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PROCUREMENT

**Dilute Nitric Acid Pretreatment and Enzymatic Hydrolysis
of Lignocellulosic Materials**

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to:

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Summary

Pretreatment of a whole-tree hybrid Poplar chipped feedstock was performed in a series of steady-state reactions, using dilute nitric acid and temperatures from 160 °C to 170 °C. Feed rates from 0.143 to 0.202 kilograms per minute were maintained.

An initial Experimental Plan for the investigation of the pretreatment process and variables, plus simultaneous saccharification and fermentation (SSF) studies of the reaction product, to yield ethanol, and additional enzymatic hydrolysis studies on the slurry, is discussed in Section 1.

Chemical analytical methods used, with modifications developed and statistical evaluations, are detailed in Section 2.

Servicing of the reactor is reported in Section 3.

The series of seven pretreatment reactions is detailed in Section 4, with material balance data as well as feedstock and product (hydrolyzate and slurry) composition analyses and calculated yields.

Section 5 contains the biological studies on the SSF generation of ethanol from the product slurry, as well as independent enzymatic hydrolysis studies carried out at 38 °C and 50 °C.

Simultaneous saccharification and fermentation (SSF) treatments were applied to the slurry product, to form ethanol in high yields. Enzymatic hydrolysis studies at 38 °C and 50 °C were also applied to the product slurry, to investigate possible optimization and interference factors in the enzymatic process.

The pretreated residues produced under the best conditions used (Reaction No 2) provided a substrate for SSF which gave maximum yields of ethanol from 91% to 92% of the theoretical yield based on the glucan content of the substrate. Yields at 96 hours were 88% to 89%.

The relatively high rates of conversion of cellulose to ethanol in SSF reactions is attributed to the small particle size produced by the mechanical action of the disintegrator and the production of a large surface available for enzymatic hydrolysis of the cellulose. The high yields and rates of ethanol production in SSF indicate that there is no special inhibitory effect in the use of dilute nitric acid as the catalyst in hydrolysis.

PREFACE

This study was directed to the hydrolysis of a Hybrid Poplar feedstock using dilute nitric acid in the pretreatment stage to remove hemicelluloses. Then cellulose, present in the pretreated residue from the first stage, was converted to ethanol by simultaneous saccharification-fermentation (SSF) or to glucose by enzymatic hydrolysis. Adaptation of the continuous flow hydrolysis reactor to the specific feedstock, preparation of prehydrolysis residues under steady-state reaction conditions and the evaluation of these as substrates in SSF and enzymatic hydrolysis have been carried out.

This study is an extension of work that has been carried out over the last two decades at the University of California Forest Products Laboratory on the production of ethanol from biomass.

In the previous work a continuous-flow hydrolysis reactor serving as a prototype that can be scaled to commercial size was developed and has been used to evaluate numerous feedstocks. One of the principal objectives of this work was to evaluate the technical feasibility of producing ethanol by SSF using the pretreatment residue from Hybrid Poplar. Based on the data obtained, the technical and economic feasibilities of using an enzymatic based hydrolysis or, the alternative, a dilute nitric acid hydrolysis process for converting cellulose in the pretreated residue from the first stage can be assessed.

The support of NREL in providing financial support in this work is gratefully acknowledged and appreciated. Also the technical consultations with D. Hsu and T. Ehrman and the administrative expertise and guidance of M. Yancy and D. Koepping have been enjoyable and professional and have greatly facilitated the work.

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The professionalism, enthusiastic participation, electronic expertise, and contribution of time by C. Herrmann are gratefully acknowledged and appreciated and have been vital for maintaining and keeping equipment operating at all times of the day, and in preparing this report.

Finally, I wish to acknowledge the professionalism, wisdom, patience, and unflagging energy and dedication that M. Merriman has provided to this project and to the overall program at UCFPL.

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Introduction

Over the past two decades, a process for the hydrolysis of lignocellulosic materials (LCM) to provide sugars and an insoluble "ligneous" residue as intermediate products has been developed at the University of California Forest Products Laboratory. The sugars are converted to high energy chemicals, especially ethanol, by appropriate fermentations. The insoluble residue is utilized as a fuel to produce power and process heat and other products such as resins or soil amendments. A staged process has been developed in which dilute nitric acid is used in pretreatment of the LCM (i.e., the pretreatment stage, stage 1 or S1) to remove hemicelluloses and reduce the remaining lignin-cellulose complex to a fine powder. The cellulose present in this fine powder can then be hydrolyzed by appropriate agents, either enzymes or hydrogen ion, to ethanol or glucose. The objective of the program has been to maximize economic returns developing products enjoying large markets in order to initiate commercial use at an early date. Then, as the technology is developed and the economic conditions become favorable, the "all ethanol" plant can become a reality and maximum quantities of ethanol can be produced (Brink 1989). The technology to realize the potential of an "all ethanol" plant is moving forward, with the potential of utilizing pentoses as well as hexoses in the production of ethanol nearing reality (Brink 1989).

We have named the process developed: hydrolysis-fermentation-combustion (HFC). Continuous Flow, demonstration scale, Hydrolysis Reactors (CFHR) have been designed, constructed and extensively used with a number of feedstocks. Hemicelluloses are converted to high yields of sugars and acetic acid in the first stage or pretreatment reactor. Cellulose is converted to glucose and its dehydration and degradation products in subsequent hydrolysis stages under more severe conditions of temperature. Although the preponderance of work on this cellulose conversion has been limited to a second stage (S2) of hydrolysis, a third stage (S3) was investigated in order to limit the degradation of glucose and increase glucose yields. One of the objectives of the work carried out under this subcontract has been to evaluate the efficacy of Simultaneous Saccharification and Fermentation (SSF) using the prehydrolyzed solids as a feedstock.

In past work evaluation of fermentability of sugars in the hydrolyzates produced has been carried out using *Saccharomyces cerevisiae* to produce ethanol and *Candida utilis* to produce Torula Yeast.

It has been anticipated that the insoluble residue and unutilized soluble products in the hydrolyzate will be used primarily as a fuel for the process. However, products may be developed from the residues which, in the introduction of this industry in the United States, could improve process economics and provide a source of materials from biomass (e.g., resins, activated carbons, soil amendments) rather than from fossil fuels.

A unique feature of the HFC process is that dilute nitric acid is used as the catalytic agent rather than the more commonly used sulfuric acid. We have found that nitric acid has a number of advantages over sulfuric acid. These include:

- * Effective catalyst at low concentrations; 0.25-0.35%.
- * Corrosion problems appear to be negligible when using 316 stainless steel; whereas exotic metals must be used with sulfuric acid to avoid corrosion.
- * The avoidance of producing insoluble calcium sulfate when lime or calcium hydroxide is used to neutralize hydrolyzates; thus eliminating a serious environmental problem.
- * Dilute nitric acid solutions are much less hazardous to plant personnel. Sulfuric acid solutions become more concentrated as water evaporates resulting in attacks on personnel and clothing; the very dilute nitric acid being used decomposes or evaporates without attack on personnel or clothing.

STATEMENT OF WORK

The work addressed in this project involved the use of the first stage demonstration scale hydrolysis reactor (CFHR) to maximize the yield of ethanol attained from the pretreated lignocellulosic residue by subsequent SSF and, simultaneously, minimize the formation of sugar degradation products in the pretreatment stage. The principal goals were, to the extent possible, to optimize conditions of dilute nitric acid pretreatment in order to effect maximum yields ethanol by SSF and of xylose with minimum degradation to furfural.

The following objectives were addressed:

- A. Demonstrate that the cellulose in the pretreated residual solids (cellulose + lignin) were converted to maximum yields of ethanol by SSF.
- B. Establish best hydrolysis conditions to achieve maximum conversion of xylan to xylose with minimum furfural production.
- C. Define material balance over pretreatment steady-state periods.
- D. Define energy balance of the pretreatment stage.

1

Experimental Plan

The objective of this program was to use dilute nitric acid, as developed in the HFC process, to hydrolyze hemicelluloses of wood. Results are to be compared with those reported from the use of dilute sulfuric acid. Conditions were sought to provide maximum conversion of cellulose in the pretreated residue to alcohol by SSF and to provide maximum xylan conversion with minimal degradation to furfural.

The experimental plan was prepared to meet these objectives and was submitted May 8, 1993.

The UCFPL hydrolysis reactor was planned to be run in a series of day-long continuous-duty runs, with several steady-state periods at several different chosen operating conditions in each run. Detailed operating conditions were collected for later analysis, and hydrolyzate and solids were collected for each steady-state period, for chemical analysis.

In operation the wood chip feedstock is delivered to a lockhopper via a screwfeeder. The chips are steamed in the lockhopper to remove air and are transferred to the top of the pretreatment reactor. Preheated acid feed is introduced to the upper end of the reactor and is also used to flush the lockhopper. The slurry of chips plus acid flows by gravity through the reactor while temperature is maintained by indirect steam heating.

At the bottom of the reactor the contents are removed through an in-line disintegrator which provides a slurry of finely divided particles of lignocellulose in the hydrolysate. This slurry is pumped through a cooling heat exchanger, a pressurized surge chamber, and finally discharged to weighed collection tanks. The slurry, collected over a steady-state interval of approximately two hours, is weighed, thoroughly mixed and a sample is taken for chemical analysis. The collected slurry is centrifuged, the solids are washed and the centrifugate plus wash and washed solids are weighed. Samples of each are taken for chemical analysis. All data points being sensed are scanned per minute and are recorded as hard copy and downloaded to the hard disc of a computer.

Data collected over steady-state intervals includes:

- *Reaction conditions; temperature, pressure, mass flow rate, weight of acid and wood feeds, weight of product slurry, disintegrator speed and power consumption, and liquid levels.

- *Chemical composition of all inputs and outputs over selected steady-state reaction intervals.

- *pH of dilute acid feed and product slurry.

- *Particle size distribution of residual solids from pretreatment.

Collected solids were stored and later treated by the SSF process, to obtain alcohol yields from each steady-state condition. Hydrolyzate liquids, containing substantial amounts of pentoses, were stored for separate fermentation by appropriate organisms, to obtain the ethanol yield from this stream.

Pretreatment Process Variables

Critical reaction parameters include:

Temperature, °C	160	170
Retention time, min.	30-60	15-30
Nitric acid concentration, %	0.2-0.3	0.2-0.3

Hemicellulose Hydrolyzate Treatments

The hydrolyzate solutions from each steady-state period, containing substantial amounts of pentoses suitable for fermentation to ethanol, were flash-evaporated to similar xylose concentrations, so that the fermentation results could be compared. This flash evaporation also served to remove some of the volatile constituents other than water (e. g., furfural). The solution was then neutralized to pH 4-5, using calcium carbonate, before preparation for fermentation tests.

2 Quality Assurance - Quality Control: QA/QC

The written procedures on quality assurance - quality control also were submitted May 8, 1993. The steps that were undertaken in meeting NREL specifications in this task were carried out through June, 1994. These steps are outlined with variances approved by NREL.

2.1 Chemical Analysis and Testing Standard Procedures

This section discusses the analytical procedures used in the course of this study. Where variances from NREL protocols were employed the reasons are given and the specific steps are identified using NREL protocol numbering.

2.1.1 *Determination of Total Solids/Moisture in Biomass. CATSP No. 001, Rev. #1, 8/19/92*

Moisture determination was done as in the NREL method, with the exception that weighing bottles were used. This was to avoid moisture pickup during weighing, as detected in some initial samples. The specific steps are as follows:

Sample to be analyzed were initially brought to a reference state by exposure to constant humidity in a 23 °C, 49% RH chamber.

Glass weighing bottles and caps were dried in a laboratory oven at 105 °C. While still in the oven the caps were placed on the bottles and the closed bottles transferred to a desiccator for cooling. No desiccant was used in the desiccator and the port to the atmosphere was closed. The bottles were allowed to cool to room temperature. This takes 20-30 minutes, and this same cooling period was used each time before weighing. Each cap was momentarily removed to relieve vacuum and then replaced. The cap and bottle tare weights were recorded in a laboratory notebook and the specimens were placed in the bottles. The cap was put in place and the gross weight recorded.

The bottles were then placed in a 105 °C oven, with the cap removed but resting at right angles on the lips of the bottles, to allow unrestricted loss of moisture. After the appropriate drying time - from a few hours to overnight depending on the original moisture content - the cap was put in place on the bottle and the dried sample was removed from the oven and placed in a desiccator for cooling as before. The same procedure was followed in weighing the sample as in determining tare weight.

All handling was done with tongs or dry tissues to avoid contamination from fingers. If there was any chance that the sample was not completely dry, it was returned to the oven and dried to constant weight.

2.1.2 *Two Stage Sulfuric Acid Hydrolysis for Determination of Carbohydrates. CATSP No. 002, Rev. #1, 8/19/92*

A few changes from the NREL protocol were employed.

1. Sample. The sample to be analyzed was conditioned at 23.0 ± 1.0 °C and 50.0 ± 2.0 % relative humidity in a paper testing room. The moisture content of one portion of the sample was determined. An amount of the sample to be analyzed calculated to contain 0.3 grams of oven-dried (OD) biomass was weighed to 0.1 mg accuracy and transferred to a 200 ml screw cap centrifuge bottle (Pyrex No. 1261). 3.0 ml of iced, 72% w/w sulfuric acid was added.

2. Sugar standards run in parallel with the samples were dried over P_2O_5 . The purity of the sugars were determined by HPLC analyses and found to be 99.4% - 99.7%.

3. After 30 °C hydrolysis and dilution with 84.00 g of water, the centrifuge tubes were capped, tightened, and weighed (to 10 mg accuracy). After autoclaving and cooling, the tubes were again weighed, to determine any water loss that may have occurred.

4. Approximately 50 ml. of supernatant from each tube was taken and stored in 100 ml prescription bottles.

5. From each (samples and standards), 10 ml of solution was transferred to a beaker and 2 ml of an i-erythritol internal reference (0.2 %) was added, and mixed. These solutions were then neutralized with powdered calcium carbonate to about pH 6. The solutions were centrifuged for 10 minutes (3500 RPM), the supernatant removed and passed through a mixed-bed ion-exchanger (0.2 ml of Biorad AG 50W-X8, 100-200 mesh in the H⁺ form, covered by 0.4 ml of Dowex 1X-8, 200-400 mesh in the carbonate form). The resin bed was washed after elution with 0.7 ml of water and the combined collected solutions mixed and saved for HPLC analysis.

6. HPLC conditions were: BioRad HPX-87P column at 85 °C with a Brownlee PPP-GU guard column, eluant water passed through a 0.45 micron filter, 0.6 ml/min flowrate. The sample size was 20 µl and a Knauer model 9800 refractive index detector was used. Peak areas and retention times were obtained for each sugar and the erythritol reference, using a Shimadzu C-R3A integrator. Untreated sugar standards were prepared over the concentration ranges required for the analyses, mixed with the erythritol reference, as above, and response factors for each sugar with respect to the internal reference were calculated and averaged. Typical recoveries of sugars processed by the acid hydrolysis procedure, as fractions of the corresponding analyses of untreated sugar standards, were:

Glucose	98.3%
Xylose	82.1%
Galactose	82.9%
Arabinose	84.6%
Mannose	95.4%

Similarly prepared correction factors have been used for feedstock and pretreatment solids analyses in this report. However from material balance studies, we believe that degradation of cellulose and hemicellulose during the analytical hydrolysis is not as great as the degradation of free sugars, thus the true recoveries are higher (and the correction factors lower) for wood determinations.

2.1.2.1 Acids by HPLC

Acids present in the hydrolysate were determined by HPLC, using a BioRad HPX-87H column, a Brownlee PPH-GU guard column, and eluted with 0.01 M sulfuric acid at a 0.6 ml/min flow rate.

2.1.2.2 Acid Insoluble Lignin

Solids remaining from centrifugation in carbohydrate determination (Step 4 above) but before calcium carbonate addition were filtered, washed, dried and weighed as Acid Insoluble Lignin. A Standard Deviation of 1.1% of the values found resulted.

2.1.2.3 *Acid Soluble Lignin*

The NREL procedure was used with the exception that 4% (w/w) sulfuric acid was used as blank and as diluent. For a typical dilution factor of 10, 2.7 ml of 4% sulfuric acid was added to a 1.0 cm spectrophotometer cell and balanced against a reference cell containing the same solution; then 0.3 ml of the sample was added and mixed and absorbance determined at 205 nm. A standard deviation of 4% of the values found resulted.

2.1.3 ***Determination of Klason Lignin in Biomass. CATSP No. 003, Rev. #1, 8/19/92***

The procedure given in section 2.1.2.2 above, for Acid Insoluble Lignin, was used in preference to the NREL procedure. This avoids the necessity of a second preparation and hydrolysis, and insures that results for Klason Lignin are fully consistent with the carbohydrate determinations of CATSP No. 002.

2.1.4 ***Determination of Acid Soluble Lignin in Biomass. CATSP No. 004, Rev. #1, 8/19/92***

Acid soluble lignin was determined on the filtrate obtained from the hydrolysis procedure of NREL No. 002, rather than NREL No. 003. The procedure is described in section 2.1.2.3 above.

2.1.5 ***Determination of Ash in Biomass. CATSP No. 005, Rev. #1, 8/18/92***

This procedure was followed with the exception that sample moisture content was done using the fired crucibles to be used subsequently for ignition. The crucibles were covered for cooling and weighing. A standard deviation of 0.11% was found from several determinations of poplar feedstock samples.

2.1.6 ***Measurements of Cellulase Activities. CATSP No. 006, Rev. #1, 8/19/92***

Cellulase activities were measured following the NREL procedure. Activities of 81. and 74. FPU/ml were found.

In order to assure accurate and reproducible filter paper specimens used as the substrate in this protocol, the following procedure was followed.

1. Specimens of Whatman No. 1 filter paper were cut from a large sheet to give strips 1.0x6.0 cm.
2. A large number of these were brought to moisture equilibration in a controlled environment room (TAPPI testing conditions), and moisture content was measured on 6 specimens (The average moisture content was 3.97%)
3. This moisture content was used to calculate the weight (52.0 mg) of a specimen equivalent to 50.0 mg oven-dried.
4. Specimens selected for enzyme assay were trimmed to give strips which were close to 52.0 mg. The oven-dried weights of the selected specimens are thought to be 50.0 ± 1.0 mg.

The Cytolase CL cellulase enzyme used throughout this study was first sterile-filtered in a laminar flow hood using 0.2 μ m SUPOR-200 polysulfone 47 mm membrane filters (Gelman) in a pressure filter.

2.1.7 ***Preparation of Dilute-Acid Pretreated Biomass. CATSP No. 007, Rev. #2, 3/17/93***

The UCFPL pretreatment procedure is substantially different from the NREL reference procedure.

Equipment. The principal item is a vertical reactor column, briefly described in the Experimental Plan section and in more detail in the CFHR Operation section (4.2.1). Wood feed to the top of the reactor is by a "VibraScrew" feeder; product slurry is centrifuged to separate hydrolysate from solids.

Chemicals. The hydrolysis acid is nitric acid, added in concentration calculated from the feedstock moisture content to yield the desired concentration in the reactor: in the range 0.25% to 0.50%.

Procedure. The detailed procedures are documented in a protocol document, used as a checklist with each run. QA/QC is controlled as described in Section 2.2, the CFHR reactor.

2.1.8 SSF Experimental Protocols. Lignocellulosic Biomass Hydrolysis and Fermentation. CATSP No. 008, Rev. #4, 6/4/93

2.1.8.1 Hydrolysis of Lignocellulosic Biomass (Enzymatic Hydrolysis at 38 °C)

A few changes to the enzyme hydrolysis procedure of NREL Procedure 008 Rev #4 p. 3 were made.

Step 1. The Cytolase CL enzyme addition was based on the measured enzyme activity of 74 FPU/ml.

Step 3. The biomass was not pressed to remove air, rather, it was mixed by swirling and refrigerated overnight.

Step 5. After biomass was added to the shake flask, only a portion (about 80%) of the DI water was added. The samples were allowed to steep overnight in a refrigerator. This soaking allowed dispersion of chunks of agglomerated biomass by swirling, and avoided probing of the chunks with a rod and the attendant chance of loss of part of the sample. After the overnight soak and dispersion, the rest of the DI water was added and the samples autoclaved.

Step 7. 4 ml samples were taken rather than the Step 10-specified 2 ml samples, to provide more sample for analysis. These samples were put into 5 ml borosilicate glass, screw-capped, centrifuge tubes (Kimble 73785-5), immediately placed into a boiling water bath for 5 1/2 minutes (rather than the specified 5 minutes) and chilled on ice.

