oven-dried exploded wood.

FIGURE C.2

EFFECT OF VARIOUS EXPLODED WOOD CONSISTENCIES AS SHOWN
BY ENZYMATIC DIGESTION

FIGURE C.2

FIGURE C.3

EFFECT OF CONSISTENCY ON ASPEN EXPLODED AT 560 psig

AS SHOWN BY ENZYMATIC DIGESTION

FIGURE C.3

FIGURE C.4

EFFECT OF INCREASING ENZYME ON SUGAR PRODUCTION

FIGURE C.4

FIGURE C.5

EFFECT OF VARIOUS ENZYME CONCENTRATIONS ON SUGAR PRODUCTION

FIGURE C.5

TABLE C .2

EFFECT OF VARIOUS EXPLODED WOOD CONSISTENCY
ON ENZYMATIC DIGESTION.

5 days			Reducing Sugars		
% Consistency	% xylose	% glucose	Day 1	Day 2	Day 3
0.5	10	30.0	44.79	53.63	58.45
1.5	6.7	36.7	28.74	35.81	39.92
2	11.5	35.0	28.67	36.88	41.79
4	10.0	31.3	25.58	30.62	34.11
5	10.0	29.0	28.22	30.18	31.75
6	11.3	29.2	28.22	30.18	31.75
8	10.0	23.8	27.03	29.28	30.77
10	10.5	24.5	26.81	30.14	30.50
15	11.9	25.0	28.11	29.31	29.39
20	11.0	11.5	22.99	22.42	22.18

TABLE C .2

TABLE C .3

EFFECT OF CONSISTENCE ON ASPEN EXPLODED AT 560 psig
AS SHOWN BY ENZYMATIC DIGESTION

Time (sec)	5% consistency		10% consistency	
	% xylose	% glucose	% xylose	% glucose
5	13.31	13.31	10.24	10.24
15	13.88	21.87	11.41	13.81
32	14.25	23.76	12.02	16.92
60	10.46	31.39	8.94	20.57
70	8.56	29.48	8.93	21.87
85	9.50	34.21	7.39	22.28
90	9.12	38.01		
100	7.60	28.51	8.46	24.04
120	7.62	33.32		
240	3.43	40.05	2.06	23.26

TABLE C .3

EFFECT OF INCREASING ENZYME CONCENTRATIONS
ON ENZYMATIC DIGESTION

<u>% enzyme to</u> <u>oven-dried</u> <u>exploded</u> <u>wood</u>	<u>%</u> <u>xylose</u>	<u>%</u> <u>glucose</u>	<u>%</u> <u>Reducing</u> <u>Sugars</u>
0.5	6.3	8.6	15.5
0.7			17.5
1.0			19.3
1.4	6.3	10.3	21.7
2.0			26.1
2.8			29.8
4.0	6.7	22.8	35.2
5.6			41.8
8.0			49.2
11.0			56.1
16.0	8.6	56.1	72.7

EFFECT OF VARIOUS ENZYME CONCENTRATIONS
ON ENZYME DIGESTION

<u>% enzyme</u>	<u>xylose</u>	<u>glucose</u>
1	1.6	3.0
2	2.0	9.0
4	2.0	5.0
8	3.0	10.0
15	3.0	17.9
30	2.6	26.9
60	1.6	29.8
90	2.0	35.9

APPENDIX D

METHOD OF RUMEN DIGESTIBILITY ASSAY

APPENDIX D: METHOD OF RUMEN DIGESTIBILITY ASSAY

All rumen digestion in this work is "in vitro cellulose digestion" (% IVCD) expressed as a percentage of the cellulose present in the sample.

Quite often the rumen and the enzymes give very different results and it is important to know this. The rumen microflora is far larger than the enzymes so that accessibility will be different, and the rumen microflora are alive making them susceptible to toxicity.

Some of the work being done in the field of ethanol production uses live organisms to convert cellulose direct to ethanol. It is essential that we have a test that gives some indication of how these organisms will perform on exploded wood. Rumen microflora will never imitate these organisms exactly, but we will continue to use the test until a replacement system is devised.