Step 10. The samples were taken using 5 ml cutoff tips and placed in centrifuge tubes as stated above, and centrifuged at 3800 RPM, the maximum speed of the IEC clinical centrifuge used, for 10 minutes. The supernatant (approximately 3-3.5 ml) was removed using a 5 ml serological pipet and stored frozen in 4 ml amber glass vials with PTFE-lined screw caps. Analysis for glucose in the supernatant was initially by the Sigma 510A procedure as described in section 2.1.8.4.4, and later by HPLC.

2.1.8.2 Inoculum Preparation

Inoculum preparation was as described in NREL Procedure 008, Rev #4, p. 5 except as follows.

Step 1. 250 ml polycarbonate baffled flasks (Nalgene 4110-0250) with nonabsorbent cotton plugs were used. Antibiotics were not used.

Step 3. Cells were transferred from YPD agar culture plates to first stage inoculum flasks using sterile loops, in place of addition of a thawed stock vial. The YPD agar culture plates were streaked from a *Saccharomyces cerevisiae* D₅A plate supplied by NREL, labelled QA YST.01A.

Step 4. Incubation of second stage flasks was for 23.5 hours and for 18.5 hours for the two series of SSF experiments, instead of 8-12 hours. Glucose values obtained were 0.0 and 0.06 g/l. The protocol calls for a residual glucose level less than 5 g/l but sufficient to insure that the cells remain in the growth phase: this was difficult to control.

2.1.8.3 Simultaneous Saccharification and Fermentation of Biomass

The procedure described in Protocol No. 008, Rev. #4, pages 6-8, was followed with minor variations as noted below.

Step 1. The shake flasks were 250 ml polymethylpentene (Corning) Erlenmeyer flasks. Cytolase CL cellulase enzyme addition was based on the current enzyme assay showing 81 FPU/ml.

Step 3. The biomass was not pressed to remove air. Rather it was mixed by swirling with most of the DI water and then left overnight in a refrigerator. The next day the biomass was dispersed by swirling. This procedure was the same as used for the enzyme hydrolysis experiments.

Step 4. The pH was adjusted to 5.0 ± 0.2 with 1% sodium hydroxide solution. The volume of alkali addition was taken into consideration by holding a few milliliters of DI water addition earlier, and then adding the rest of the water (to 100.0 ml total working volume) after pH adjustment.

Step 8. Sterile technique was followed in all steps except that the CO₂ traps employed were not autoclavable. They were, however, cleaned and exposed to UV light in a laminar flow hood for several hours before use.

Four ml samples were taken. The same procedures described above for recovery of supernatant during 38 °C enzyme hydrolysis were used in SSF. While the samples were always centrifuged and the supernatant removed within one hour of sampling, the supernatants were not sterile-filtered at this time. The supernatant was carefully removed with a 5 ml serological pipet without disturbing the pellet. If any disturbance was noted, the supernatant was returned to the centrifuge tube and the sample was spun down again.

Step 11. Microscope slides were prepared for each fermentation flask and observed at 400x in a light microscope. No signs of any contamination were observed at any time.

The pH's of the 168 hour SSF slurry samples were recorded. The pretreated solids and α -cellulose samples varied between pH 4.43 and pH 4.67. The hybrid poplar feedstock (-40 mesh) which was largely unaffected by the SSF treatment gave pH 4.84 and pH 4.87.

Step 14. To calculate the ethanol yield the ethanol concentration at zero time is subtracted from the ethanol concentration at the end of SSF. We have also corrected for the ethanol which would be produced from the glucose present at zero time. This glucose present in the zero time samples was found to be relatively large and clearly included that which had been formed by rapid enzyme hydrolysis of the pretreated solids before the sample was analyzed or frozen. To get a better estimate of the glucose truly present at zero time, i.e. glucose added with the enzyme, YP medium and inoculum, these sources were analyzed for glucose directly

by using the Sigma 510A method. The corrections applied for the two series of SSF experiments are listed below. The concentrations are those present in the shake flasks.

Source	SSF Series	
	1	2
	glucose, g/l	
Inoculum	0	0.006
10x YP medium	0.0112	0.0112
Enzyme solution	0.0221	0.0221
Total glucose	0.0333	0.0393
Ethanol equivalent, g/l	0.017	0.0201
Ethanol in inoculum, g/l	2.09	2.15
Zero time ethanol, g/l	2.11	2.17

The ethanol in the inoculum is the average of all flasks used in a series, as determined by HPLC.

2.1.8.4 Sample Analysis

2.1.8.4.1 HPLC

Comparison was also made between SSF results for glucose between the Sigma method 510A, and the HPLC method. Values from both methods are reported in Tables 5.5 to 5.13, Products from SSF. It was found that HPLC values for glucose concentrations below 1 g/l are seriously in error, compared to the more sensitive Sigma 510A method.

2.1.8.4.2 Gas Chromatography

The gas chromatograph used was a Varian Series 200 with flame ionization detector and a freshly packed, 6 foot 1/8 inch Poropak Q column at 175 °C. A Varian model 477 integrator was used to provide peak integral values. Two ethanol standard preparations were evaluated: one standard set was prepared from Sigma E-2385 (Lot 13H8275) Ethanol Standard, to appropriate dilutions as specified in NREL CATSP 008. A second standard was prepared from absolute ethanol, to the appropriate dilutions. Spreadsheet calculations and plots showed a linear response over the range of up to 25 g/l with a very slight zero-point error, probably due to integrator baseline error.

Sample dilutions were made using larger than the NREL-recommended quantities: 0.250 ml of unknown or standard was mixed with 9*0.250 ml of isopropanol internal reference (1 g/l). Injections into the GC were of 2, 3, 4, or 5 microliters, using a Hamilton 10 µl syringe. Satisfactory agreement with injector volume was found.

Determinations of ethanol by gas chromatography (standardized from Sigma E-2385 ethanol standard ampoules), in comparison with those found in the HPLC runs, are consistently slightly lower, as seen in the Tables 5.5 to 5.13, Products from SSF. The HPLC values are in close agreement with values found with the YSI analyzer (using the ethanol membrane and procedure), so the HPLC values have been used.

2.1.8.4.3 YSI Glucose Analyzer

The YSI analyzer was not available at the time the fermentation and enzyme hydrolysis reactions were done, however a comparison of the Sigma 510 and the HPLC values with the YSI results was done after receipt of the YSI analyzer. Enzyme hydrolysis samples were reanalyzed on the YSI analyzer. Almost all of the 38 °C YSI values were very close to those by HPLC. The slightly lower values by YSI may be a result of the 3 month period between the original HPLC analyses and the reanalysis. All analytical values are presented in Tables 45, 46, 47, 48, and 49.

Glucose standards were prepared with concentrations of 1.0 g/l, 2.5 g/l, 10.0 g/l, and 25.0 g/l. Sample sizes for injection in the YSI procedure of 10 µl, 20 µl, 30 µl, 40 µl, 50 µl, and 60 µl were used (except: only 10 ml for the 25 g/l solution) to test for optimum sample size to be used in analyses. Coefficients of variation (CV's) were obtained from 0.5% to 2.79%. The interpretation was that for glucose concentrations up to 10 g/l sample sizes of 30 to 40 µl are to be used, and for higher concentrations sample sizes of 10 to 20 µl are to be used.

YSI Ethanol Analysis

The YSI analyzer was also used for ethanol determination on a limited number of samples from SSF. Results are shown in Table 36 for reaction 2-1. The YSI results essentially duplicate those by HPLC.

2.1.8.4.4 Glucose by the Sigma 510A Method

A sensitive reagent method, Sigma method 510A, was used for glucose determinations in the absence of an available YSI analyzer recommended by the NREL procedures. The method is specific for glucose and involves quantitative formation of hydrogen peroxide from glucose, in the presence of a glucose oxidase reagent, followed by peroxide oxidation of an o-dianisidine reagent in the presence of peroxidase, to give a brown color measured spectrophotometrically at 450 nm.

The protocol used was that of the Sigma Chemical Company, Procedure 510A, as revised July 1990. It was used for SSF and for 38 °C and 50 °C enzyme hydrolysis analyses. To be applicable for nominal glucose concentrations of 0 to 3 g/l it employs a 20-fold dilution step for both samples and standards. The actual concentration being analyzed is 0.0005 to 0.15 g/l.

Glucose standard solutions (purchased from Sigma Chemical) were:

- 1 g/l Sigma No. 635-100
- 10 g/l Sigma No. 14-11

From these standards other working standards were prepared by dilution to 0.1, 0.2, 0.3, 0.4, 2.0 and 3.0 g/l. Each time hydrolysis or fermentation samples were analyzed a set of at least 3 glucose standards were also run, using concentrations similar to concentrations of the samples. A minimum detectable nominal glucose concentration was found to be approximately 0.01 g/l. This extreme sensitivity was found to be most useful with SSF samples where the glucose concentration at any given time was very low, owing to the rapid conversion of glucose to ethanol. For analysis of enzyme hydrolyzates the glucose concentrations rapidly exceeded the linear range and the samples therefore required up to 80-fold dilution rather than the standard 20-fold. It was later found that HPLC analysis of glucose gave more reliable results at higher concentrations of glucose (> 2 g/l) than the Sigma 510A method. This may occur because of the large dilution factors that need to be applied to the original analytical value and the possibility of exceeding the linear absorbance range of 0 to 0.8 AU.

In this work the Sigma 510A values were used only for SSF analyses where glucose concentrations were generally less than 1.0 g/l. HPLC values were used for enzyme hydrolyzate samples. Analytical values for both methods are given in tables in the appropriate sections of this text.

Linear regression applied to Sigma 510A analyses of glucose standards run in conjunction with a series of successive determinations on SSF samples gave the results shown in the table below (I = intercept, S = slope, r = correlation coefficient):

S	I	R
0.2258	0.0072	0.9991
0.2625	-0.0105	0.9989
0.24	0.0025	0.9972
0.2217	0.0018	0.9997
0.253	0.0017	0.9996
0.235	-0.003	0.9997
0.2444	-0.0044	0.9997
0.2439	-0.0021	0.9997
0.2336	-0.0054	0.9995
0.2361	-0.0024	0.9995
0.2363	-0.0009	0.9935
0.2448	-0.0046	0.9995

Glucose in the sample is determined from the relationship:

$$A_{450} = (\text{Glucose g/l}) * S + I,$$

rearranged to:

$$\text{Glucose g/l} = (A_{450} - I)/S$$

Since standardizations of the Sigma 510A method were done with each set of analyses, the history of the glucose calibration values can be used as a trend line to assure stability of the enzyme-based preparations. This has been done in Figure 1, giving the glucose calibration values found, vs. analysis set, over a period of months. It is seen that some learning occurred, in that the later calibrations differ less from the average than the earlier, and that some slight calibration differences are seen in the runs, but there is no long-term drop in sensitivity of the method or preparations.

2.1.8.5 Dry Cell Weight Determination

Dry cell weights were determined by drying at 80 °C and weighing as in NREL procedure No. 008, Rev. #4, p. 12. For rapid determination of inoculum cell mass the weights were correlated with spectrophotometric absorption at 600 nm on a diluted sample. For *Saccharomyces cerevisiae* (D_5A), this correlation is shown in Figure 2. To determine the dry cell mass in the inoculum, the indicated cell mass is multiplied by the dilution factor used to bring the absorbance reading within the range of 0.10 - 0.50 AU. Typically, a dilution factor of 50 was required. Absorbance was measured against a reference cell containing DI water.

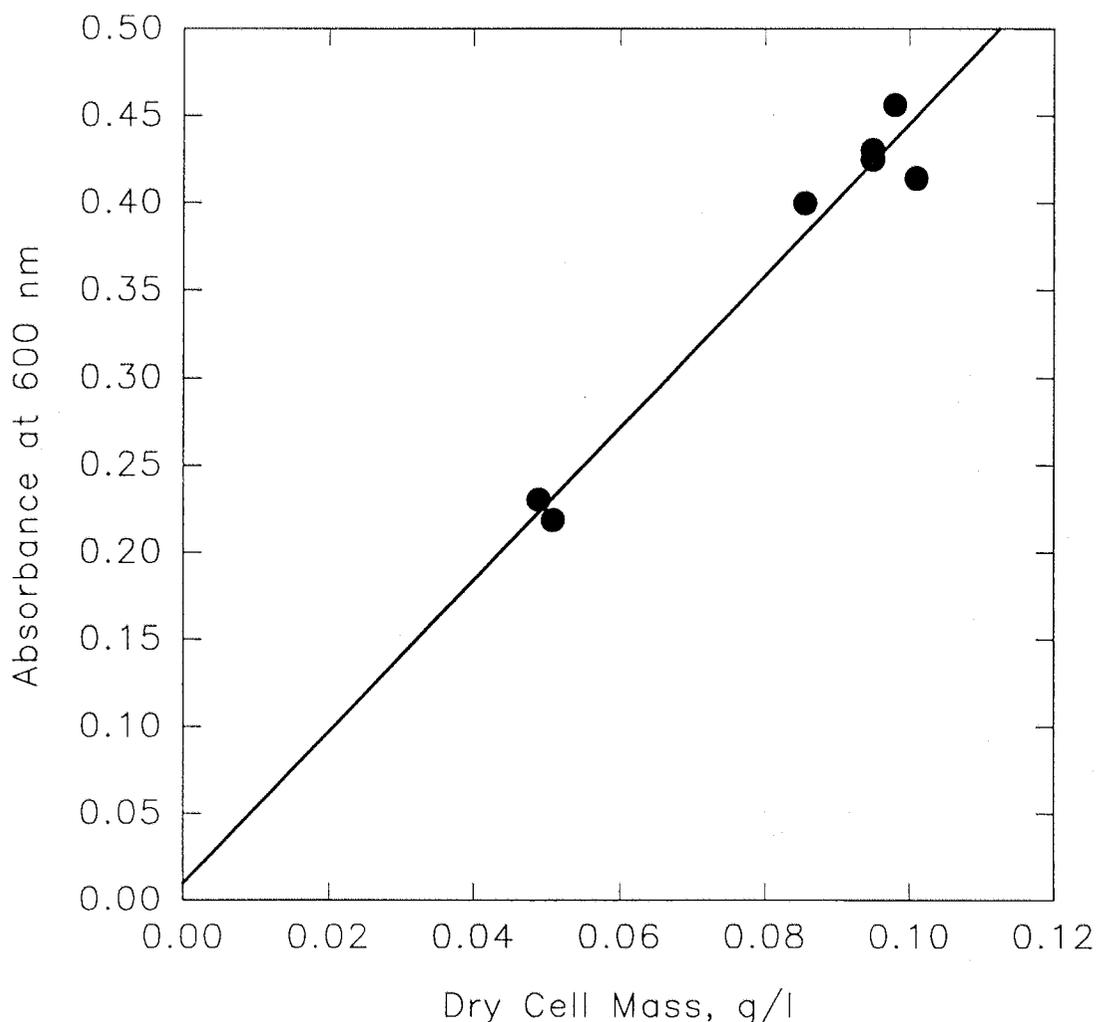


Figure 2. Dry Cell Mass Calibration, *Saccharomyces cerevisiae* (D_5A).

2.1.8.6 Culture Preservation

We were unable to use the -70 °C long-term storage procedure suggested. Instead we periodically prepared fresh YPD agar culture plates by streaking *S. cerevisiae* D_5A cells from previous plates.

2.1.9 Enzymatic Saccharification of Lignocellulosic Biomass. CATSP No. 009, Rev. #1, 8/11/93

A few changes to the 50 °C enzymatic hydrolysis procedure of NREL Procedure No. 009 were introduced.

Step 5.2. All samples were weighed to contain exactly 0.1000 g. of glucan. These included wet pretreated solids, air dried hybrid poplar feedstock (-40 mesh), and dry α -cellulose. The samples were added to glass culture tubes 20x125 mm (Kimble 455066-20125) with white-rubber-lined screw caps rather than 20 ml. scintillation vials.

Step 5.5. After the citrate buffer, tetracycline and cycloheximide were introduced, all of the required water except for 1.000 ml, was added to each culture tube and the biomass was allowed to soak overnight in a refrigerator. The next day the biomass was dispersed by swirling the tube contents, the tube was heated to 50 °C, the enzyme was added, and the wall of the tube was washed with the reserved 1.000 ml of water.

Step 5.7. The culture tubes were placed in a tube rack, which was held by an adjustable support mounted in the incubator shaker. Preliminary tests showed that good agitation was achieved when the tube rack was held at an angle of 19° to the shaker table and the orbital speed was 120 RPM. Under these conditions the contents did not wet the tube cap or all of the tube wall. This setup was used as an alternative to the "Roto-Torque" rotor specified by NREL.

Step 5.8. Larger samples were taken than the 0.3 to 0.5 ml specified. At zero time 1.000 ml slurry samples were taken (using a 1000 μ l micropipet and 1 ml cutoff tip). At other sampling times 0.800 ml samples were taken. These samples were transferred to 5 ml glass centrifuge tubes (Kimble 73785-5) with PTFE-lined screwcaps and immediately placed into a boiling water bath for 5 minutes followed by an icewater bath. This step was adapted from CATSP No. 008, Revision 4, in order to terminate enzyme hydrolysis activity.

The tubes were centrifuged for 10 minutes at 3800 RPM, the maximum speed of the IEC clinical centrifuge used. The supernatants (about 0.5 - 0.6 ml) were removed using Pasteur pipets and a Brinkman pipet helper, and transferred to 4 ml amber vials (with PTFE-over-whiterubber-lined screwcaps). The samples were kept frozen until analyzed, by HPLC and Sigma 510A methods.

The 50°C enzymatic hydrolysis was carried out in two series of reactions, one with 4 biomass samples (in duplicate) and the other with 5 biomass samples. In the first series sampling was at zero time, 24, 48, 72, 96, 144 and 196 hours. In the second series only zero time, 24, 48, 72 and 96 hour samples were taken, since the first series had shown that the glucose plus cellobiose maximum yields occurred before 96 hours.

2.2 Continuous Flow Hydrolysis Reactor (CFHR)

The reactor was cleaned, seals replaced as necessary, and valves serviced.

2.2.1 Instrument Calibration

Calibrations of all measurement sensors are described below. In all cases calibration measurements were taken through the data-collection system to final printout, as well as by notebook recording of panel meters.

2.2.1.1 Temperatures

All thermocouples were removed from the reactor ports but not from their electrical connection, and tested at room temperature and in an oil-bath over a temperature range to cover and exceed the operating temperature range. In a few cases this identified defective thermocouples which were replaced, or poor electrical connections which were cleaned. Linear regressions of the data over the operating temperature range 150 to 170 show R^2 values in the range 0.996 to 0.998.

2.2.1.2 Pressures

Pressure is not a critical parameter, since steam pressure is a function of temperature. The pressure measured at the Milroy pump outlet was calibrated, and adjusted, by use of an Ashcroft Dead Weight Tester, Type 300, and standard weights. Reactor pressure sensors were calibrated at operating temperature from this calibrated meter.

2.2.1.3 Mass Flows

Mass balance calculations required measurements of input flows, the feedstock and the dilute nitric acid, and the output slurry flow.

2.2.1.3.1 Feedstock

Feedstock entry was measured by a balance position-counter, transmitted digitally to the data-collection system.

The Vibra Screw feeder, which is used for feedstock entry to the lockhopper, is mounted on an electric balance. The electrical balance adjusts mechanically, by a reversible motor driving a screw of the balance mechanism. This screw is coupled to a digital encoder, which sends pulses representing addition of 10 grams on one wire, and pulses representing decrease of 10 grams on another, to an interpreting card added to the data collection system. This card, constructed during this project based on a previously used design, contains an up/down counter and a 16 bit D-A converter, whose output is read by the data collection unit. Performance of the data chain was confirmed by calibration using 25 kg standardized weights, and recording the voltage obtained, to generate a calibration factor. During runs, notebook entries of an independent display of the electrical balance measurements were made after each lockhopper feedstock input.

2.2.1.3.2 Dilute Acid

Dilute acid consumption was measured by weight: the weighframe was calibrated as reported below for the slurry-collection weighframes.

2.2.1.3.3 Slurry

Slurry flow was measured by a Micromotion flowmeter. This was in turn calibrated after adjustment, to agree with the Milroy pump settings, and confirmed by weighing timed water collection samples.

Slurry collection weigh-frames were calibrated using standardized 25 kg weights (themselves checked on a reliable balance) over the collection weight range. Weights were measured through the digital data-collection system, so that any discrepancy could be mathematically corrected either in the data-collector or in later calculations.

A slurry intermediate holding-tank (of capacity ca. 5 liters) was equipped with a level sensing device (Robertshaw model 161) : this was calibrated by filling with water and weighing on draining, stepwise.

2.2.1.4 Power

Disintegrator power to the 3 HP motor was recorded as calibrated input to a Parametrics controller, as converted by an Exeltronics Watt Transducer (Model XL34-2K5A2) from Dynametr current and voltage sensors to a 0-1 V DC signal corresponding to 0-10 KW input. No correction for motor or coupling efficiency was attempted.

2.2.2 Reactor Protocol

A 5 page protocol checklist was used with each run, to insure that valves and controls were correctly set and recorded, and that the established startup and shutdown procedures were followed, and recorded. In addition detailed notebook records were maintained at each operating station, by the person responsible for that station. This in part supplements the automatic data-collection system, and in part is redundant confirmation.

2.2.3 Data Collection

Analog (as voltage) and digital data from 9 thermocouples, 3 pressure sensors, 2 flow sensors, 2 liquid-level sensors, 4 weighframes, and the disintegrator speed and power measures, as well as time values and woodfeed signals were digitized in an AutoGraph 800 recording system. This system has a resolution of 14 bits plus sign, and the accuracy is rated at 0.03% of reading plus 0.012 percent of range. It is programmed in a local language, allowing sampling scheduling, mathematical conversions and display formatting. The output was taken in serial ASCII format, and read to a laboratory general-purpose computer by a conventional communications program (PC-VT), stored on the computer's hard-disk and printed on a line printer located at the main reactor control station. An alternative to the communications program was also used at times, to read the data into the computer, plot it graphically, record on the hard-disk and print on the line printer.

Critical data were also recorded in laboratory notebooks, at the main control station, the wood feed station, the acid feed station, the slurry-centrifuge station, and the computer control station.

The partially redundant data were later compared to verify correctness and to allow interpolation of time values less than one minute (the AutoGraph sampling period).

3

Maintenance and Repair of the CFHR

This essential task involved reconfiguration of the S1 reactor, repair of certain reactor components, and maintenance of this and other equipment to be used. In the study preceding this one the CFHR was configured to introduce a slurry of straw. As a first step in this study the CFHR had to be reconfigured to introduce particulate wood (feedstock) through the lockhopper. This lockhopper had been designed and extensively used in previous work. The configuration used in this work is shown schematically in Figure 1. This reconfiguration and additional maintenance required for the CFHR included:

1. Repair of the 4-inch ball valve on the lockhopper.
2. Replacement of the Moyno pump stator.
3. Replacement of the data logger with a computer system for receiving, displaying, providing hard copies of data in real time, and storage of data.

Each part of the CFHR was given thorough attention in an effort to place all equipment items into good operating condition after having not been used for some time. This work proved to be more extensive than anticipated and substantially exceeded the time and budget that had been allocated for this purpose.

All devices used for measurement were carefully calibrated.

4

Pretreatment Reactions

Seven steady-state reactions were carried out for which complete data has been developed. These are described in this section.

4.1 Feedstock

NREL selected and provided a hybrid Poplar feedstock for this project.

4.1.1 Procurement

The feedstock had been chipped (with bark), and then screened to provide -2 +6 mesh accepts. The accepted fraction was placed in plastic bags that, in turn, were placed in 55 gallon fiberboard drums. These drums were shipped by truck to UCFPL.

4.1.2 Preparation and Storage

Upon receiving, the contents in each fiberboard drum were processed using an 18-inch Sweco oscillating screen to remove, on the average, 8.3% of +2 mesh material. This was done only to assure uniform delivery of chips from the Vibra Screw feeder used to introduce this feedstock into the pretreatment reactor. Results obtained in screening the 50 drums are shown in Table 1. After screening an average of 95 pounds of screened chips at an average moisture content of about 58% were returned to plastic bags which were tightly closed. Each bag was placed in a drum. These drums were then placed in frozen storage. Each time the supply in the laboratory had to be replenished, four to eight drums were withdrawn from frozen storage about two weeks before needed. After thawing the drums were placed in cold storage at 4°C until used in shakedown runs or in pretreatment reactions.

At the time this material was prepared for storage, a composite sample of the feedstock was prepared by taking approximately 250 g. of accepts from each of 20 of the 50 drums that had been prepared. This gross sample was then air dried to 10 % moisture, thoroughly mixed, and approximately 200 g. were comminuted in a Wiley mill fitted with a screen to produce -40 mesh material. Oversized material was reprocessed until all of the sample taken for comminution had been reduced to -40 mesh. This material was air dried in a constant humidity room at 50 ± 2 % humidity and 72 ± 2 degrees F. This air dried sample was then placed in an amber colored glass bottle with a tight fitting cover, thoroughly mixed, and retained as the representative analytical sample in the humidity room, to be used throughout this project.

4.1.3 Particle Size Distribution

Several drums were selected for initial screening and moisture content determination. Results of the screening and analysis is presented in Table 1.

4.1.4 Chemical Characterization

The chemical composition of the Hybrid Poplar was determined. Duplicate 0.3 g samples of the stock analytical sample of feedstock, prepared as described in section 4.1.2, were weighed out to 0.1 mg and hydrolyzed using the Two Stage Sulfuric Acid Hydrolysis for Determination of Carbohydrates, CATSP No. 002, with modifications approved by NREL. This protocol is discussed in Section 2.1.2. Simultaneously, duplicate samples were weighed out for moisture content determination.

Table 1. Feedstock Drum Analysis: Particle Size, Accepts/Rejects, Moisture Content.

Drum	Weight (pounds)	- 10 mesh	+ 2 mesh	Rejects	Accepts	Accepts Moisture	Rejects Moisture
1	100.9	1.5	10.5	12.0	88.9	55.4	55.3
2	100.7			12.7	88.0	57.1	
3	100.7			5.6	95.1		
4	100.8			2.2	98.6		
5	106.7			16.2	90.5		
6	101.6			10.0	91.6		
7	104.9	0.9	4.6	5.4	99.5	58.8	57.5
8	99.6			11.0	88.6		
9	101.6			10.0	91.6		
10	99.1			7.0	92.1		
11	103.1			1.0	102.1		
12	97.6	0.1	2.0	2.1	95.6		
13	95.6	0.1	1.5	1.6	94.1	58.5	
14	90.6	0.1	6.5	6.6	84.1		
15	97.6	0.1	1.0	1.1	96.6		
16	95.6	0.1	1.5	1.6	94.1		
17	95.6	0.1	1.0	1.1	94.6		
18	97.6	0.1	9.0	9.1	88.6	58.0	
19	102.2	0.1	5.0	5.1	97.1		
20	100.2	0.1	6.0	6.1	94.1		
21	101.2	0.1	1.0	1.1	100.6		
22	100.7	0.1	11.0	11.1	89.6		
23	104.7	0.1	13.0	13.1	91.6		
24	100.6	0.1	11.4	11.5	89.1		
25	101.6	0.1	2.9	3.0	98.6		
26	103.6	0.1	0.9	1.0	102.6		
27	108.1	0.1	14.4	14.5	93.6		
28	99.2	0.1	7.0	7.1	92.1		
29	98.9	0.3	7.0	7.3	91.6		
30	99.9	0.3	2.0	2.3	97.6		
31	100.4	0.3	8.5	8.8	91.6		
32	99.9	0.3	9.5	9.8	90.1		
33	101.4	0.3	0.5	0.8	100.6		
34	99.4	0.3	9.5	9.8	89.6		
35	104.4	0.3	5.5	5.8	98.6		
36	100.9	0.3	1.0	2.3	98.6		
37	99.9	0.3	5.0	5.3	94.6		
38	100.9	0.3	8.5	8.8	92.1		
39	101.6			1.0	100.6		
40	98.6			8.0	90.6		
41	106.1			5.0	101.1		
42	97.6			8.0	89.6		
43	101.1			7.5	93.6		
44	100.6			1.0	99.6		
45	100.6			4.0	96.6		
46	101.6			3.0	98.6		
47	101.6			3.5	98.1		
48	91.1			11.0	80.1		
49	111.6			9.0	102.6		
50	101.6			2.0	99.6		

Duplicate determinations of the monosaccharides present in each of the hydrolyzates prepared were carried out by HPLC. The values of the five monosaccharides present in the feedstock determined in each of the four HPLC runs and the mean value of these analyses are given in Table 2. In addition the correction factors determined according to the protocol noted are given in the table with the corrected percentage of each monosaccharide expressed on the basis of the unextracted feedstock. The method used in results obtained and reported, 04/06/94, was substantially improved (May - September). Accordingly, these earlier results have not been included in the results given here.

Table 2. Feedstock (Hybrid Poplar) Monosaccharides.

Hydrolysis Sample. HPLC No.	1 1 %	1 2 %	2 1 %	2 2 %	Average %	Std. Dev. %	Corr. Factor
Glucose: analysis	43.43	43.41	42.60	42.67	43.03	0.50	
Corrected	47.94	47.91	47.02	47.10	47.49		1.100
Xylose: analysis	13.98	13.06	13.66	12.73	13.36	0.69	
Corrected	17.10	15.97	16.70	15.57	16.34		1.220
Galactose: analysis	1.65	0.94	2.19	1.28	1.51	0.59	
Corrected	1.81	1.03	2.41	1.41	1.67		1.100
Arabinose: analysis	1.71	1.03	2.17	1.59	1.62	0.59	
Corrected	2.13	1.29	2.72	1.99	2.03		1.250
Mannose: analysis	3.20	1.97	2.95	2.86	2.75	0.59	
Corrected	3.54	2.18	3.25	3.16	3.03		1.104

The monosaccharidic composition of the feedstock has been expressed as glycans in Table 3 based upon the corrected percentage of the corresponding monosaccharide given in Table 2. The corrections applied were obtained according to CATSP No. 002. The summation of the total polyglycans, the acid insoluble residue ("Klason lignin"), the acid soluble lignin obtained using an adsorption coefficient of 110, and ash, all determined analytically in this analysis, amounted to over 96% of the unextracted feedstock on an oven dried (O.D.) basis. The values of acetyl and anhydroglucuronic acids previously determined and reported (Brink et al. 1993) comprise distinct constituents of the feedstock which are not accounted for in the other four types of components quantified in Table 3. Accordingly, they are given as independent items in this table. In the analytical procedure a small amount of degradation of sugars to 2-furfural and to 5-hydroxymethylfurfural occurs. Therefore, the analysis for 2-F and 5-HMF has been calculated in terms of additional sugar content present in the wood but not found in the hydrolysate.

Another independent class of constituents, which are independent of the components comprising cell wall constituents, is the extremely diversified but always present fraction of the feedstock known as extractives or extraneous materials. These have not been determined but reference is made to the extractives content found in hybrid poplar wood and bark by Blankenhorn et al. and summarized in the reference given by

Brink et al., (1993). Specifically the extractives content of wood, bark, and wood/bark (i.e., whole tree material) ranged from 5.0%-6.7%, 22.7%-31.9%, and 8.0%-10.2%, respectively.

The low value for the glucan and high value for acid insoluble residue, found for this poplar, may be attributed to the fact that whole tree chipping was carried out in the preparation of this feedstock; specifically, the inclusion of bark will produce the trends noted. These trends can be exacerbated by juvenile growth and by the characteristics of a particular clone or hybrid that is involved (Blankenhorn et al. 1985).

The explanation of these results is almost certainly the use of whole tree chips, i. e., the feedstock contains both wood and bark. In addition, the short rotation fast growing hybrid poplars grown for biomass utilization processes represent juvenile wood which is known to differ both chemically and in fiber properties from mature wood. Blankenhorn et al. (1985) have compared chemical content values of ash, lignin, extractives, holocellulose and alpha cellulose for several poplar clones over tissue ages of 1 to 8 years old. Wood, bark, and wood plus bark fractions were evaluated. They concluded that significant differences existed within and among clones. However, the factors affecting chemical composition were principally the tissue class, i. e., wood, bark, or wood/bark. The age of the tissue was second and the parentage effects were of less importance compared with wood analyses of the hybrid poplar furnished to UCFPL.

Range of values for 7 poplar clones; 8 year old specimens

	Wood	Bark	Wood/bark	UCFPL results
Extractives, %	5.0- 6.7	22.7-31.9	8.0-10.2	8.0
α-cellulose, %	41.8-44.8	21.5-35.1	31.9-41.1	55.7
Lignin, %	17.0-21.9	28.0-38.8	18.4-29.3	28.3
Ash, %	0.8- 1.3	6.0- 7.5	1.5- 2.0	2.1

It appears that our values for extractives, acid insoluble lignin, and ash are consistent with those of Blankenhorn et al. for wood/bark mixtures.

Thus, a range within which an analytical value for this class of components would be expected to fall, given in Table 3 for purposes of discussion, is most conservative. The summation of all the components for which an analytical value has been obtained is 102.3%, unextracted, O.D. feedstock basis. It is predicted that this value will be from 3% to 6% higher with the addition of the value for extractives. Accordingly, there are values given for constituents in this table which are too high. It is not appropriate to correct the percentages of all components to 100% by deducting an amount from each component proportional to the amount of that component in the analysis given. It is our opinion that the correction factors applied to each sugar given in this analysis are excessive.

Table 3. Hybrid Poplar Feedstock Composition (corrected).

As Monosaccharides	Mean % ¹	As Polyglycan	% ¹
Hexose		Hexosan	
Glucose	47.49	Glucan	42.74
Mannose	3.03	Mannan	2.73
Galactose	1.67	Galactan	1.50
Subtotal	52.2	Subtotal	47.0
Pentose		Pentosan	
Xylose	16.34	Xylan	14.38
Arabinose	2.03	Arabinan	1.79
Subtotal	18.37	Subtotal	16.17
Total Sugars	70.6	Total Polyglycan	63.2
Lignin			
Acid Insoluble			30.4
Ash			
Acid Soluble			1.59
Ash			1.16
Subtotal			96.32
Acetyl			3.11
Anhydroglucuronic Acids			2.86
Subtotal			102.29
Extractives			3 to 6
Summation			105-108

¹ Based on OD sample milled to -40 mesh (American Standard Screen).

It is well known that 72% sulfuric acid at 20 to 30°C will initiate dehydration reactions immediately. On the other hand, the oligomers first formed by sulfuric acid at this concentration are not hydrolyzed. To hydrolyze the oligomers the acid is diluted to 4% and hydrolysis is carried out at elevated temperatures for given periods of time depending upon the temperatures used. Thus, the degradation of polysaccharides under the condition of hydrolysis specified in CATSP No., 002 and used in this work is not expected to be as high as the degradation of the monosaccharides subjected to the same conditions. As observed in the correction values the rate of degradation of xylose is higher than that of glucose, the two correction values of greatest significance in this work.

The results obtained using the corrected values in Tables 2 and 3 are given in the following sections. A set of tables comparable to those given in the following section based upon the sugar analysis made without corrections but based on the summative analysis concept is given in the appendix without discussion.

4.2 Dilute Nitric Acid Pretreatment

A protocol was developed for operation of the CFHR in advance of the reactions that were carried out under steady-state conditions. This protocol was similar to that used in previous work. A substantial amount of physical work is required in certain of the operations required routinely through out a steady-state reaction. This requirement would be eliminated in a fully automated plant. The skill of the eight person operating team continued to increase over the three month period that the CFHR was run. The team acquired a fair level of skill by the time the steady-state reactions were carried out in the period from December 27, 1993, to January 20, 1994.

4.2.1 CFHR Operation.

A schematic diagram of the pretreatment (i.e. S1) reactor is presented in Figure 3 showing the configuration that was used in this work.

The feedstock was supplied to the bin of a Vibra Screw feeder as required. This feeder was mounted on a weigh frame (0.01 kg sensitivity). A spiral auger conveys the feedstock from the bin to a funnel mounted on the top of the lockhopper. In operation, the wood chip feedstock was delivered intermittently to the lockhopper via the Vibra Screw feeder during the lockhopper cycle. This segment of the cycle required about 30 seconds in a total of 150 seconds used in Reactions 1 - 4 or 180 seconds used in Reactions 5 - 7.

Upon completion of the feeding segment of the lockhopper cycle the upper valve of the lockhopper was closed and the feedstock charge was purged with low pressure steam (about 20 psig) to remove air. The low pressure, steam purging segment of the cycle was terminated, high pressure steam was introduced and the feedstock charge was transferred to the top of the pretreatment reactor.

Dilute nitric acid introduction was obtained as the cumulative loss of weight of the acid feed tank supplying acid to the pump up stream of the heat exchanger which preheats this feed before introduction into the reactor. Dilute nitric acid, prepared from concentrated nitric acid (70% w/w) in an acid preparation tank, is transferred as needed to the acid feed tank. The acid feed tank is mounted on a weighing frame which weighs to 0.1 lb. The dilute acid is metered using a high pressure piston pump to a tube in jacket heat exchanger. Heat is supplied using high pressure steam. A temperature recorder-controller receives an analog signal from the control thermocouple mounted downstream of the heat exchanger. The temperature recorder-controller controls the air supply (3 -15 psig) operating the air actuated valve providing steam to the heat exchanger. The temperature of the preheated acid leaving the heat exchanger can be manually set to provide a temperature sufficiently above the control temperature to allow for slight heat losses taking place in the piping system supplying the preheated acid to the reactor.

Preheated acid feed was introduced continuously either to the upper end of the reactor when the bottom valve was closed or to flush the lockhopper when the bottom valve was open.

The slurry of chips plus acid flowed as a plug, by gravity, through the pretreatment reactor.

Temperature control was achieved by three independent means.

Gross temperature control was achieved by preheating the acid feed that was introduced into the top of the reactor to the predetermined target temperature of the reaction being carried out.

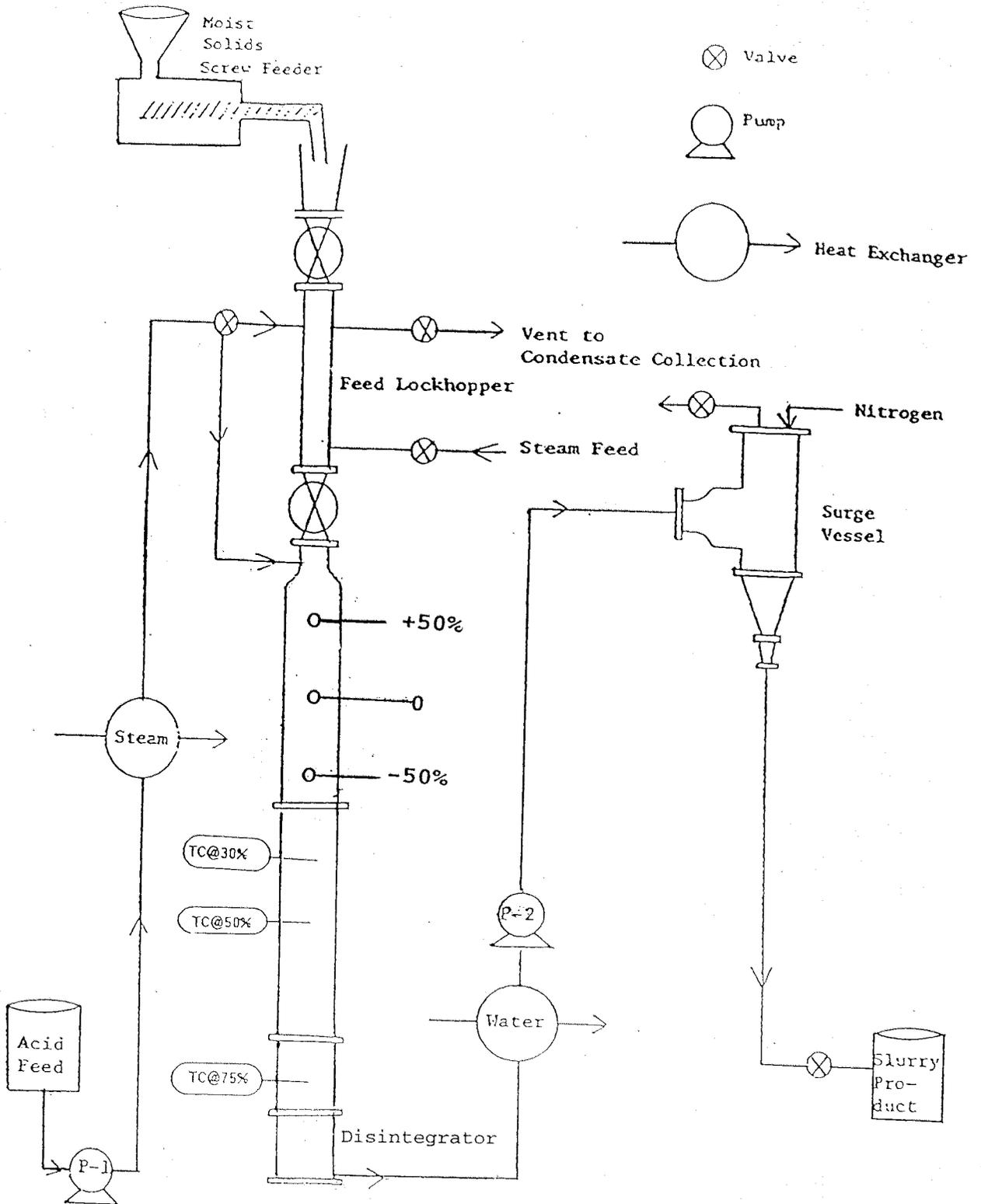


Figure 3. Schematic CFHR used in pretreatment .