The assays are being carried out by MacDonald College, McGill University in Montreal.

In vitro Rumen Fermentation Procedure for Cellulose Digestibility Determination

The procedure adopted for all the fermentation runs was that reported by Donefer et al. (1960) with slight modification, and is performed as follows:

a) source of rumen ingesta

A rumen-fistulated steer fed ad libitum a diet consisting exclusively of good-quality alfalfa hay, cobalt-iodized salt and water available at all times, served as the source of rumen ingesta.

b) preparation of inoculum (Phosphate Buffer Extract)

About six litres of rumen ingesta were collected and the liquid mechanically pressed out through two layers of cheesecloth and discarded. A sample of 1,800 grams of solid ingesta was mixed with 1,500 ml pH 7 phosphate buffer (PB). After moderate mixing, the mixture was re-pressed and the resultant liquid, designated as phosphate buffer extract (PBE), was filtered through four layers of cheesecloth into a preheated thermos container and transported to the laboratory.

Prior to making the extract, the phosphate buffer solution (1.059 g Na_2HPO_4 + 0.436 g KH_2PO_4 per litre) was preheated to 45-48°C (to compensate for the drop in temperature to approximately 40°C during extracting procedure) and 25 ml of a 20% Na_2CO_3 solution (saturated) was added to the buffer. CO_2 gas was bubbled through the buffer solution until the pH returned to 7, as measured by a pH meter.

c) preparation and dispensation of basal medium

All the basal medium (Table D.1), except iron and calcium was premixed in a 2-litre Erlenmeyer flask in quantities sufficient for the inoculation of 40 conditioned fermentation tubes (heated to 40°C, saturated with CO_2 , and adjusted to pH 7). Following this, 800 ml of the PBE inoculum and 20 ml of the iron and calcium solution were added to the flask containing the basal medium, and the total mixture adjusted to 2 litres with distilled water. The flask was then placed on a magnetic stirrer and attached to a Brewer Automatic Pippette which

dispensed 50 ml of the mixed medium and inoculum to each fermentation tube (into which the substrate had been pre-weighed).

d) in vitro system and substrates

The in vitro system used consisted of 32 fermentation tubes (90 ml, Pyrex No. 8260), each fitted with a one-hole rubber stopper (No. 6) through which was inserted a glass gas delivery tube whose tip extended to approximately 0.4 cm from the bottom of the fermentation tube. The gas delivery tube was attached by means of rubber tubing to a gas manifold with 32 outlets, each fitted with a needle valve adjusted to deliver 160 bubbles per minute. The gas manifold was connected to a tank of carbon dioxide fitted with a gas pressure regulator. Gas was exhausted by way of the clearance between the pouring lip of the tube and the rubber stopper. Fermentation tubes were maintained at a temperature of 40°C in a water bath. Total liquid volume of each tube was 50 ml. Each tube in the in vitro system contained approximately 200 mg of cellulose, provided by 460 to 600 mg of modified (by chemical and/or physical means) or unmodified oat straw. The actual amount of substrate was dependent on the cellulose content of the substrate materials as determined by analysis prior to the fermentation runs. Standard substrate was included in most of the runs in order to test repeatability of results between runs. In all runs Avicel (a commercially prepared, purified cellulose) was a standard substrate.

e) initiation and termination of the fermentation run

The addition of the inoculum and basal medium mixture to the substrates initiated the fermentation run. Two drops of mineral oil were added to each tube in order to prevent foaming, after which the tubes were connected

to a CO₂ gas supply and placed in the water bath, kept at 40°C. The tubes were shaken hourly for the first eight hours of fermentation to effect adequate mixing of the substrate and inoculum. At the end of 24 hours, the tubes were removed from the water bath, substrate materials at the sides of the tubes were washed down with distilled water, and the tubes were immediately centrifuged at 2,200 r.p.m. for eight minutes. The supernatant was discarded and the residue was immediately analyzed for cellulose, or refrigerated for subsequent cellulose analysis.