The temperature of the steam introduced in the high pressure segment of the lockhopper cycle is used to increase the feedstock charge to the nominal temperature of the reaction being carried out. In normal operation a pressure in excess of the autogenic pressure is required to open the bottom discharge ball valve of the lockhopper so that the feedstock charge can be transferred into the top of the reactor.

The high water content of the hybrid poplar feedstock, 55% to 57%, required a higher steam temperature to provide the heat transfer necessary to heat this feedstock to reaction temperature compared to the feedstocks previously used that had moisture contents of 45% or less. This increment of heat transfer increases as the moisture content of the feedstock is increased. Moreover, the time in the lockhopper cycle in Reaction Nos. 1 through 4, about 30 seconds, was not increased over that used in previous work. Thus, the rate of heat transfer had to be increased.

To achieve the increased heat transfer required for this feedstock, compared to previous feedstocks run, a higher temperature was required in the high pressure steam segment of the lockhopper cycle.

The higher moisture content of the Hybrid Poplar feedstock had further detrimental effects in the lockhopper operation; specifically on, (a) acid penetration of the particulate feedstock and (b) the distribution of nitric acid between that portion entering into the slurry conveyed through the reactor and that portion distilled with the steam purge at the beginning of the lockhopper cycle. These effects are considered later in this discussion. It should be noted that acid penetration could be avoided in this work (partially) as well as in a commercial operation by preimpregnation of the feedstock with the dilute acid and in a commercial operation by use of a different design of the system introducing feedstock to the pretreatment reactor.

Finally, temperature of the slurry in the reactor was trimmed by indirect steam heating (not shown) through steam coils tracing the reactor sections. However, this means of control had a slow response time that, with manual control, allowed the temperature to drift slightly over a period of time before a correction made in steam pressure would have the desired effect. Instrumental rather than manual control of these systems would provide even greater precision in control of temperature than that achieved and reported. A control of $\pm 1^\circ\text{C}$ was sought.

At the bottom of the reactor the contents were removed through an in-line disintegrator which provided a slurry of finely divided particles of lignocellulose in the hydrolysate. The particle size achieved can be controlled by the size of the holes in the screen through which the particles must pass and the rpm at which the disintegrator is operated. These variables were held constant in this study.

The slurry produced was pumped through a cooling heat exchanger to quench the hydrolysis reaction, a Moyno pump to control rate of flow, a pressurized surge chamber, and finally discharged to slurry collection tanks mounted on weigh frames (0.1 lb sensitivity). The total time in the reactor system consists of two parts; i.e., the residence time required for flow through the isothermally heated reaction zone in the pretreatment reactor and the time required to flow through the cooled discharge system from entry into the tubular heat exchanger to discharge into the slurry collection tank.

The slurry, collected over a steady-state interval was weighed at one minute intervals and the cumulative weight was recorded. At the end of the steady-state reaction the slurry in the collection tank was thoroughly mixed and a sample was taken for chemical analysis.

The collected slurry was centrifuged and the solids were washed with 0.25% acid. Dilute acid rather than water was used to prevent biological degradation of the solids on storage. The centrifugate plus wash and

washed solids were weighed. Samples of each were taken for chemical analyses and placed in cold or frozen storage.

Input and output streams including; feedstock, dilute acid, and slurry were weighed at one minute intervals and individual or cumulative data were recorded on the hard disk of a computer. A hard copy of controlling parameters was printed at the control station of the CFHR. In addition, critical data were displayed on digital meters and by other means on the control panel. Steady-state conditions monitored, recorded (27 functions) and controlled included temperatures (12 scanned successively as desired and 8 recorded on hard copy), four pressures, feedstock introduction, acid feed, rate of slurry discharge, rate of disintegration, liquid levels in the reactor and slurry levels in the slurry surge tank.

4.2.2 CFHR Reaction Conditions.

Parameters under steady-state conditions are given in Table 4 for the seven reactions. The major controlling parameters are temperature, residence time in the isothermally heated pretreatment reactor, nitric acid concentration and feed rate, and feedstock rate. Pressure is dependent upon temperature and slurry production rate is dependent upon input rates.

The mean temperatures recorded at depths; i.e., immersion, of 30%, 50% and 75% in the slurry column moving by plug flow through the reactor are given in Table 4. Also, the standard deviation for each temperature is given. In addition the overall mean temperature of the reaction, calculated from the temperatures at the three levels in the reactor, is given. This temperature for Reaction 1 was 170.7 °C. and the standard deviation was 0.91. The three temperatures recorded for Reaction 1 are illustrated in Figures 4 - 6. Similar figures can be prepared for each reaction. All thermocouples were calibrated using Bureau of Standards, standard thermometers both before and after the steady-state runs were made. The center thermocouple, 50% immersion, was used to control the temperature to which the acid feed was preheated.

Reactor pressure is the pressure sensed in the vapor space at the top of the reactor just below the bottom 4-in. plug valve of the lockhopper. The pressure in the vapor space during the Reaction 1 steady-state is presented in Figure 7. The pressure peaks through 16:46 occur at 3 minute intervals and are attributed to the use of higher than normal high pressure steam in the lockhopper to overcome a temperature at the 75% immersion level (Figure 3) that, during the reaction, was perceived to be low and decreasing. As this temperature stabilized the pressure of the steam used in the lockhopper was decreased slightly and the cyclical high pressure peaks were no longer produced.

The data given in Table 4 for dilute nitric acid include the nitric acid concentration in % (w/w) in the acid feed tank, the average rate (kg/min) at which this acid was continuously fed through P1 to the heat exchanger, and the calculated nitric acid concentration (% w/w) that would have been present in the slurry of the reactor had this acid been completely mixed with no losses.

The cumulative rate at which the acid was fed in Reaction 1 is illustrated in Figure 8 as the decrease in weight of the acid feed tank. A slight increase in rate is apparent in the time interval from 16:49 to 17:04. This is attributed to the reactor being operated with the wood level above the control point for the liquid level and an effort to maintain steady-state conditions in the reactor by increasing acid flow slightly in this time interval.

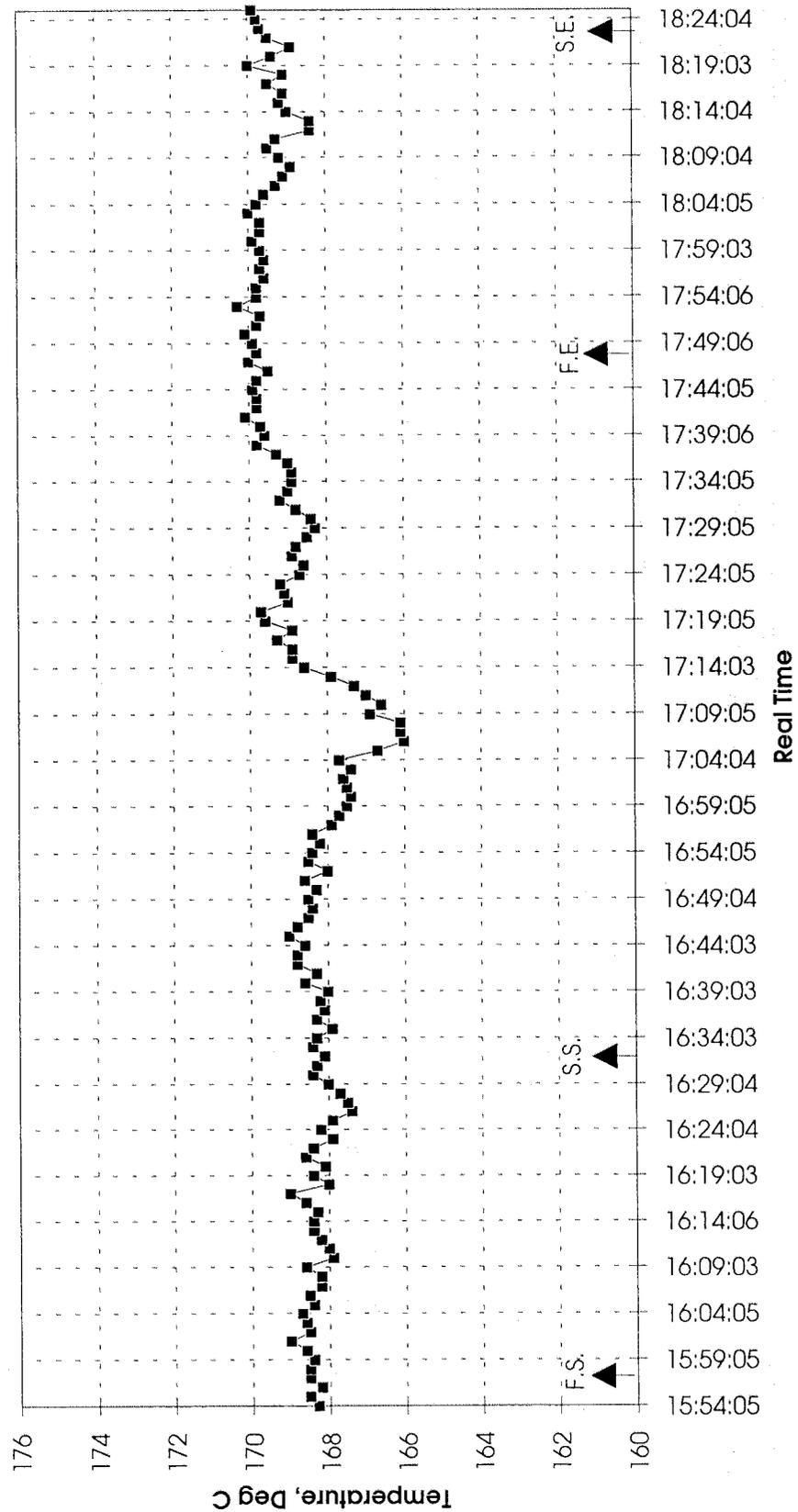


Figure 4. Top temperature, at 30% slurry depth. Reaction No. 1.

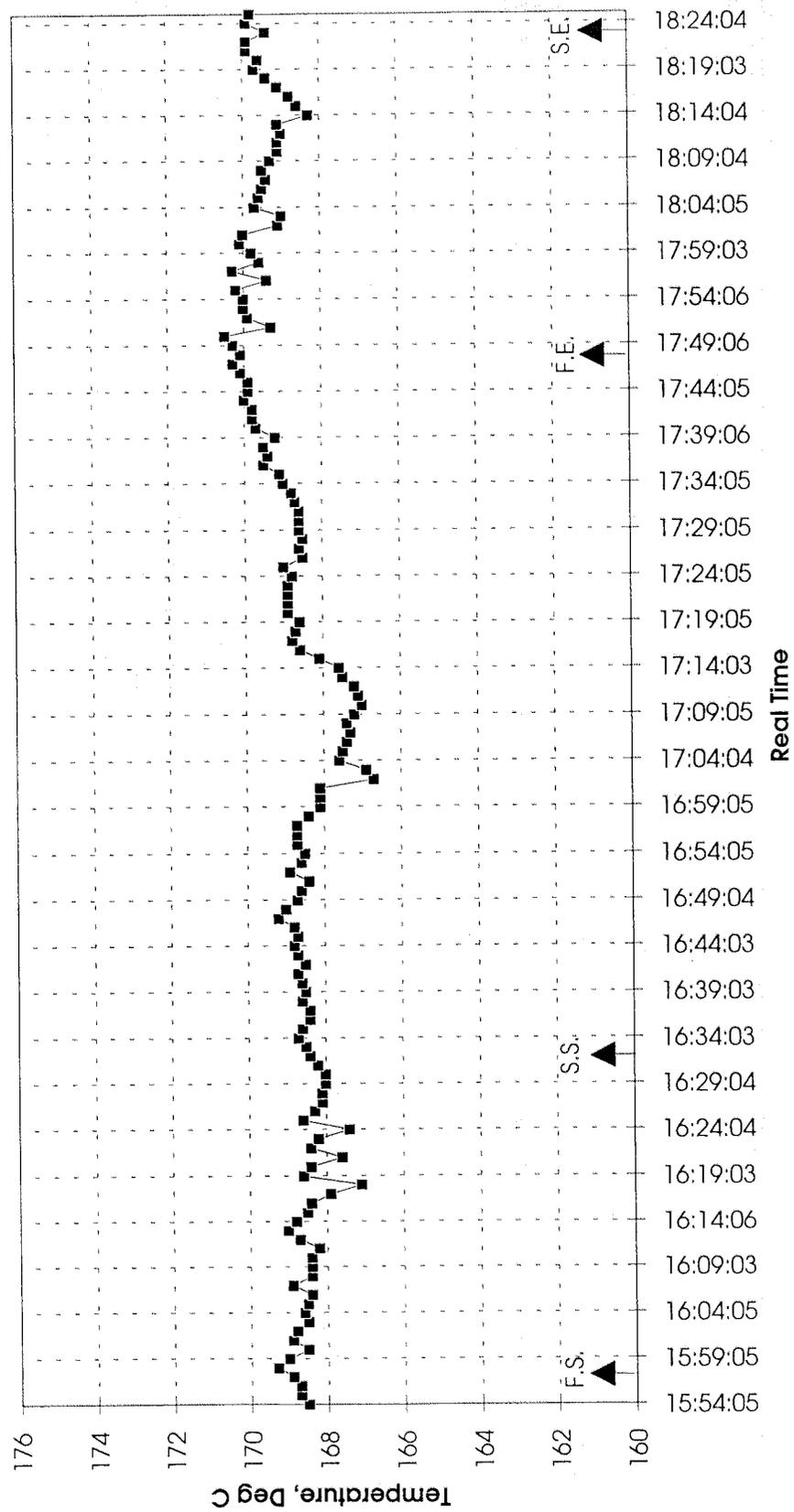


Figure 5. Center (control) temperature, at 50% slurry depth. Reaction No. 1.

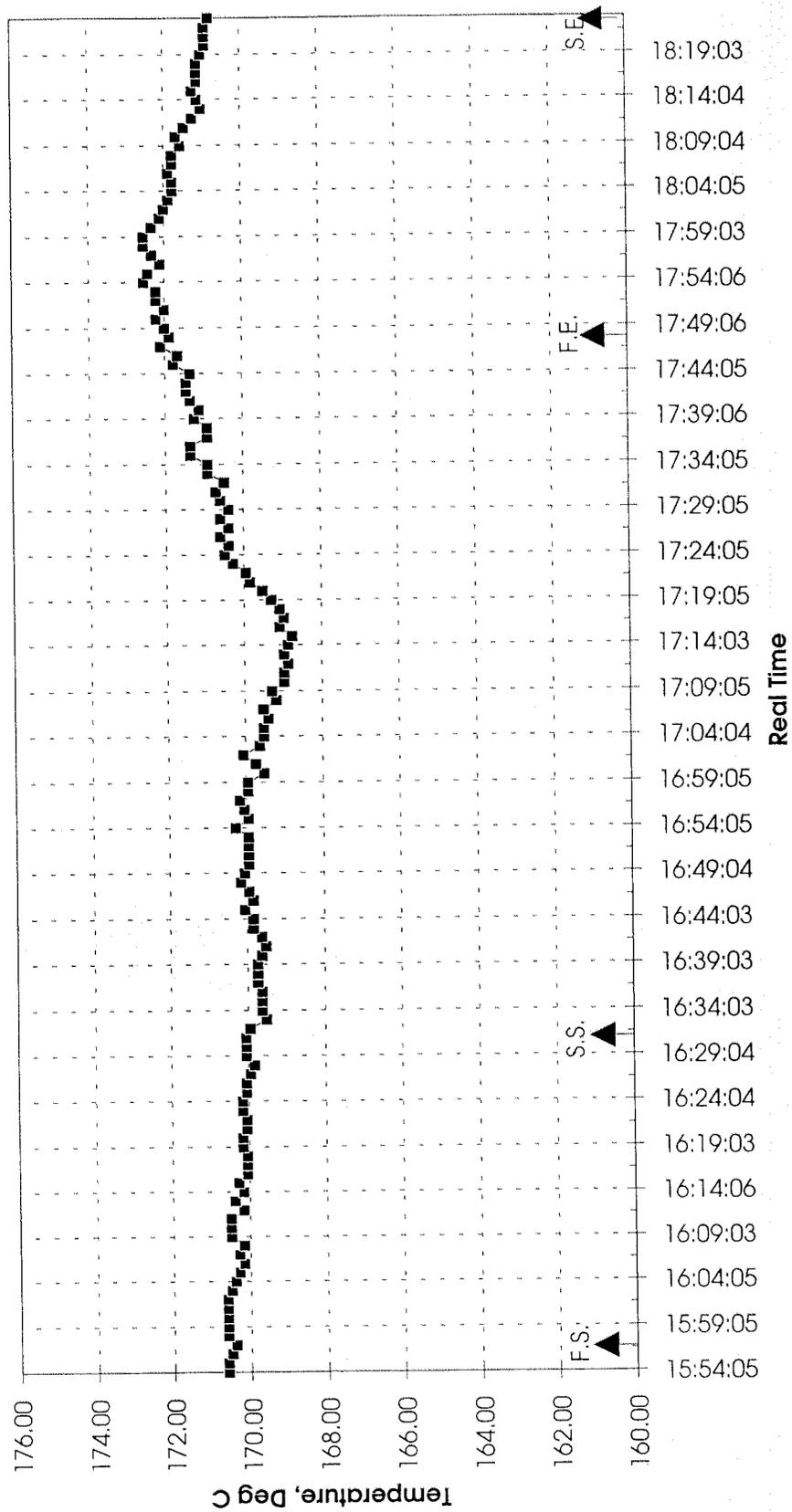


Figure 6. Bottom temperature, at 75% slurry depth. Reaction No. 1.

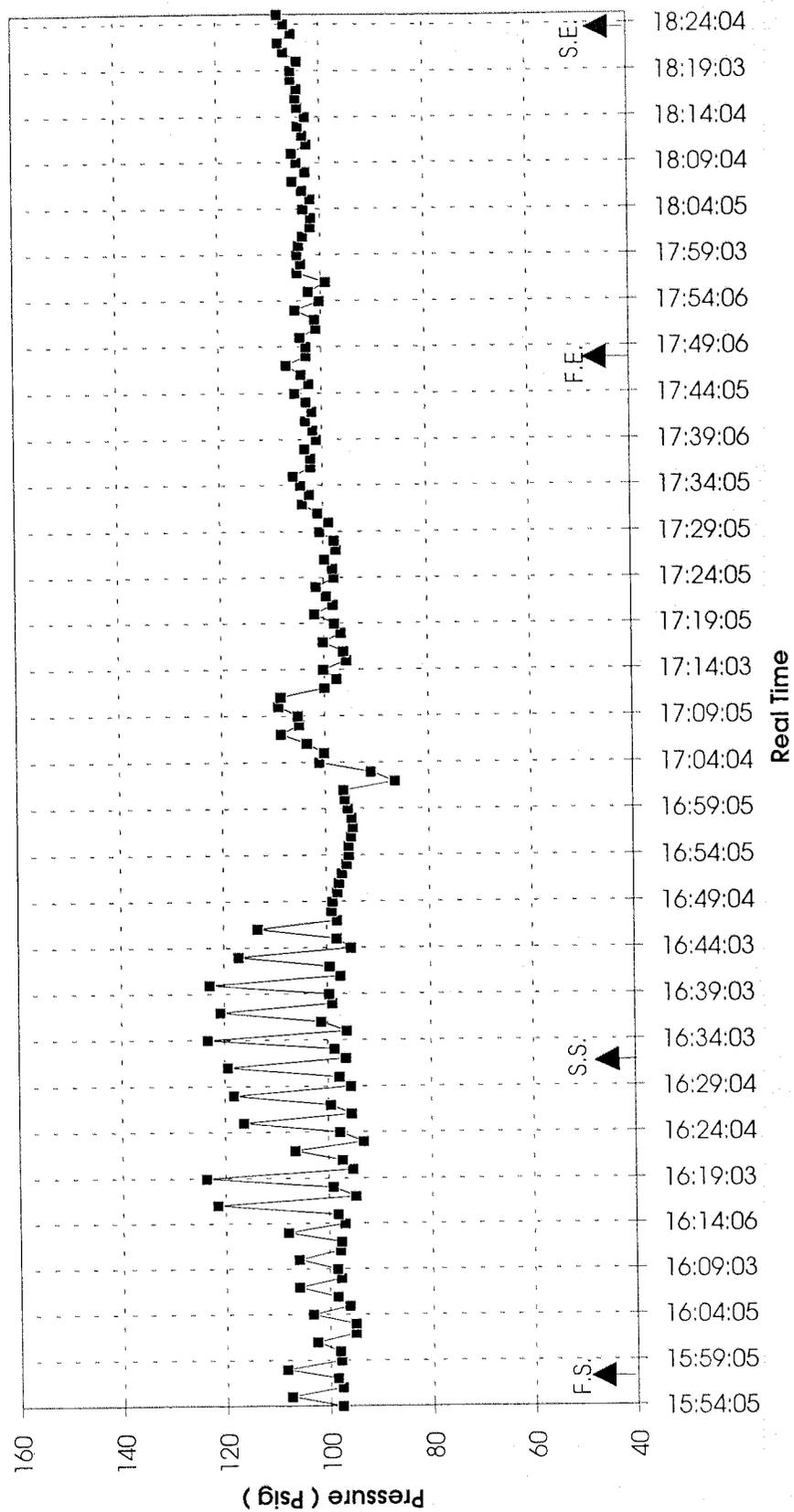


Figure 7. Reactor pressure (vapor phase). Reaction No. 1.

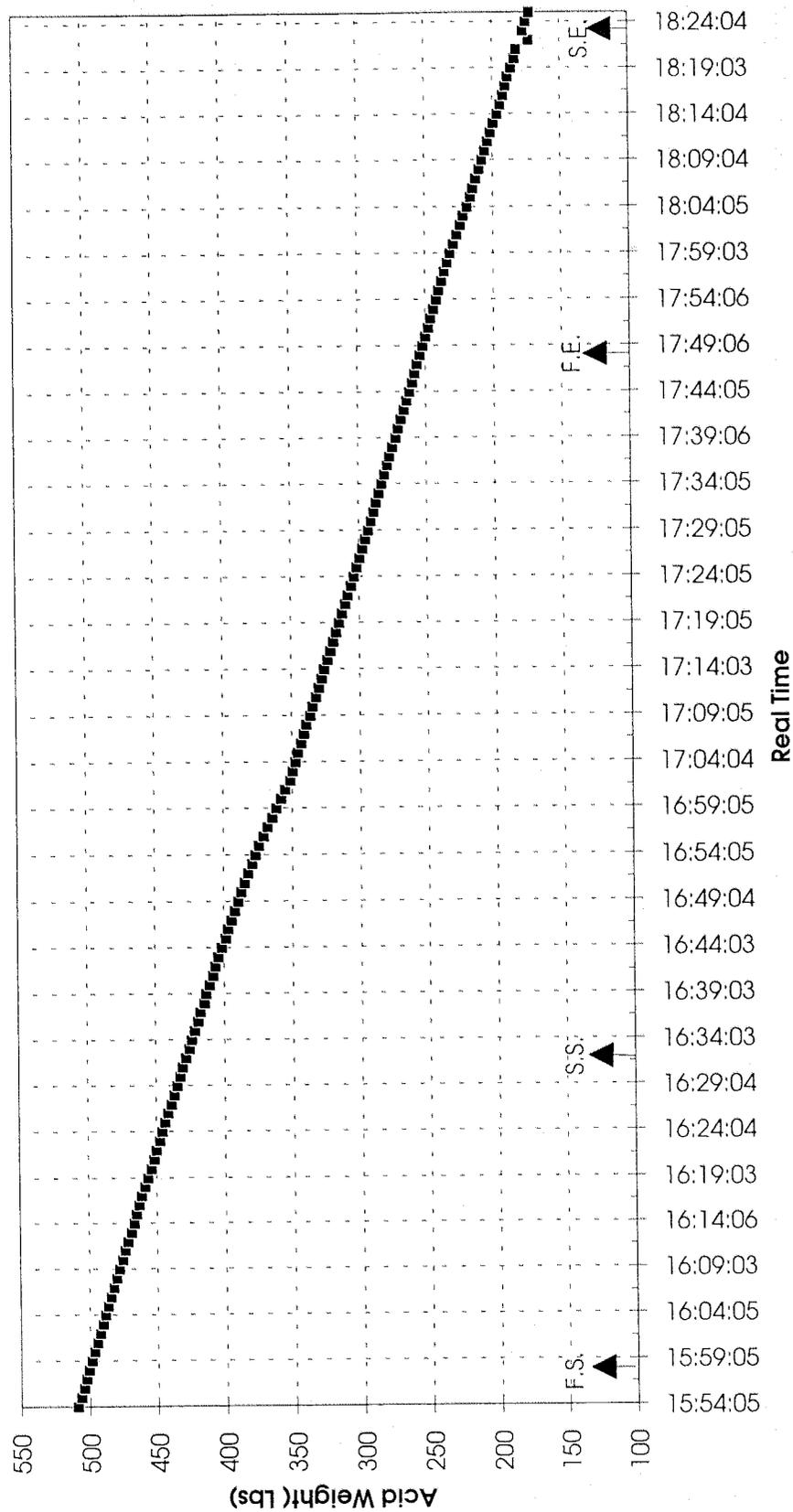


Figure 8. Cumulative introduction of dilute nitric acid. Reaction No. 1.

Table 4. Steady-state Reaction Parameters.

Reaction	Report	Units	1	2	3	4	5	6	7
Code	HP-S1-		7 I-3	8 I-1	9 I-2	10 I-1	11 I-1	12 I-1	12 I-2
Temp. Average		°C	170.7	170.7	171.1	172.5	160.4	161.2	161.9
At Reactor Depths									
	30%	°C	170.6	170.9	171.8	172.4	161.5	163.0	163.8
	50%	°C	171.3	170.6	171.2	172.6	160.6	161.1	162.0
	75%	°C	170.1	170.6	170.5	172.4	159.2	159.4	159.5
Standard Deviations									
	at 30%	°C	0.8	1.1	1.1	1.3	1.1	0.9	0.7
	50%	°C	0.7	0.7	0.9	1.4	0.9	0.8	0.6
	75%	°C	0.7	0.6	0.8	1.0	0.8	0.4	0.4
Pressure		psig	101.0	104.0	125.0	125.0	81.3	92.8	97.5
		SD	6.9	12.0	11.0	9.0	10.4	9.7	10.5
		kPa	698	722	862	864	561	640	672
Nitric Acid Feed	Conc.	% w/w	0.384	0.432	0.432	0.519	0.711	0.869	0.869
Nitric Acid	Rate	kg/min.	2.20	2.33	2.22	2.34	1.41	1.38	1.45
HNO ₃ in Reactor	Conc.	% w/w ¹	0.25	0.35	0.35	0.45	0.55	0.55	0.55
Feedstock Rate	Average	kg/min.	0.156	0.193	0.182	0.441	0.321	0.316	0.446
Residence Time	at T.	mins.	25.3	27.0	24.8	25.1	36.9	37.5	36.4
Total Steady-state	at T.	mins.	111	47	111	74	136	136	77
Disintegrator		RPM	611	588	622	607	677	681	743
S1 slurry	Rate	kg/min	1.324	1.134	1.355	1.340	0.919	0.904	0.930

¹ Calculated based upon the water in the dilute acid plus the feedstock plus predicted condensation of steam in the lockhopper.

The calculated nitric acid concentration in Table 4 is that which would have been produced if the dilute nitric acid added had been completely mixed with the water in the feedstock and the water produced by condensation of steam in the lockhopper. This concentration is reported in Table 4 as a calculated value. The calculated nitric acid concentrations were not attained for the following interrelated reasons. First, the high water content of the feedstock (55% -57% green basis) reduced the volume of void spaces present in the chips compared to the moisture contents (45% or less, green basis) of feedstocks previously used. This diminished the amount of dilute acid which can rapidly penetrate the chips by condensation of steam occupying the cell lumen and other voids following deaeration in the low pressure steaming cycle. Thus, acid penetration of the chips was accomplished more by relatively slow diffusion of acid into the water present in the void spaces than was the case in previous work. Secondly, also due to the high water content, a relatively high temperature was required to bring the feedstock to temperature in the high steam pressure segment of the lockhopper cycle. This high pressure steam overheated the lockhopper and the reactor walls in the vapor space above the feedstock slurry as discussed under temperature control in section 4.2.1. Thirdly, it became more difficult to relieve the high steam pressure at the end of the lockhopper cycle through the vent valve when the lower ball valve was closed. This is the segment in the cycle when the maximum reaction pressure used in the lockhopper must be reduced rapidly to atmospheric pressure as a necessary requirement to open the top ball valve of the lockhopper which initiates a new

cycle. Also, the vent valve is opened to vent steam during the low pressure steaming segment of the lockhopper cycle.

The venting problem persisted even though maintenance steps were taken to tighten the packing and to improve the closure of the air actuated steam vent valve. This venting problem became more apparent during the high pressure steaming segment of the cycle as the sequence of steady-state reactions was carried out. Because of the need for higher pressure steam than previously used, as discussed in section 4.2.1, a second corrective measure was taken before Reaction 5 was carried out. Thus, to assure the pressure reduction required in the high pressure steam venting segment, the lockhopper cycle was increased from 150 to 180 seconds. The added cycle time, placed largely on the high pressure venting segment, helped but did not solve the venting problem. Further, this problem became more pronounced as the concentration of acid in the dilute nitric acid feed was increased.

On review of the data from the steady-state reactions it has become clear that vapor from the reactor as well as the lockhopper leaked past the vent valve when it was closed. The replacement of the steam vent valve, which had been scheduled as a maintenance job, would eliminate this problem. As a consequence of the faulty operation of this valve, vapor containing nitric acid was vented into the heat exchanger-condensate tank collection system. This accounts for the high concentration of nitric acid found in the condensate from venting steam and the unusually high pH of all of the slurries produced as shown in Table 5.

There are two times that are considered in obtaining mass balances. There are the total time a reaction is at steady-state and the residence time a unit of feedstock is in the reactor at temperature.

To obtain the total time at steady-state, the exact time at the start and the completion of slurry collection were recorded and determined to less than 30 seconds. Slurry collection, then, is the basis for the determination of the steady-state interval in minutes and the mass balances pertaining to that steady-state interval for each reaction.

Residence time of the slurry in the pretreatment reactor was calculated for each reaction based upon two variables. One is the volume of the slurry in the reactor. The slurry moves through the reactor by plug flow without mixing until it reaches the disintegrator. This reactor volume is calculated as liters at the mean temperature of the reactor. The other is the rate of slurry production over the steady-state interval expressed in liters per minute at the mean reaction temperature.

Total steady-state interval times and the reactor residence times at the mean reaction temperatures are given in Table 4.

Cumulative slurry production in kg, rate of slurry production in kg/min, pH of the slurry, acid insoluble (pretreated) residue as a % based on the weight of the slurry, rpm of the disintegrator and disintegrator work in kWh per O.D. tonne of feedstock are given in Table 5.

Table 5. Slurry Production Summary.

Reaction No.	Weight kg	Rate kg/min	pH	Solids %	Disintegrator kWh/OD tonne	RPM	Std. Dev.
1	147.0	1.3	2.4	2.7	53.7	611	103
2	54.6	1.2	2.4	3.3	46.0	588	112
3	150.4	1.4	2.4	3.1	64.9	622	96
4	98.8	1.3	2.4	2.9	57.3	607	90
5	125.0	0.9	2.4	3.2	150.0	677	71
6	122.9	0.9	2.3	3.7	169.0	681	70
7	71.6	0.9	2.4	6.1	155.0	743	734

Cumulative slurry production is illustrated in Figure 9 for Reaction 1. Slurry collection for this reaction was initiated at 16:32 and was terminated at 18:23 to provide a steady-state interval of 111 minutes. Introduction of the feedstock to provide this slurry was started at 15:57:30 and ended at 17:48:30. In the figures given for Reaction 1 in which the parameter is plotted vs real time; i.e., Figures 4 through 12, the slurry start and end times and the feedstock start and end times are designated as SS, SE, FS and FE, respectively. Also, each of these times is marked by an arrow with the appropriate symbol times.

These times are summarized in Table 6.

Table 6. Starting and Ending Times for Slurry and Feedstock.

Reaction No.	Slurry		Feedstock	
	Start	End	Start	End
1	16:32	18:23	15:57.5	17:48.5
2	19:23	20:07	18:46.5	19:30.5
3	18:50	20:41	18:16.6	20:07.6
4	22:28	23:42	22:51.1	23:08.1
5	17:30	19:46	16:40.1	18:56.1
6	20:27	22:43	19:36.8	21:52.8
7	11:32.5	00:49.5	10:43.3	00:00.3

The acid concentration in the each slurry produced has been calculated from the pH of the slurry assuming an activity of the dilute nitric acid of 1.0. These are approximately 0.024% for reactions 1, 3 and 4; 0.026% for Reaction 2; and 0.028% for Reactions 5, 6 and 7.

Feedstock introduced to produce the product slurry was determined as the mass of feedstock added to the reactor in an interval of time initiated before the start of slurry collection and ending at the same interval of time before the termination of slurry collection. This interval of time, required for a given feedstock sample introduced into the lockhopper to issue as the slurry product in the slurry collection tank, is comprised of two segments. These are the times that; the slurry resides at reaction temperature in the reactor and the time for the slurry to pass through the heat exchanger-flow control pump-slurry surge tank and associated tubing system before slurry discharge to the slurry collection tank.

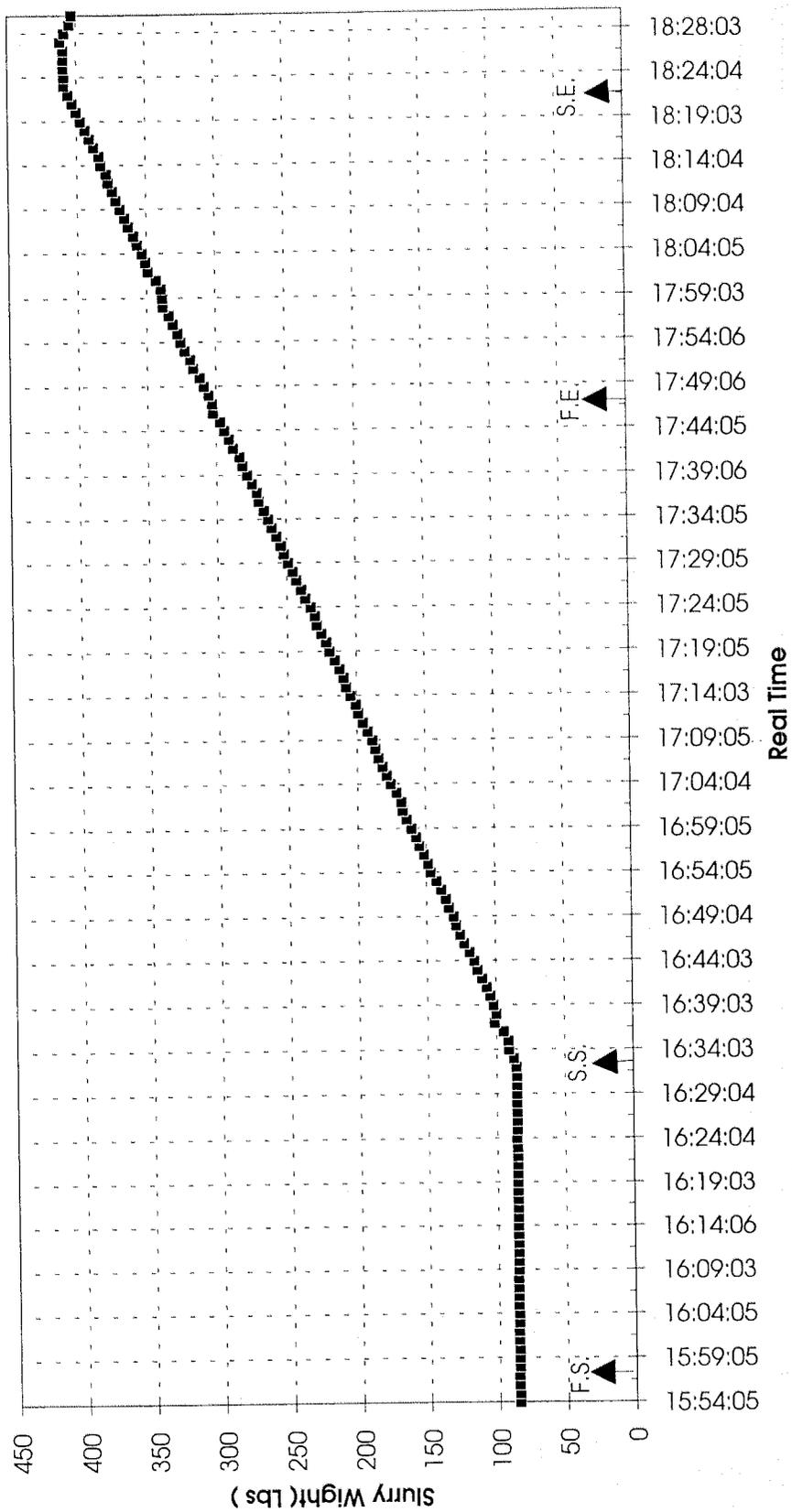


Figure 9. Cumulative slurry production. Reaction No. 1.

For Reaction 1, the residence time in the reactor at the mean reaction temperature was 25.3 minutes and the residence time in the discharge system at a temperature of about 40°C was 8.9 minutes. Thus, the interval of time for a unit of feedstock to be discharged from the system was 34.2 minutes based upon the relative volumes of the two systems and the residence time in the reactor for this reaction. These times are unique for each reaction. The real time interval during which wood was feed to provide the slurry collected in the steady-state interval of reaction 1 was from 15:57.5 to 17:48.5. Accurate correlation of the feedhopper cycle with the slurry discharge cycle and of the liquid levels in the reactor and slurry surge vessel are required to accurately establish the feedstock feed with the slurry produced.

The cumulative feedstock introduction into the reactor in reaction 1 is illustrated in Figure 10.

The rate of feedstock introduction over each steady-state interval is calculated from the cumulative feedstock introduced into the reactor and the total time for that interval. These rates for the steady-state reactions carried out are given in Table 4 and are expressed as kg/min.

The two rapid increases in weight shown in Figure 10 are due to additions of feedstock to the feedstock bin. Considerable care was taken to assure that these additions were made during the time interval when the Vibra Screw feeder was not in operation.

When feeding was interrupted for an extended period of time, the same feedstock weight was observed. The causes of such interruptions are varied. One common one is to use a rate of feedstock introduction that is too high.

The rpm at which the disintegrator was operated in Reaction 1 is presented in Figure 11. The frequent and substantial changes in the rpm was a function of the operation that has not been positively identified. One likely possibility is that fine particles are washed into the annular space between the bearing surface and the shaft of the disintegrator. This space was purged with water using a high pressure metering pump with the intention of forcing a small flow of water into the disintegrator. Although there is a close tolerance in the design of this annular space, the fine particles of acid insoluble residue could be forced into this space by greater pressure in the reactor. This phenomenon appeared to take place in cycles of about 4 to 5 minutes. As the pressure built up in the water purge, the particles could be washed out of the annular space. At this point, by removal of the particulate material causing a building up friction, the rpm of the shaft would increase and the cycle would be repeated.

The power required by the disintegrator in each reaction is given in Table 5 and illustrated in Figure 12 for reaction 1. This power is obtained as the difference between that required to run the disintegrator at temperature in the presence and the absence of feedstock. The rpm used to disintegrate the feedstock and the power consumed are discussed in section 4.5.

4.2.3 Steady-state Intervals

The results obtained in conducting the steady-state reactions described in section 4.2.2 are presented for each reaction as the material balance obtained, the composition of the acid insoluble or pretreatment residue produced, the particle size distribution of the pretreatment residue, and the pretreatment liquor composition. Based on these results the hexosan conversion, yield, unconverted and recovery and the pentosan conversion, yield, unconverted and recovery are summarized.