Cellulose Analysis

All cellulose analyses in this research were done by the procedure reported by Crampton and Maynard (1938), slightly modified by Donefer et al. (1960).

a) acid digestion

The acid digestion mixture was prepared by mixing 650 ml acetic acid (99.7% v/v), 80 ml concentrated nitric acid, and 150 ml distilled water. Using an automatic pipette (Machlett), 25 ml of the acid digestion mixture was dispensed into each fermentation tube. A glass stirring rod was inserted in each tube and the contents were well mixed, with the stirring rods left in the fermentation tubes during the entire digestion period.

The tubes were analyzed in series of eight, with the tubes placed in a stainless steel wire basket and immersed in a boiling water bath for a period of 30 min. Contents of the tubes were mixed every 10 min. At the end of the digestion period, the tubes were removed from the boiling water bath and allowed to cool for five minutes.

b) filtration

After cooling for five minutes, 25 ml of 95% ethanol was added to each tube, the tube contents mixed with the stirring rod, immediately followed by quantitative transfer of the contents into filtering crucibles (Selas - coarse porosity), using a polyethylene wash bottle containing 95% ethanol to wash down the sides of the tubes. Filtration was aided by applying suction. The precipitate in the crucible was then washed with approximately 10 ml each of acetone and ethyl ether in succession.

c) drying and ashing

The crucibles were next dried in a vacuum oven at a temperature of approximately 95°C for about four hours, after which they were cooled in a dessicator and weighed. They were then ashed overnight in a muffle furnace (600°C), cooled in a dessicator and reweighed.

d) calculation of cellulose content

The cellulose content of either the initial unfermented substrate or of the fermentation residue was calculated as the loss in weight on ashing the oven-dried, acid-digested material, using the following formula:

$$\text{Cellulose content} = \text{Wt. dry crucible and contents, minus} \\ \text{Wt. ashed crucible and contents}$$

$$\text{Cellulose (\%)} = \frac{\text{Wt. of cellulose}}{\text{Wt. of substrate}} \times 100$$

e) calculation of cellulose digestibility

Cellulose digestibility was calculated as the difference between initial cellulose content and cellulose residue, and expressed as a percentage of initial cellulose content. The following formula was used:

$$\text{Cellulose digestibility (\%)} \\ = \frac{\text{Wt. initial cellulose} - \text{Wt. cellulose residue}}{\text{Wt. initial cellulose}}$$

TABLE D.1

COMPOSITION OF IN VITRO BASAL MEDIUM AND INOCULUM

Solution	Volume used per tube (ml)	Amount used per tube (mg)
Mineral Mixture	10.0	(see ¹)
Iron and Calcium		
FeCl 6H ₂ O, (4.4 mg/ml)	0.5	2.200
CaCl ₂ 2H ₂ O, (5.29 mg/ml)	---	2.645
Glucose (100 mg/ml)*	0.5	50
Urea (126 mg/ml)	0.5	63
Biotin (10 mg/ml)	1.0	10 mg
PABA (100 mg/ml)	0.25	25 mg
n-Valeric acid (5 mg/ml)*	3.0	15
Casein hydrolysate-enzymatic ² (20 mg/ml)*	2.5	50
Na ₂ CO ₃ (200 mg/ml)	1.5	300
Phosphate buffer extract ³ (inoculum)*	20.0	(see ³)

¹ Na₂HPO₄, 5.65 g; NaH₂PO₄H₂O, 6.27; KCl, 2.15 g;

NaCl, 2.15 g; MgSO₄.7H₂O, 0.582 g; and Na₂SO₄, 0.75 g/litre.

² Nutritional Biochemicals Corporation.

³ Na₂HPO₄, 1.059 g; KH₂PO₄, 0.436 g/litre.

*Prepared prior to each fermentation run.

APPENDIX E

METHOD OF SUGAR ASSAY BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

APPENDIX E: METHOD OF SUGAR ASSAY BY HIGH PRESSURE
LIQUID CHROMATOGRAPHY

High pressure liquid chromatography provides a fast, simple method for carbohydrate analysis. The HPLC system we use is essentially the system described by J.K. Palmer (Analytical Letters 8 (3) 215-224 (1975)). An Altex Model 300 liquid chromatograph is equipped with a Waters differential refractometer detector; attenuation 128 - $\frac{1}{4}$, and a Linear Instruments Model 252A integrating chart recorder. The column is a 25 cm. Brownlee Labs 10 μm NH_2 bonded phase, with a 3 cm. NH_2 bonded phase pre-column attached to it. Water: acetonitrile is the mobile phase, usually in a 10:90 ratio at a flow rate of 2.5 ml/min. All samples are extracted with ether and filtered through a 0.45 μm Millipore filter prior to injection onto the column. The ether extraction removes furfural which could bind irreversibly to the amino groups on the packing material.

During the first few days of operation of the system described above a 10 - 20% baseline drift over 15 minutes was observed. This variation was due to changes in room temperature. Since the room temperature proved very difficult to control an ambient circulating water bath was connected to the refractive index detector to buffer the temperature fluctuations. The addition of the bath nearly eliminated baseline drift.

With the system in a stable environment standard curves for glucose, xylose, galactose, mannose, cellobiose and arabinose were generated. After these samples the first of the enzyme hydrolyzed exploded wood samples were examined. These samples contained two major components: xylose and glucose. The two sugars were identified by comparing their retention times to standards and by running spiked samples under various

conditions of solvent and flow rate. The non-enzyme hydrolyzed samples were similar but contained smaller quantities of glucose and traces of cellobiose.

After a few weeks of problem-free analysis using NOVO celluclast 250 s, some difficulties were encountered when we began using a NOVO celluclast 400 s. Samples treated with this enzyme showed significant quantities of galactose. The appearance of galactose on the HPLC scans made accurate glucose determinations difficult because these two carbohydrates do not separate well under the described operating conditions. Initially it appeared as though the enzyme was producing galactose from glucose. Since this seemed extremely unlikely, an experiment was designed to determine the source of the galactose. The enzyme was provided with several substrates including glucose, galactose, cellobiose and cellulose. These systems, along with a blank containing no substrate, were allowed to digest for the normal 24 hours at 50°C. The HPLC analysis of the digested samples indicated the galactose was not produced by the enzyme but was intrinsic to the enzyme solution. This problem was overcome by using NOVO 100 L instead.

This enzyme batch was preserved with glycerol and potassium sorbate. These two compounds separate well from xylose and glucose but not from cellobiose.

This HPLC system initially provided several problems but now it is working efficiently and providing reliable sugar assays.

A standard curve and a typical run are shown in Figures E.1 and E.2.

FIGURE E.1

HPLC RUNS

STANDARD CURVE WITH 10 mg/ml OF XYLOSE AND GLUCOSE

FIGURE E.2

TYPICAL EXPERIMENTAL CURVE

APPENDIX F

YEAST GROWTH STUDIES

APPENDIX F: YEAST GROWTH STUDIES

It has been observed that the water soluble component of exploded wood tends to inhibit yeast growth and alcohol production.

A standard yeast toxicity assay was therefore developed to allow us to identify combinations of gun treatment parameters that minimize the production of the inhibiting substances (toxins).

Bioassay Method

The test employed Saccharomyces cerevisiae (bakers' yeast). Toxin solutions were obtained from exploded wood samples by slurrying the sample in water, then filtering. The slurries were made up to give a standard 5% of oven-dry exploded wood on total weight. The slurry temperature at the time of filtering was 20°C in some cases and 50°C in others. 50°C is of interest because it is the temperature at which the enzymic saccharification of exploded wood is likely to be conducted.