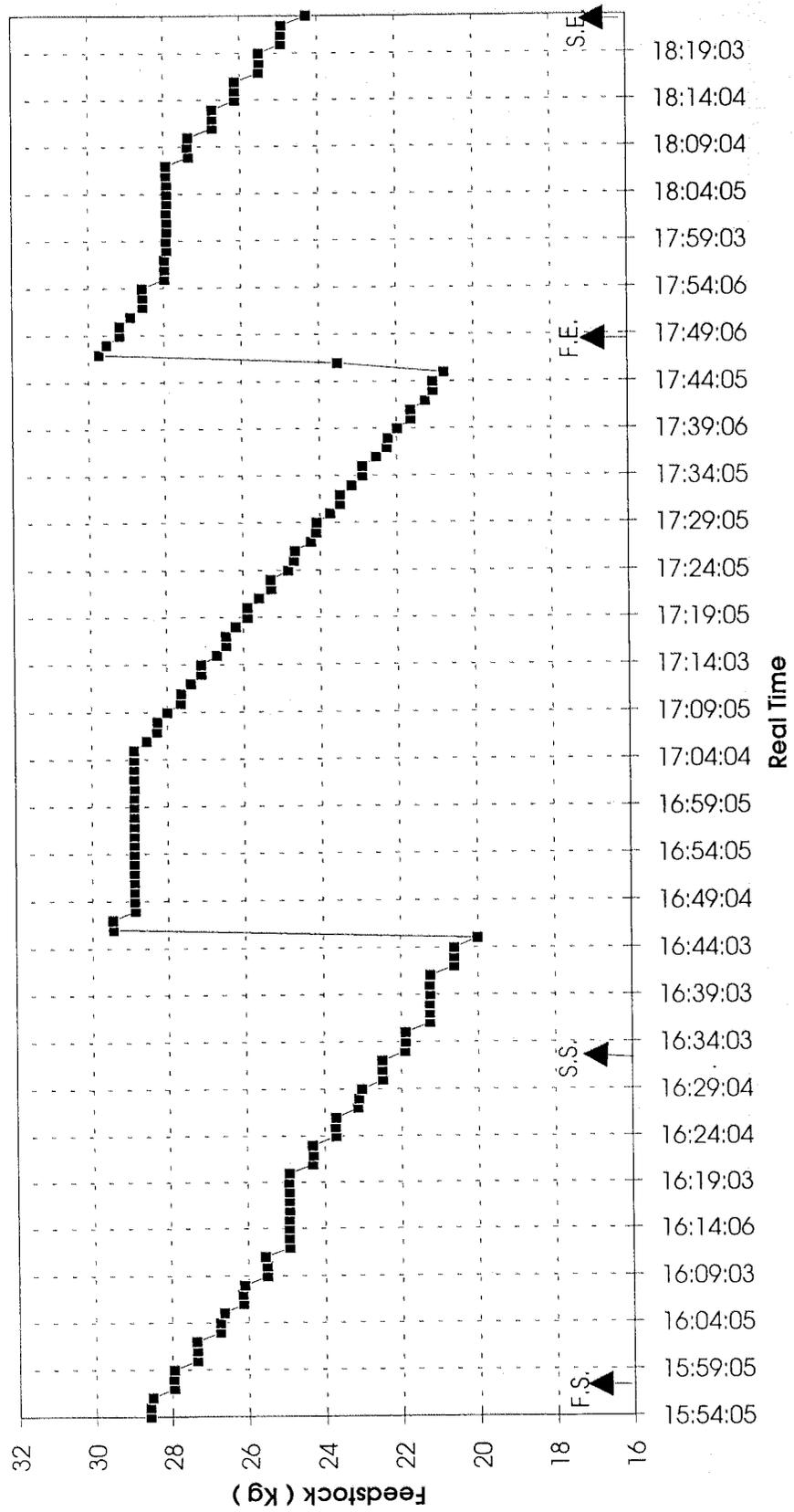


Figure 10. Feedstock introduction. Reaction No. 1.

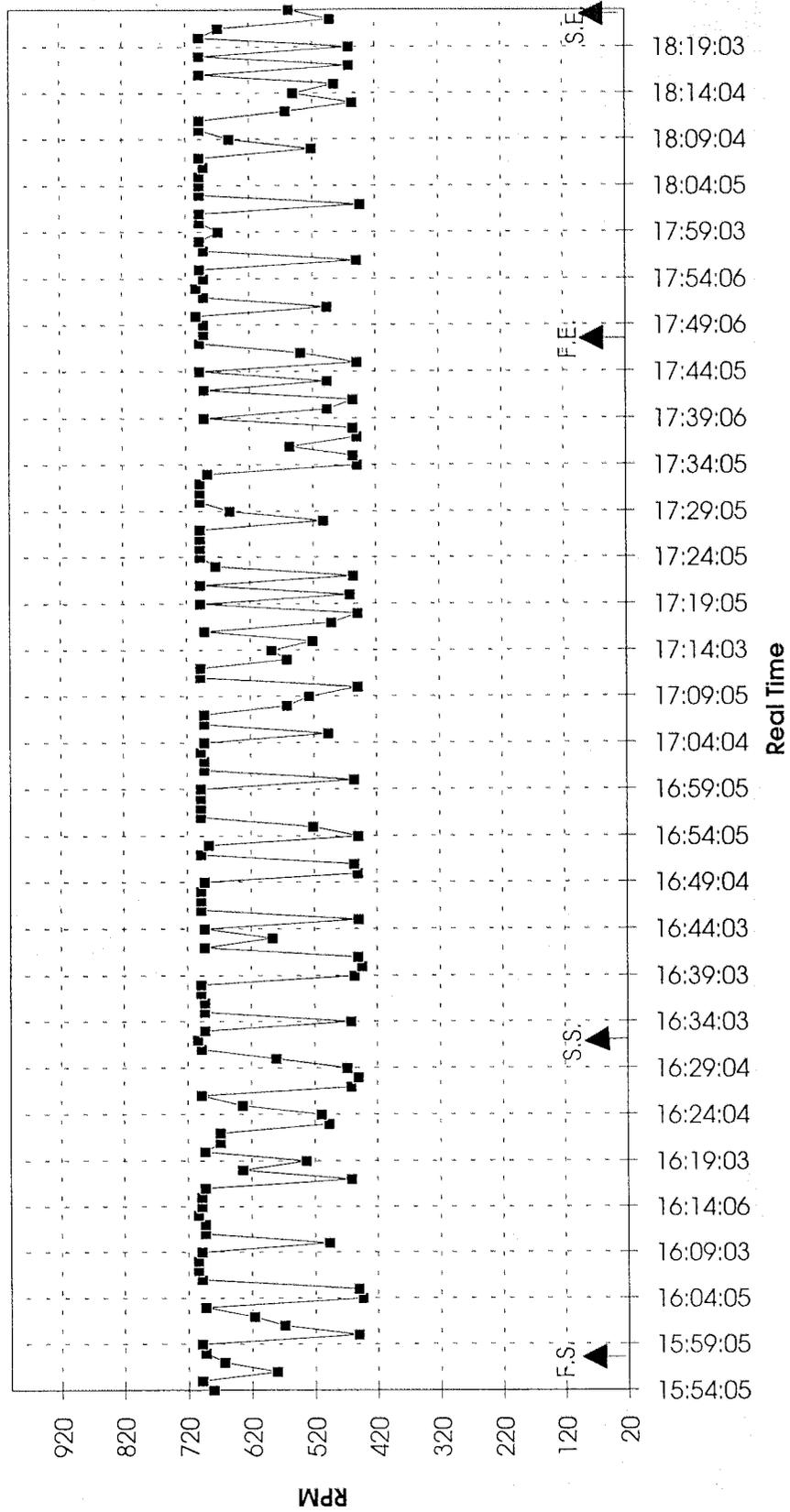


Figure 11. Disintegrator RPM. Reaction No. 1.

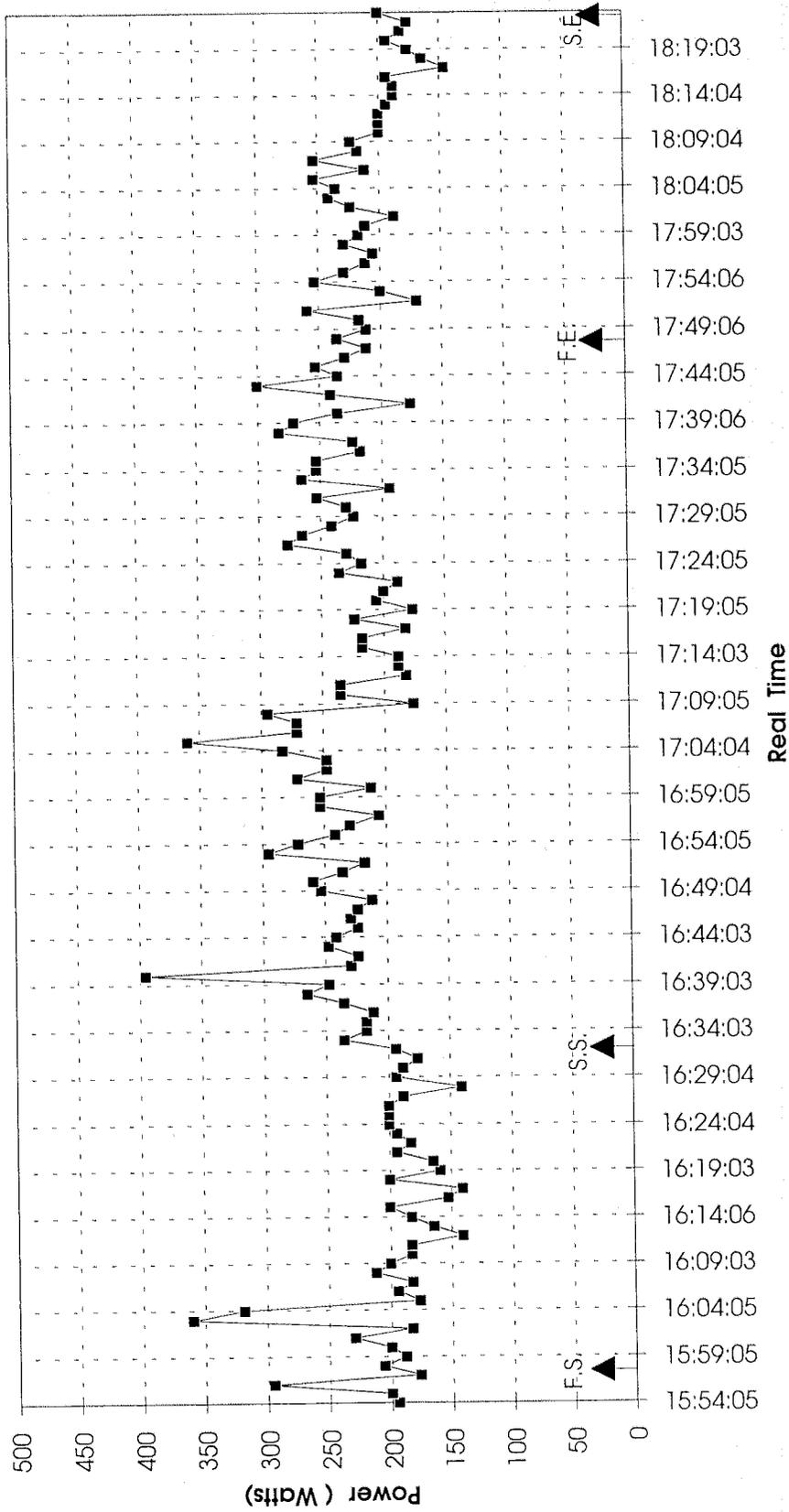


Figure 12. Disintegrator power. Reaction No. 1.

4.2.3.5 Mass Balances

Material balances obtained in the steady-state reactions 1 through 7 are given in Tables 7 through 13, respectively.

The total amounts of inputs, feedstock and dilute nitric acid are given and calculated on a rate basis. The moisture content of the feedstock and the concentration of the acid provide the data required to calculate the water and solids input in each of these streams and the mass rates. Similar data is provided for the slurry output.

Table 7. Material Balance, Reaction 1.

Average Temperature (°C):		170.6	
Residence Time in the reactor [at T]:		25.62 mins.	
Steady-state interval		111 mins.	
Input:	%	Lb	Kg
Feedstock			
Total:		38.2	17.32
Rate (mass/ min):		0.344	0.156
Moisture Content	55.4		
Water		21.1	9.59
OD Feedstock		17.00	7.73
OD Feed Rate (mass/min):		0.154	0.07
Dilute Nitric Acid			
Total:		244	110.8
Acid Feeding Rate (mass/min) :		2.20	0.998
Concentration of Acid :	0.384		
Water, total:		243	110
Water (mass/min):		2.19	0.994
HNO ₃ (total)		0.938	0.425
Rate (mass/min.):		0.0084	0.0038
Output			
Slurry:			
Flowrate (@ 25°C) (mass/min):		324	147
		2.92	1.32
Insoluble Solids:	2.66	8.62	3.91
Hydrolyzate			
Total	97.3	315	143
Soluble solids	2.13	6.71	3.04
Water		309	140.1
Water gain in lockhopper		44.4	20.1
Rate (mass/min.)		1.55	0.705
Total soluble+insoluble solids		15.3	6.95
Recovery		90	

The input stream that cannot be calculated by direct measurement is the steam that condenses in the lockhopper and enters into the slurry produced at this point. However, by accounting for the water in the two input streams and one output stream the amount of water introduced as condensate can be calculated as a difference. This difference is reported in each of the material balance tables as the water gain in the lockhopper. The total water gain and the rate, mass/min, are given in these tables. From these data the recovery of the mass introduced into the reactor is calculated as a percent of the mass input.

In general, the recovery of mass in these steady-state reactions was lower than expected. Thus, based on the increment of water added to the input mass due to hydrolysis good recoveries should be in the range from 105% to 110%.

Table 8. Material Balance, Reaction 2.

Average Temperature (°C):	171.3		
Residence Time in the reactor [at T]:	27.34 mins.		
Steady-state interval:	44 mins.		
Input:	%	Lb	Kg
Feedstock			
Total:		18.77	8.52
Rate (mass/ min):		0.427	0.194
Moisture Content	55.4		
Water		10.4	4.72
OD Feedstock		8.37	3.80
OD Feed Rate (mass/min):		0.19	0.086
Dilute Nitric Acid			
Total:		96.4	43.7
Acid Feeding Rate (mass/min) :		2.19	0.994
Concentration of Acid :	0.432		
Water, total:		96.0	43.5
Water (mass/min):		2.18	0.99
HNO3 (total)		0.416	0.189
Rate (mass/min.):		0.0095	0.0043
Output			
Slurry:			
Flowrate (@ 25°C) (mass/min):		120.4	54.6
		2.74	1.24
Insoluble Solids:	3.3	3.97	1.80
Hydrolyzate			
Total:	96.7	116.5	52.8
Soluble solids	2.64	3.07	1.39
Water		113.4	51.43
Water gain in lockhopper		6.98	3.17
Rate (mass/min.)		0.159	0.072
7.2.3 Total soluble+insoluble solids		7.05	3.20
Recovery		84.2	

Only one reaction fell in this range. A number of reasons can be given for this. Probably more than one is involved.

Some of the possible explanations are:

- * The relatively short steady-state intervals that were carried out with corresponding effects on the recovery of material due to any decrease in flow rates.
- * The low concentration of pretreatment solids in the slurries produced. This is due, in part, to the shakedown of a system that requires some additional instrumental controls to provide an assured uniform operation. In particular, a satisfactory method is required for the detection of the solids level present in the reactor. Also, the operating team was new and a level of expertise was being acquired which allowed operation at a higher solids level.

Table 9. Material Balance, Reaction 3.

Average Temperature (°C):		171.26	
Residence Time in the reactor [at T]:		25.04 mins.	
Steady-state interval:		111 mins.	
Input	%	Lb	Kg
Feedstock			
Total:		44.54	20.203
Rate (mass/ min):		0.401	0.182
Moisture Content	57.1		
Water		25.4	11.5
OD Feedstock		19.1	8.67
OD Feed Rate (mass/min):		0.172	0.078
Dilute Nitric Acid			
Total:		247	112.1
Acid Feeding Rate (mass/min) :		2.23	1.01
Concentration of Acid :	0.432		
Water, Total:		246	112
Water (mass/min):		2.22	1.01
HNO3 (total)		1.07	0.484
HNO3 (mass/min.):		0.0096	0.0044
Output			
Slurry:			
Flowrate (@ 25°C) (mass/min):		332	150.4
Insoluble Solids:	3.1	10.28	4.66
Hydrolyzate			
Total	96.9	321	146
Soluble solids	2.49	7.98	3.62
Water		313	142.12
Water gain in lockhopper		41.8	19.0
Rate (mass/min.)		0.377	0.171
Total soluble+insoluble solids		18.3	8.28
Recovery		96	

Table 10. Material Balance for Reaction 4.

Average Temperature (°C):		171.55	
Residence Time in the reactor [at T]:		25.36 mins.	
Steady-state interval:		74 mins.	
Input	%	Lb	Kg
Feedstock			
Total:		32.6	14.807
Rate (mass/ min):		0.441	0.2
Moisture Content	57.1		
Water		18.6	8.45
OD Feedstock		14	6.35
OD Feed Rate (mass/min):		0.189	0.086
Dilute Nitric Acid			
Total:		172.9	78.43
Acid Feeding Rate (mass/min) :		2.34	1.06
Concentration of Acid :	0.519		
Water, total:		172	78.02
Water (mass/min):		2.32	1.054
HNO ₃ (total)		0.897	0.407
Rate (mass/min.):		0.0121	0.0055
Output			
Slurry:			
Flowrate (@25°C) (mass/min):		218	98.8
		2.94	1.34
Insoluble Solids:	2.9	6.32	2.87
Hydrolyzate			
Total:	97.1	211	95.9
Soluble solids	2.52	5.33	2.42
Water		206	93.523
Water gain in lockhopper		15.5	7.05
Rate (mass/min.)		0.21	0.095
Total soluble+insoluble solids		11.6	5.28
Recovery		83	

Table 11. Material Balance for Reaction 5.

Average Temperature (°C):		161.3	
Residence Time in the reactor (at T):		37.36 mins.	
Steady-state interval:		136 mins.	
Input	%	Lb	Kg
Feedstock			
Total:		43.6	19.78
Feeding Rate(mass/ min):		0.321	0.145
Moisture Content	57.08		
Water		24.9	11.3
OD Feedstock		18.7	8.49
OD Feeding Rate (mass / min):		0.138	0.062
Dilute Nitric Acid			
Total:		192	87.16
Acid Feeding Rate (mass/min):		1.41	0.641
Concentration of Acid :	0.711		
Water, total:		191	86.5
Water (mass/min):		1.40	0.636
HNO3 (total)		1.37	0.62
Rate (mass/min.)		0.0101	0.0046
Output			
Slurry:		276	125
Flowrate (@ 25°C) (mass/min):		2.03	0.919
Insoluble Solids:	3.23	8.9	4.04
Hydrolyzate			
Total	96.8	267	121
Soluble Solids	0.952	2.54	1.15
Water		264	119.8
Water gain in lockhopper:		48.4	22.0
Rate (mass/min.)		1.18	0.534
Total soluble+insoluble solids		11.4	5.19
Recovery		61	

Table 12. Material Balance for Reaction 6.

Average Temperature (°C):		160.7	
Residence Time in the reactor [at T]:		37.95 mins.	
Steady-state interval		136 mins.	
Input	%	Lb	Kg
Feedstock			
Total:		42.96	19.49
Rate (mass/ min):		0.316	0.143
Moisture Content	57.08		
OD Feedstock		18.4	8.36
OD Feed Rate (mass/min):		0.136	0.061
Dilute Nitric Acid			
Total:		188	85.41
Rate (mass/min) :		1.38	0.628
Concentration of Acid :	0.869		
Water (total):		187	84.7
Water (mass/min):		1.37	0.623
HNO3 (total)		1.64	0.742
Rate (mass/min.):		0.0120	0.0055
Output			
Slurry:			
Flowrate (@ 25°C (mass/min):		271	123
		1.99	0.904
Insoluble Solids:	3.67	9.94	4.51
Hydrolyzate			
Total:	96.3	261	118
Soluble solids	3.63	9.48	4.3
Water		251	114.1
Water gain in lockhopper		40.3	18.3
Rate (mass/min.)		0.296	0.134
Total soluble+insoluble solids		19.4	8.81
Recovery		105	

Table 13. Material Balance for Reaction 7.