Ten ml of the toxin solution were added to a sterile 50 ml Ehrlenmeyer flask together with 10 ml of sterile double-strength malt extract broth ("Difco"). The inoculum was 0.5 ml of a yeast suspension grown up overnight in malt extract broth. Since the preparation of the assay broth involved diluting the toxin solution to half-strength, the effective toxin concentration to which the yeast were exposed corresponded to that found in a 2.5% exploded wood slurry. For every series of toxicity assays, as control was run in which distilled water substituted for the toxin extract.

The assays were incubated anaerobically at 28°C. At predetermined intervals, a representative 5-ml aliquot was removed from each flask in the series. The aliquots were centrifuged in "Servall" thick-walled tubes for 10 min at 10,000 r.p.m., after which the supernatants were discarded. The pellets were washed once in the centrifuge using distilled water, then resuspended in more water. The turbidity of the resulting suspensions was read at 440 nanometers in a Pye Unicam spectrophotometer. The turbidity was a measure of the concentration attained by the yeast in the time allotted and was therefore inversely proportional to the toxicity of the broth.

Results and Discussion

The accompanying graphs show yeast concentration as a percent of the control, plotted against the period for which the aspen wood had been heated in the gun prior to explosive decompression.

In general, the graphs show that for practical holding times, yeast growth declined as the heating time increased, demonstrating the accumulation of toxins during the heating period. The net accumulation stops, however, and eventually reverses itself at longer times as shown in Graph F.3 for the case of treatment with 430 psig steam. This seems to indicate that a relatively slow neutralization of toxins is also occurring during the heating period. The accumulation and subsequent decay of toxicity may be related to the accumulation and subsequent disappearance of depolymerized lignin observed by Wyman,¹ since several lines of evidence (see below) indicate that the smallest of the depolymerized lignin fragments, namely simple phenols, contribute to the toxicity of steam-treated wood.

The graphs also show that for very short heating times, yeast growth consistently appears to exceed that of the control. This is probably an artifact. A certain amount of turbidity is associated with the toxin extract when the heat treatment time is very short. The turbidity is due to colloidal lignin that passes the medium filters used in preparing the extracts. This turbidity is negligible by 40 seconds at 560 psig.

Graph F.2 indicates that for testing purposes a 20-hour incubation is to be preferred over longer incubation because the resulting curve of growth versus heating time is steeper, indicating that the test is more sensitive under these conditions. Graph F.1 indicates that extraction of toxins should be done at 20°C, again because the test is rendered more sensitive. The reason why yeast growth is so much better when the sample is extracted at 50°C is not known but a contributing factor may be accelerated inactivation of the toxic compounds at the higher temperature. Experiment shows that if the toxin extract is boiled for five minutes, yeast growth in a subsequent toxicity assay increases from 13% to 62% of the control.

The chemical identity of the toxins is not known, but we have established that they may be largely eliminated not only by boiling but also by extraction with ethyl ether. Ether extraction improves yeast growth from 13% to 84%. The toxins are therefore probably phenolics broken out of the lignin molecule and/or quinones formed by air-oxidation of the phenolics, although furfural derived from the hemicellulose component cannot be ruled out as a possible toxin.

The Masonite process is superficially similar to the Iotech process and it is significant that it also produces occasionally a toxic, water-soluble component. Investigation of the soluble fraction (marketed as "Masonex") by Galloway² revealed that the toxins were mostly simple phenols.

Conclusions and Future Work

Simply varying the heating time does not appear to be a promising method of avoiding toxicity because impractically long or short times would be necessary (see Graph III.3). We are therefore trying out the various detoxification methods recommended for the treatment of Douglas fir hydrolysates³ to see if they are effective on exploded wood. The Iotech process resembles the wood hydrolysis process to the extent that wood is exposed to wet heat in the general vicinity of 200°C while at low pH. It seems reasonable, therefore, to proceed on the assumption that the toxins generated in the Iotech process are similar to those generated during wood hydrolysis.

Our toxicity assay will involve adding inorganic salts, yeast extract and sugar to water extracts of exploded wood, plus a heavy inoculum of Clostridium tropicalis. Ethanol production and glucose consumption will be monitored as indicators of yeast activity.