Average Temperature (°C):		161.9	
Residence Time in the reactor [at T]:		36.82 mins.	
Steady-state interval		77 mins.	
Input	%	Lb	Kg
Feedstock			
Total:		34.37	15.59
Rate (mass/ min):		0.446	0.202
Moisture Content	57.08		
Water:		19.6	8.9
OD Feedstock		14.8	6.69
OD Feed Rate (mass/min):		0.192	0.087
Dilute Nitric Acid			
Total:		112	50.80
Acid Feed Rate (mass/min) :		1.45	0.66
Concentration of Acid :	0.869		
Water, total:		111	50.4
Water (mass/min):		1.44	0.654
HNO ₃ (total)		0.973	0.441
Rate (mass/min.):		0.0126	0.0057
Output			
Slurry:			
Flowrate (@ 25°C) (mass/min):		158	71.6
		2.05	0.93
Insoluble Solids:	6.08	9.60	4.36
Hydrolyzate			
Total:	93.9	148	67.3
Soluble solids	3.28	4.86	2.21
Water		143	65.1
Water gain in lockhopper		12.8	5.82
Rate (mass/min.)		0.167	0.076
Total soluble+insoluble solids		14.5	6.56
Recovery		98	

4.2.3.6 Pretreated Solids Compositions

The mass and compositions of the pretreated solids obtained in the steady-state intervals are presented in Tables Nos. 14-20. These solids were the feedstocks used in SSF, and 38°C and 50°C enzymatic hydrolyses described in Section 5. The analyses of these materials was carried out using the same protocol used in the analysis of the hybrid poplar feedstock, Tables 2 and 3, section 4.1.3. In these analyses corrections were made according to NREL CATSP No. 002. to account for the loss of xylose and glucose in the hydrolysis step.

Table 14. Pretreated Solids Composition, Reaction 1.

				%	Lb	Kg
Pretreated Solids					8.62	3.91
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	58.59	5.05	2.29	Glucan	52.7	4.55 2.06
Mannose	0	0	0	Mannan	0	0 0
Galactose	0	0	0	Galactan	0	0 0
Subtotal	58.59	5.05	2.29	Subtotal	52.7	4.55 2.06
Pentose				Pentosan		
Xylose	1.76	0.152	0.068	Xylan	1.55	0.136 0.062
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	1.76	0.152	0.068	Subtotal	1.55	0.136 0.062
Total Sugars	60.4	5.20	2.36	Total Glycan	54.3	4.68 2.12
Acid Insoluble Residue					40.4	3.48 1.58
Acid Soluble Lignin					0.89	0.077 0.035
Ash					1.46	0.126 0.057

The mass has been used to establish gross material balances. The composition of the pretreated solids produced has been used as a component in the determination of the percentage conversions, yields, unconverted glycan and recovery of the monosaccharide as monosaccharide plus the corresponding glycan in summary tables to be presented.

The summary of the chemical compositions of feedstock, pretreated solids and alpha cellulose are presented in Table 21. The analysis of the feedstock is discussed in section 4.1.3.

The values obtained for the pretreated solids are uniform with certain variations which are noted below.

The glucan content varies with the narrow limits of 52.2% to 54.4% with the exception of reaction 2 at 50.5%. This uniformity is expected under the processing conditions used. The residual xylan content demonstrates the efficacy of hemicellulose hydrolysis in reactions 4, 3 and 2, respectively. Also, the absence of mannose and galactose demonstrates the complete removal of the glucomannans that are present in minor amounts in the feedstock.

The higher content of xylan in the reactions at 160°C provides evidence that the residence times in these reactions were low and/or the pH in the reactor was not sufficiently low. These parameters have been discussed previously and the steps that need to be taken in optimizing the reactions are clear.

Table 15. Pretreated Solids Composition, Reaction 2.

				%	Lb	Kg
Pretreated Solids					3.97	1.80
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	55.0	2.13	0.970	Glucan	49.5	1.92 0.870
Mannose	0	0	0	Mannan	0	0 0
Galactose	0	0	0	Galactan	0	0 0
Subtotal	55	2.13	0.970	Subtotal	49.5	1.92 0.870
Pentose				Pentosan		
Xylose	1.07	0.041	0.019	Xylan	0.96	0.037 0.017
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	1.07	0.041	0.019	Subtotal	0.96	0.037 0.017
Total Sugars	56.1	2.17	0.99	Total Glycan	50.5	1.96 0.89
Acid Insoluble Residue					39.9	1.55 0.70
Acid Soluble Lignin					1.09	0.043 0.020
Ash					1.35	0.054 0.024

The ash contents of the residues is surprising and indeed troubling. It indicates that appreciable amounts of silica or a mineral insoluble under the conditions of pretreatment are present. This could be due to a high mineral content in the fines that were shown to vary appreciably from drum to drum of feedstock or it could be due to a mineral content of the bark. Analyses should be undertaken to find an explanation of these values that might suggest steps that to be taken to minimize the ash content.

The acid insoluble lignins or more properly, the acid insoluble residues are uniform, varying from 38.9% to 41.2%.

This summary of composition of the pretreatment residues produced shows that the process being used produces a product that can be readily duplicated with reasonable controls.

Table 16. Pretreated Solids Composition, Reaction 3.

					%	Lb	Kg
Pretreated Solids						10.30	4.66
Sugars							
As Monosaccharides	%	Lb	Kg	As Polyglycan			
Hexose				Hexosan			
Glucose	58	5.96	2.70	Glucan	52.2	5.37	2.43
Mannose	0	0	0	Mannan	0	0	0
Galactose	0	0	0	Galactan	0	0	0
Subtotal	58	5.96	2.70	Subtotal	52.2	5.37	2.43
Pentose				Pentosan			
Xylose	1.06	0.109	0.049	Xylan	0.95	0.098	0.044
Arabinose	0	0	0	Arabinan	0	0	0
Subtotal	1.06	0.109	0.049	Subtotal	0.95	0.098	0.044
Total Sugars	59.1	6.07	2.75	Total Glycan	53.2	5.46	2.48
Acid Insoluble Residue					40.5	4.17	1.89
Acid Soluble Lignin					1.03	0.106	0.048
Ash					2.46	0.253	0.115

Table 17. Pretreated Solids Composition, Reaction 4.

				%	Lb	Kg
Pretreated Solids					6.32	2.87
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	57.8	3.65	1.66	Glucan	52	3.29 1.49
Mannose	0	0	0	Mannan	0	0 0
Galactose	0	0	0	Galactan	0	0 0
Subtotal	57.8	3.65	1.66	Subtotal	52	3.29 1.49
Pentose				Pentosan		
Xylose	0.99	0.063	0.028	Xylan	0.89	0.056 0.026
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	0.99	0.063	0.028	Subtotal	0.89	0.056 0.026
Total Sugars	58.8	3.71	1.68	Total Glycan	52.9	3.34 1.52
Acid Insoluble Residue				41.2	2.60	1.18
Acid Soluble Lignin				1.22	0.077	0.035
Ash				2.29	0.145	0.066

Table 18. Pretreated Solids Composition, Reaction 5.

				%	Lb	Kg	
Pretreated Solids					8.90	4.04	
Sugars							
As Monosaccharides	%	Lb	Kg	As Polyglycan			
Hexose				Hexosan			
Glucose	55	4.89	2.22	Glucan	49.5	4.40	2.00
Mannose	0	0	0	Mannan	0	0	0
Galactose	0	0	0	Galactan	0	0	0
Subtotal	55	4.89	2.22	Subtotal	49.5	4.40	2.00
Pentose				Pentosan			
Xylose	3.05	0.271	0.123	Xylan	2.75	0.244	0.111
Arabinose	0	0	0	Arabinan	0	0	0
Subtotal	3.05	0.271	0.123	Subtotal	2.75	0.244	0.111
Total Sugars	58	5.16	2.34	Total Glycan	52.2	4.65	2.11
Acid Insoluble Residue					39.8	3.54	1.61
Acid Soluble Lignin					1.07	0.095	0.040
Ash					2.01	0.179	0.081

Table 19. Pretreated Solids Composition, Reaction 6.

				%	Lb	Kg
Pretreated Solids					9.94	4.51
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	58.2	5.78	2.62	Glucan	52.3	5.20 2.36
Mannose	0	0	0	Mannan	0	0 0
Galactose	0	0	0	Galactan	0	0 0
Subtotal	58.2	5.78	2.62	Subtotal	52.3	5.20 2.36
Pentose				Pentosan		
Xylose	2.35	0.237	0.107	Xylan	2.14	0.213 0.097
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	2.38	0.237	0.107	Subtotal	2.14	0.213 0.097
Total Sugars	60.5	6.02	2.73	Total Glycan	54.5	5.42 2.46
Acid Insoluble Residue					41.2	4.10 1.86
Acid Soluble Lignin					1.19	0.118 0.054
Ash					2.3	0.229 0.104

Table 20. Pretreated Solids Composition, Reaction 7.

				%	Lb	Kg
Pretreated Solids					9.60	4.36
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	57.6	5.53	2.51	Glucan	51.9	4.98
Mannose	0	0	0	Mannan	0	0
Galactose	0	0	0	Galactan	0	0
Subtotal	57.6	5.53	2.51	Subtotal	51.9	4.98
Pentose				Pentosan		
Xylose	2.44	0.234	0.106	Xylan	2.20	0.211
Arabinose	0	0	0	Arabinan	0	0
Subtotal	2.44	0.234	0.106	Subtotal	2.20	0.211
Total Sugars	60.1	5.77	2.62	Total Glycan	54.1	5.19
Acid Insoluble Residue					38.9	3.74
Acid Soluble Lignin					1.12	0.108
Ash					2.09	0.201

Table 21. Chemical Composition of Hybrid Poplar Feedstock, Pretreated Solids and Alpha Cellulose (using NREL hydrolysis correction), in percent of sample weight.

Sample	Hybrid Poplar	Pretreated Solids, Reaction Number							Alpha cellulose
		1	2	3	4	5	6	7	
Glucose	47.5	58.6	55.0	58.0	57.8	55.0	58.2	57.6	102.4
Mannose	3.03	0	0	0	0	0	0	0	0
Galactose	1.67	0	0	0	0	0	0	0	0
Xylose	16.34	1.76	1.07	1.06	0.99	3.05	2.35	2.44	2.91
Arabinose	2.03	0	0	0	0	0	0	0	0
Total Monosac.	70.6	60.4	56.1	59.1	58.8	58.0	60.5	60.1	105.3
Glucan	42.8	52.7	49.5	52.2	52.0	49.5	52.3	51.9	92.2
Mannan	2.73	0	0	0	0	0	0	0	0
Galactan	1.5	0	0	0	0	0	0	0	0
Xylan	14.4	1.55	0.942	0.933	0.871	2.68	2.07	2.15	2.56
Arabinan	1.79	0	0	0	0	0	0	0	0
Total Glycans	63.2	54.3	50.5	53.1	52.9	52.2	54.4	54.0	94.7
Ash	1.16	1.46	1.35	2.46	2.29	2.01	2.3	2.09	
Lignins									
Acid Insoluble	30.4	40.4	39.9	40.5	41.2	39.8	41.2	38.9	
Acid Soluble	1.59	0.89	1.09	1.03	1.22	1.07	1.19	1.12	
Total	96.3	97.1	92.8	97.1	97.6	95.1	99.1	96.1	94.7

4.2.3.7 Particle Size Distribution of Pretreated Solids

The reduction of feedstock solids by the disintegrator provides the particle size distribution of slurry solids given in Table 22. This classification was carried out using a wet screening method and U. S. Standard screens. The uniformity of the particle sizes obtained demonstrates the reproducibility of the size reduction obtained using the disintegrator design. Thus, the particle sizes of the products prepared at 160°C and 170°C are the same even though the xylan contents of the pretreated solids at 160°C are significantly higher. It is clear that the diameter of the perforations in the disintegrator screen controls the particle size of the product produced. The relative uniformity of the pretreatment residue size classes produced in the 7 steady-state reactions carried out demonstrates that the size reduction is achieved by an increase of the work done. This is described in section 4.5.

The size reduction achieved appears to be a major factor in the superiority of the results obtained in hydrolysis and fermentation discussed in Section 5. This superiority is attributed to two factors.

First, pretreated residues produced at 170°C contained less hemicellulose and were disintegrated with lower power than those produced at 160 °C. Secondly, the particle size achieved provided a substrate that was readily available for enzymatic hydrolysis.

The parameters of disintegration were held constant in this study. Clearly, the parameters of pretreatment that maximize removal of hemicelluloses and minimize the power requirement of disintegration need to be optimized to produce a product at a particle size that will minimize rate and maximize ethanol production

Table 22. Particle Size Distribution, Prehydrolyzed Solids.

Reaction No:	1	2	3	4	5	6	7
Mesh Fraction	Airdry Weight %						
(+40)	14.1	20.3	13.2	13.1	13.4	21.2	14.8
(-40+80)	19.2	18.1	18.5	21	21.8	18.3	22.1
(-80+120)	6.1	5.1	8.1	9.1	6.1	5.4	5.3
(-120+140)	5.5						
(-120+170)		7.1	6.9	4.4	7.2	5.5	4.5
(-140+170)	3.1						
(-170)	52	49.4	53.3	52.4	51.5	49.6	53.3
Total	100	100	100	100	100	100	100

Table 23. Hydrolysate Analysis: Reaction 1

				%	Lb	Kg		
Hydrolysate Produced				2.13	315	143.1		
Soluble Monosaccharides	%	Lb	Kg	As Glycan				
Hexose				Hexosan				
Glucose	0.152	0.479	0.217	Glucan	0.137	0.431	0.195	
Mannose	0.100	0.316	0.143	Mannan	0.090	0.284	0.129	
Galactose	0.084	0.265	0.120	Galactan	0.076	0.239	0.108	
Subtotal	0.336	1.060	0.481	Subtotal	0.302	0.954	0.433	
Pentose				Pentosan				
Xylose	0.435	1.372	0.622	Xylan	0.383	1.208	0.548	
Arabinose	0.050	0.159	0.072	Arabinan	0.044	0.140	0.063	
Subtotal	0.485	1.531	0.694	Subtotal	0.427	1.347	0.611	
Total Sugars	0.821	2.590	1.175	Total Glycan	0.729	2.300	1.044	
Acid Soluble Lignin	0.288	0.909	0.412					
Ash								

4.2.3.8 Pretreatment Liquor Composition

The compositions of the pretreatment liquors obtained in the steady-state intervals are presented in Tables Nos. 23 through 29. Because of the urgency of other work being carried out during the three months following the period of time that steady-state reactions were completed, all samples of slurry and hydrolysate that were obtained were placed in either cold or frozen storage for analysis at a later date. Upon analysis of these materials it was found that furfural was no longer present in any of the samples. Thus, this information was lost. In future work, the analysis of the hydrolysate will be carried out assigning it highest priority to avoid losing labile materials.

Evidence was developed that sugars in acid hydrolyzates, xylose in particular, undergo losses on cold and, perhaps frozen storage at unacceptable rates.

Analyses of the pretreatment hydrolyzates produced in the reactions are presented in Tables 23 through 29. The data given is parallel to that given in the analyses of the pretreatment residues. The gross amounts of hydrolyzates produced and the solubles solids (% w/w) are from the material balance tables. The composition of the hydrolysate is given as a percentage (w/w) of each component analyzed. The ratio of sugars present appear to be normal but the concentrations are definitely low.

The low concentration of sugars in the hydrolysate is a direct consequence of producing slurries using a liquor:feedstock ratio that is too high; 10.9:1, in reaction 7.

Table 24. Hydrolysate Analysis: Reaction 2

					%	Lb	Kg
Hydrolysate Produced					2.64	116.5	52.8
Soluble Monosaccharides	%	Lb	Kg	As Glycan			
Hexose				Hexosan			
Glucose	0.152	0.172	0.078	Glucan	0.137	0.155	0.070
Mannose	0.133	0.151	0.068	Mannan	0.119	0.136	0.062
Galactose	0.091	0.104	0.047	Galactan	0.082	0.093	0.042
Subtotal	0.376	0.427	0.194	Subtotal	0.338	0.384	0.174
Pentose				Pentosan			
Xylose	0.622	0.706	0.320	Xylan	0.547	0.622	0.282
Arabinose	0.056	0.064	0.029	Arabinan	0.049	0.056	0.025
Subtotal	0.678	0.770	0.349	Subtotal	0.597	0.678	0.307
Total Sugars	1.054	1.197	0.543	Total Glycan	0.935	1.062	0.482
Acid Soluble Lignin	0.365	0.415	0.188				
Ash							

It was anticipated, based on reactions routinely carried out and repeatedly replicated in previous work, that in this project slurries would be produced with insoluble solids approaching 10% (w/w) and with hydrolyzates containing 2% to 3% of xylose. This was accomplished by using a liquor:wood ratio of 6:1.

This was an objective of this study but had to be abandoned before it was attained in January, 1994, in order to proceed with the chemical and microbiological evaluations of the products that had been produced in the steady-state reactions discussed. Improvements were made toward reaching this goal by increasing the insoluble solids contents of the slurries produced from a high in reaction 6 of 3.67% to 6.03% in reaction 7, the last reaction of the series.

The production of slurries at higher concentrations will be achieved by optimizing the operation of the CFHR.

4.2.4 Storage of Pretreatment Products

The storage of the pretreatment products is now recognized as a most critical factor. It was our belief that using cold storage or frozen storage would retard changes in composition to the point that analytical samples could be kept for months or longer. We have relied upon frozen storage as being adequate. This was reinforced by the belief that, in combination with a low pH, microbiological of the samples would be avoided. The low temperature was expected to reduce chemical degradation to the point that storage could be carried out over weeks if not months without significant changes in the types of materials under consideration.

Table 25. Hydrolysate Analysis: Reaction 3.

	%	Lb	Kg		%	Lb	Kg
Hydrolysate Produced					2.49	321	146
Soluble Monosaccharides	%	Lb	Kg	As Glycan			
Hexose				Hexosan			
Glucose	0.150	0.481	0.218	Glucan	0.135	0.433	0.196
Mannose	0.152	0.487	0.221	Mannan	0.136	0.438	0.199
Galactose	0.093	0.300	0.136	Galactan	0.084	0.270	0.122
Subtotal	0.395	1.270	0.575	Subtotal	0.355	1.140	0.517
Pentose				Pentosan			
Xylose	0.528	1.700	0.769	Xylan	0.465	1.490	0.677
Arabinose	0.070	0.224	0.102	Arabinan	0.061	0.197	0.090
Subtotal	0.598	1.920	0.871	Subtotal	0.526	1.690	0.767
Total Sugars	0.992	3.190	1.450	Total Glycan	0.881	2.830	1.280
Acid Soluble Lignin							
Ash							

Storage of feedstock is included in this discussion for the reason that long time storage must be relied upon to maintain material in good condition over considerable lengths of time as a program is being carried out. Experience has indicated that storage at 4°C should not be extended beyond two months and preferably one month to avoid changes. It appears that frozen storage of green material can be extended for years if the material is carefully packaged and sealed in material having low permeability to water vapor so that sublimation from the package is reduced to a minimum. If long term storage is to be carried out the moisture content of the feedstock should be reduced below its fiber saturation point (about 28%, dry basis). This terminates microbiological degradation and chemical changes become trivial. Also, physical changes in the cell structure that may be incurred on normal air drying will be avoided and biomass can be stored over long periods of time. We employ all three types of storage to meet the requirements of retaining feedstock over long periods and meeting time requirements of the work being carried out.

Storage of the pretreatment residue has been carried out by completing its preparation in the centrifugation step by about two displacement washes with dilute nitric acid. This removes most of the soluble components in the hydrolysate adhering to the centrifuged solids and provides a product at about 40% solids content. In addition the low pH of this product prevents microbiological decay. This product is sealed in plastic bags and can be retained in cold storage for years. Also, it can be stored at ambient temperature for months without deterioration. However, a systematic study of this has not been made.

Table 26. Hydrolysate Analysis: Reaction 4.