A second line of work will involve growing various strains of *Candida*, *Torula* and *Saccharomyces* in the presence of the water extract of exploded wood in order to obtain a strain resistant to the toxins.

REFERENCES

1. J.H. Tora and M. Wyman, "Delignification of Hardwood by autohydrolysis and Extraction", TAPPI 61 (6) 1978; 47-50.
2. D. Galloway, personal communication.
3. R.H. Leonard and G.J. Hajny, "Fermentation of Wood Sugars to Ethyl Alcohol", Ind. Eng. Chem., 37 (4) 1945; pg. 390.

FIGURE F.1

EFFECT OF COOK TIME ON TOXICITY OF EXPLODED ASPEN SOLUBLES

DIFFERENT EXTRACTION TEMPERATURES

FIGURE F.1

FIGURE F.2

EFFECT OF COOK TIME ON TOXICITY OF EXPLODED ASPEN SOLUBLES.

DIFFERENT INCUBATION TIMES

FIGURE F.3

EFFECT OF COOK TIME ON TOXICITY OF EXPLODED ASPEN SOLUBLES

430 psig (235°C/455°F)

FIGURE F.3

TABLE F.1

EFFECT OF COOK TIME ON TOXICITY
OF EXPLODED ASPEN SOLUBLES

Time (sec)	Extent of Yeast Growth (% of control)	
	44 hr. incubation 50°C extract	48 hr. incubation 20°C extract
5	115	
15	114	
32	90	100
60	88	
70	79	39
90	80	28
100	79	29
120	71	26
220		22
240	71	

TABLE F.2EFFECT OF COOK TIME ON TOXICITY
OF EXPLODED ASPEN SOLUBLES

Time (sec)	Extent of Yeast Grown (% of control)	
	20 hr.	44 hr.
5	100	115
15	95	114
32	77	90
60	60	88
70	54	79
90	51	80
100	51	79
120	48	71
240	44	71

TABLE F.3EFFECT OF COOK TIME ON TOXICITY
ON EXPLODED ASPEN SOLUBLES

Time (sec)	% of control 20°C extract 20 hr. incubation
30	104
60	69
120	64
240	26
300	19
360	23
480	23
960	30
1800	60

APPENDIX G

THEORETICAL PRODUCTS FROM HYDROLYSIS OF ASPEN WOOD

SUMMARY OF COMPONENTS OF ASPEN WOOD (SOLVENT EXTRACTED) ,
AND THE PRODUCTS PRODUCED THEORETICALLY ON HYDROLYSIS*

Component	Amount of Cmpt. in 100g of dry wood (grams)	Glucose	Lignin	Xylose	Biuronic Acid	Acetic Acid	Mannose	Galactose	Ara- binose	Galact- uronic Acid
Cellulose	48	53.33								
Lignin	21		18.9 2.1							
O-Acetyl-4-0- methylglucurono- xylan.....	24			17.96	4.52	5.59				
Glucomannan	3	1.11					2.22			
Pectin, Starch, ash etc: (total of 4.0) Anydrogalactose	2.0							2.22		
Anhydroarabinase	0.5								0.57	
galacturonic anhydride	1.1									1.21
ash	0.4									
Total	100.0	54.44	21.0	17.96	4.52	5.59	2.22	2.22	0.57	1.21

* assuming no decomposition of the sugars to furfural or other decomposition products

- ** Biuronic acid 4.52: The weight of uronic acid equivalent (no methyl group) to this would be 2.58 grams if it were hydrolyzed to xylose and methyl glucuronic acid. The weight of xylose also produced would be 2.00 grams.

APPENDIX H

WEIGHT LOSS CORRECTION CHART

FIGURE H.1

WEIGHT LOSS CORRECTION CHART - TO CORRECT EXPLODED BIOMASS TO
ORIGINAL BIOMASS

FIGURE H.1

SAMPLES OF WOOD CHIPS
AND EXPLODED WOOD

WOOD
CHIPS

EXPLODED
WOOD

PARTIAL VIEW OF
THE IOTECH PILOT PLANT