				%	Lb	Kg	
Hydrolysate Produced				2.52	211	95.9	
Soluble Monosaccharides	%	Lb	Kg	As Glycan			
Hexose				Hexosan			
Glucose	0.145	0.306	0.139	Glucan	0.130	0.275	0.125
Mannose	0.136	0.287	0.130	Mannan	0.122	0.259	0.117
Galactose	0.093	0.197	0.089	Galactan	0.084	0.177	0.080
Subtotal	0.373	0.790	0.358	Subtotal	0.336	0.711	0.322
Pentose				Pentosan			
Xylose	0.585	1.237	0.561	Xylan	0.515	1.089	0.494
Arabinose	0.064	0.135	0.061	Arabinan	0.056	0.118	0.054
Subtotal	0.649	1.372	0.622	Subtotal	0.571	1.207	0.548
Total Sugars	1.022	2.162	0.980	Total Glycan	0.907	1.918	0.870
Acid Soluble Lignin	0.361	0.763	0.346				
Ash							

Storage of hydrolyzates has proved to be more difficult than originally thought to be the case. In previous work analyses were conducted shortly after the hydrolyzates were produced; usually within the week. It was known that furfural was probably lost in countercurrent work when hydrolyzates were recycled to the stage 1 reactor. Also, it was known that storage of prehydrolysis liquors over years at ambient temperature would result in the loss of furfural and an appreciable loss in sugar concentration. However, in this work it is now known that a storage of 3 months under refrigeration and in frozen storage will result in a complete loss of furfural from hydrolyzates.

Thus, it is imperative to analyze hydrolyzates within hours of their preparation, if possible, and certainly within a week of their preparation.

4.2.5 Kinetics

Kinetics will be discussed in the report on Subcontract No. XAW-4-14292-01 with information gained in the study of switchgrass and, possibly cornstover.

Table 27. Hydrolysate Analysis: Reaction 5.

					%	Lb	Kg
Hydrolysate Produced					0.952	267	120.9
Soluble Monosaccharides	%	Lb	Kg	As Glycan			
Hexose				Hexosan			
Glucose	0.125	0.332	0.151	Glucan	0.112	0.299	0.136
Mannose	0.101	0.269	0.122	Mannan	0.091	0.242	0.110
Galactose	0.099	0.264	0.120	Galactan	0.089	0.237	0.108
Subtotal	0.324	0.864	0.392	Subtotal	0.292	0.778	0.353
Pentose				Pentosan			
Xylose	0.484	1.290	0.585	Xylan	0.426	1.136	0.515
Arabinose	0.075	0.200	0.091	Arabinan	0.066	0.176	0.080
Subtotal	0.559	1.490	0.676	Subtotal	0.492	1.310	0.595
Total Sugars	0.883	2.350	1.068	Total Glycan	0.784	2.090	0.948
Acid Soluble Lignin	0.362	0.965	0.438				
Ash							

4.3 Material Balance Closure

Mass balance closure involves correlation of the information presented in the Material Balance tables 7 - 13, the Pretreated Solids Composition, Tables 14 - 20, and the Hydrolysis Analysis tables 23 - 29. This has been done in Table 21, Chemical Composition of Hybrid Poplar Feedstock, Pretreated Solids and Alpha Cellulose; Table 30 Hexosan Conversion, Yield, Unconverted and Recovery; and Table 31, Pentosan Conversion, Yield, Unconverted and Recovery.

Conversion is: 100 minus the amount of glycan recovered in the pretreatment residue expressed as a percentage of the glycan in the feedstock.

Yield is: the amount of the monosaccharide, expressed as glycan, recovered in the hydrolysate expressed as a percentage of the glycan in the feedstock.

Unconverted glycan is the amount of glycan in the prereacted solids expressed as a percentage of the glycan in the feedstock. The percentages of converted glycan plus unconverted glycan equal 100%.

Recovery of glycan is the sum of glycan in the hydrolysate, expressed as glycan, plus the glycan in the prereacted solids expressed as a percentage of the glycan in the feedstock.

The conversion of glucan seen in Table 30, was substantially higher than expected. This, at least in part, is attributed to the use of correction of sugars in the determination of sugars by CATSP No. 002. It is well known that sugars are degraded readily by 72% sulfuric acid under the conditions of the Klason lignin

Table 28. Hydrolysate Analysis: Reaction 6.

					%	Lb	Kg
Hydrolysate Produced					3.63	261	118
Soluble Monosaccharides	%	Lb	Kg	As Glycan			
Hexose				Hexosan			
Glucose	0.187	0.488	0.221	Glucan	0.168	0.439	0.199
Mannose	0.141	0.368	0.167	Mannan	0.127	0.331	0.150
Galactose	0.116	0.301	0.137	Galactan	0.104	0.271	0.123
Subtotal	0.444	1.157	0.525	Subtotal	0.399	1.042	0.473
Pentose				Pentosan			
Xylose	0.681	1.777	0.806	Xylan	0.599	1.560	0.709
Arabinose	0.094	0.246	0.112	Arabinan	0.083	0.217	0.098
Subtotal	0.775	2.023	0.918	Subtotal	0.682	1.780	0.808
Total Sugars	1.220	3.180	1.440	Total Glycan	1.080	2.820	1.280
Acid Soluble Lignin	0.372	0.972	0.441				
Ash							

determination which is the basis of the hydrolysis used. Polysaccharides, on the other hand are depolymerized to oligomers which are substantially more resistant to acid degradation. Dilute acid hydrolysis is required to form the glycoses. The glycan content of the feedstocks are too high. This will give conversions, (100 minus unconverted glycan) that are, also, too high.

The conversion of mannan and galactan are expected since the monosaccharides represented by these glycans are actually present in the feedstock as glucomannans or trivial amounts of other polysaccharides. It is well established that the glucomannans are readily and completely hydrolyzed.

The low yields of glucose are expected since the amount of glucomannans present in angiosperms is low and the hydrolysis of cellulose, under the conditions used is also low.

Because of the low percentage content of mannose and galactose in the feedstock, the concentration of each is low and the accuracy of the determination of each diminishes. With conversion of the hemicelluloses containing mannan and galactan complete, the unconverted glycans are essentially zero and the yields are high. However, with the application of substantial conversion factors in the determination of the monosaccharides in the feedstock, it follows that the yields of these sugars will be low and likewise the recovery will be low. The recovery of galactan is better than that of mannose.

In Table 31 trends similar to those in Table 30 are observed. The major sugar produced is xylose. The conversion of xylan to xylose is particularly important since it is a measure of the efficiency of the pretreatment reaction, it is a measure of the power requirements to disintegrate the prereacted residue, and it is correlated with the best results obtained in enzymatic hydrolysis. Also, the recovery of xylose at this

Table 29. Hydrolysate Analysis: Reaction 7.

					%	Lb	Kg
Hydrolysate Produced					3.28	148	67.29
Soluble Monosaccharides	%	Lb	Kg	As Glycan			
Hexose				Hexosan			
Glucose	0.239	0.355	0.161	Glucan	0.215	0.319	0.145
Mannose	0.178	0.264	0.120	Mannan	0.160	0.238	0.108
Galactose	0.132	0.196	0.089	Galactan	0.119	0.176	0.080
Subtotal	0.550	0.815	0.370	Subtotal	0.495	0.734	0.333
Pentose				Pentosan			
Xylose	0.737	1.093	0.496	Xylan	0.649	0.962	0.436
Arabinose	0.112	0.166	0.075	Arabinan	0.098	0.146	0.066
Subtotal	0.849	1.259	0.571	Subtotal	0.747	1.108	0.502
Total Sugars	1.400	2.070	0.941	Total Glycan	1.240	1.840	0.835
Acid Soluble Lignin	0.556	0.825	0.374				
Ash							

point greatly simplifies the overall processing of the feedstock by being able to separate the operations in which fermentation of the hexoses and pentoses take place.

A reason for the low yields of the monosaccharides, other than that induced by the application of the sugar correct factor is being sought. This subject will be considered in an independent report based on the data given in the Appendix.

4.4 Energy Balance

The power to stir the slurry and disintegrate the feedstock is given in Table 5. It is greater by a factor of approximately 4 than previously observed. The reasons for this include a low acid concentration in the slurry resulting in reduced cleavage of cellulosic bonds and, in reactions 5 - 8, leaving a relatively high percentage of hemicelluloses in the acid insoluble residues. The increase in power required in reactions 5 - 8 over that required in reactions 1 - 4 is solely attributed to this factor. This is demonstrated in Figure 13 which under otherwise similar reaction conditions indicates a linear dependence of power consumption on hemicellulosic content of the pretreatment residue being disintegrated.

Thus, the physical strengths of the acid insoluble residues were not reduced to the extent they should have been by hydrolysis before being subjected to disintegration and discharged from the reactor. This is reflected also in the residence times of the reactions being 14 percent longer than expected at 170°C and at least 16% shorter than would be expected to reduce the xylan content of the acid insoluble residues at 160°C to the level expected under more optimum conditions reaction conditions. The fact that slurry concentrations achieved only about 30 to 35% of that expected would also increase the power per unit of feedstock treated. Thus, it is anticipated that increasing the slurry concentration would result in only a

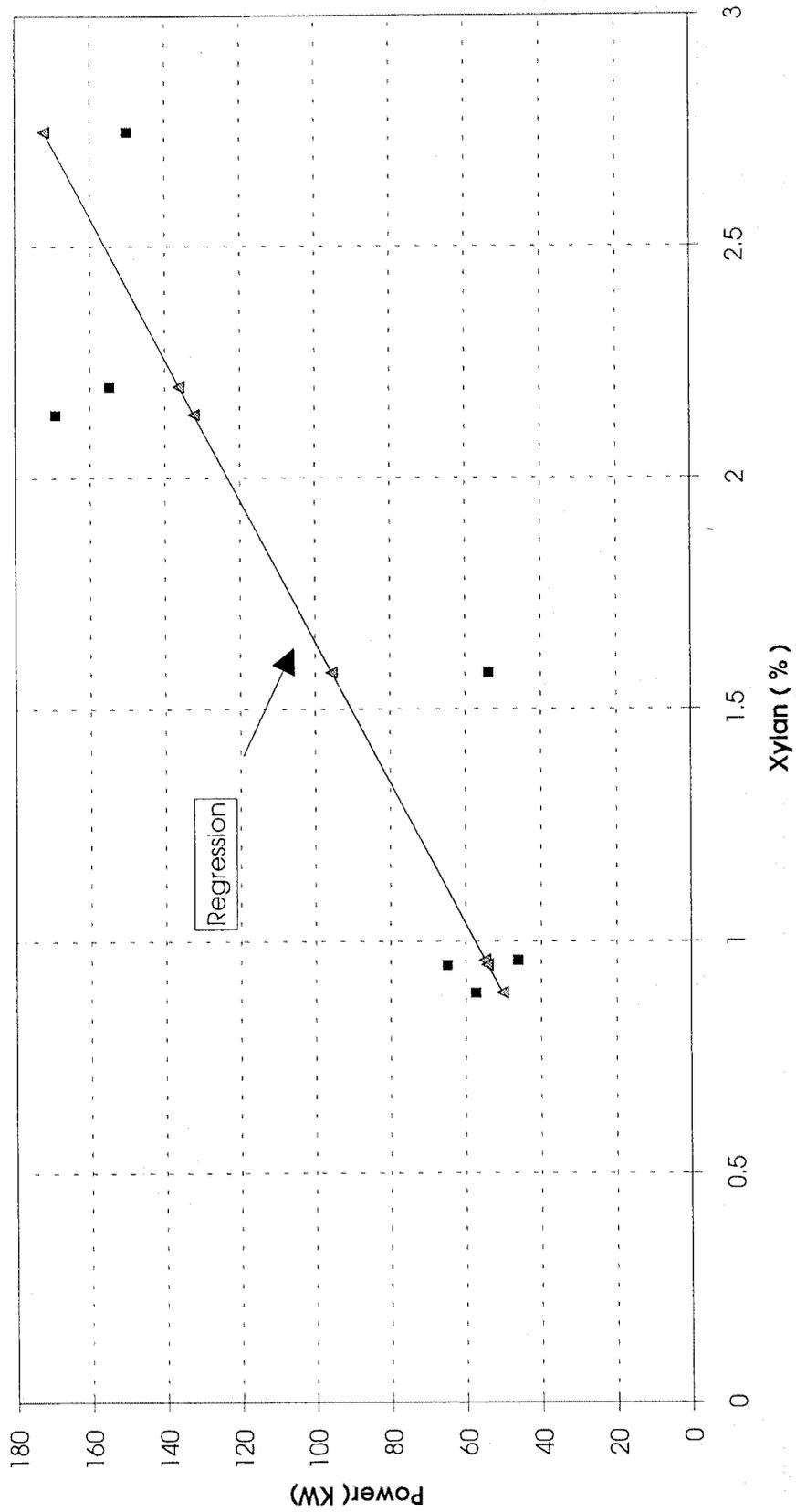


Figure 13. Power consumption as a function of xylan content of pretreated residues.

Table 30. Hexosan Conversion, Yield, Unconverted and Recovery.

React- ion	Hexosan Feedstock Kg	Hydrolyzate Kg	Solids Kg	Converted %	Yield %	Unconverted %	Recovery %	
1	glucan	3.30	0.195	2.06	37.6	5.91	62.4	68.3
	mannan	0.211	0.129	0	100	61.2	0	61.1
	galactan	0.116	0.108		100	93.2	0	93.2
	total	3.52	0.324	2.06	41.4	9.23	58.7	68.0
2	glucan	1.73	0.070	0.870	49.8	4.06	50.3	54.3
	mannan	0.111	0.062	0	100	55.7	0	55.6
	galactan	0.061	0.042		100	69.5	0	69.5
	total	1.84	0.132	0.870	52.8	7.15	47.2	54.4
3	glucan	3.70	0.196	2.43	34.3	5.30	65.7	71.0
	mannan	0.236	0.199	0	100	84.2	0	84.2
	galactan	0.130	0.122		100	94.0	0	94.0
	total	3.94	0.395	2.43	38.3	10.0	61.8	71.8
4	glucan	2.71	0.125	1.49	45.0	4.60	55.0	59.6
	mannan	0.173	0.117	0	100	67.8	0	67.8
	galactan	0.095	0.080		100	84.2	0	84.2
	total	2.89	0.242	1.49	48.3	8.39	51.7	60.0
5	glucan	3.63	0.136	2	45	3.74	55	58.8
	mannan	0.231	0.105	0	100	45.4	0	45.4
	galactan	0.128	0.108		100	84.4	0	84.4
	total	3.86	0.240	2	48.3	6.23	51.7	56.0
6	glucan	3.57	0.199	2.36	34	5.58	66	71.6
	mannan	0.228	0.150	0	100	65.8	0	65.8
	galactan	0.126	0.123		100	97.9	0	97.9
	total	3.8	0.349	2.36	37.9	9.19	62.1	71.3
7	glucan	2.86	0.145	2.26	21	5.07	79	84.1
	mannan	0.182	0.108	0	100	59.2	0	59.2
	galactan	0.101	0.080		100	79.6	0	79.6
	total	3.04	0.253	2.26	25.7	8.31	74.3	82.6

Table 31. Pentosan Conversion, Yield, Unconverted, Recovery.

Reaction	Pentosan Feedstock	Hydrolysate Solids	Converted as pentosan	Yield	Unconverted	Recovery as pentosan	
	Kg	Kg	Kg	%	%	%	
1 Xylan	1.14	0.548	0.062	94.6	48.2	5.42	53.6
Arabinan	0.141	0.063		100	44.8	0	44.8
total	1.28	0.610	0.062	95.2	47.8	4.82	52.6
2 Xylan	0.596	0.282	0.017	97.2	47.3	2.84	50.2
Arabinan	0.074	0.025		100	34.4	0	34.4
total	0.67	0.310	0.017	97.5	45.9	2.53	48.4
3 Xylan	1.27	0.677	0.044	96.5	53.1	3.49	56.6
Arabinan	0.158	0.090		100	56.5	0	56.5
total	1.43	0.770	0.044	96.9	53.5	3.1	56.6
4 Xylan	0.93	0.494	0.026	97.3	52.9	2.74	55.6
Arabinan	0.116	0.054		100	46.3	0	46.3
total	1.05	0.550	0.026	97.6	52.2	2.43	54.6
5 Xylan	1.25	0.515	0.111	91.1	41.3	8.88	50.1
Arabinan	0.155	0.080		100	51.4	0	51.4
total	1.4	0.590	0.111	92.1	42.4	7.9	50.3
6 Xylan	1.23	0.709	0.097	92.1	57.7	7.85	65.5
Arabinan	0.153	0.098		100	64.3	0	64.3
total	1.38	0.810	0.097	93	58.4	6.99	65.4
7 Xylan	0.984	0.436	0.096	90.3	44.3	9.72	54.1
Arabinan	0.122	0.066		100	54.1	0	54.1
total	1.11	0.500	0.096	91.4	45.4	8.65	54.1

nominal increase in the power required for disintegration. Further, and most importantly, no opportunity was developed to study this variable in the few steady-state runs that could be made. It is fully expected that under conditions that are more optimum the power will be significantly reduced to values in the range of 10 to 20 KWH per O.D. ton previously obtained using a very similar feedstock.

Energy considerations for this process must be considered in view of the overall process from, at least, delivery of feedstock to a plant site to ethanol or other products, f.o.b. the plant site. These considerations will be made in the following contract on this project under subcontract XAW-4-14292-01.

5

SSF and Enzymatic Hydrolysis

5.1 Processing and Storage of Pretreated Solids

Pretreated solids from all reactions were separated from the prehydrolyzate by centrifugation and the cake was washed with 0.25% nitric acid solution to displace the prehydrolyzate. The combined prehydrolyzate plus washes were stored in food-grade polyethylene drums, and the centrifuged solids were stored wet in plastic bags for future use.

Aliquots from the one-gallon slurry samples taken from material collected over selected steady-state intervals were filtered on Buchner funnels using Whatman No. 1 filter paper. The filtrates were collected and used for chemical analysis and the solids were washed with DI water until the pH of the filtrate was greater than pH 4.3. The pretreated and washed solids from selected intervals of Reactions 1-7 were used for SSF and enzymatic hydrolysis at 38°C and 50°C. The washes were discarded. Samples of the hybrid poplar feedstock and an α -cellulose were run for comparison. The chemical analyses of the selected pretreated solids, the feedstock and α -cellulose are shown in Table 21.

5.2 Enzyme Assay

The cellulase enzyme selected by NREL, Cytolase CL lot No. 17-92262-09 Drum 1, was received from Environmental BioTechnologies, Inc. on July 20, 1993. It was kept at 4°C until used for enzyme assay, SSF and enzymatic hydrolysis. It was assayed for cellulase activity following CATSP No. 006, revision 1, Measurement of Cellulase Activities, as described in section 2.1.6. The chronology of enzyme assays and the analyses using enzymes is shown below.

Date	Analysis	Enzyme Activity Determined or used, FPU/ml
March 30, 1994	First enzyme assay	81
April 2-9, 1994	SSF 1st series	81
April 22-29, 1994	SSF 2nd series	81
May 20, 1994	Second enzyme assay	74
May 25-27, 1994	Enzyme hydrolysis at 38°C, 1st series	74
June 6-7, 1994	Enzyme hydrolysis, 38°C, 2nd series	74
June 14-21, 1994	Enzyme hydrolysis at 50°C, 1st series	74
June 24-28, 1994	Enzyme hydrolysis, 50°C, 2nd series	74

These cellulase activities are similar to those reported (NREL 1994) for early 1994.

The analytical data for the two enzyme assays are given in Table 32. Glucose liberated from filter paper by cellulase was plotted against different enzyme dilution factors. Enzyme activity is calculated from the dilution factor which releases 2.0 mg of glucose under specified conditions. This dilution factor was determined graphically. Sugar concentrations are based on spectrophotometric analysis of sugar standards using DNS color reagent run at the same time as the enzyme assay.

Table 32. Cytolase CL Cellulase Enzyme Assay: March 30, 1994

Enzyme Assay No.	Absorbance@ 540nm	Glucose(mg/0.5ml)	Enzyme Dilution Factor
1-1	0.527	2.49	178.6
1-2	0.589	2.78	178.6
2-1	0.458	2.17	208.3
2-2	0.484	2.29	208.3
3-1	0.356	1.69	238.1
3-2	0.337	1.60	238.1

Calculated FPU/ml = 81

Glucose Std. No.	Absorbance@ 540nm	Glucose(mg/0.5ml)	
1	0.523	2.50	Linear regression gives r = 0.9978
2	0.360	1.65	
3	0.203	1.00	

Cytolase CL Cellulase Enzyme Assay: May 20, 1994

Enzyme Assay No.	Absorbance@ 540nm	Glucose(mg/0.5ml)	Enzyme Dilution Factor
1-1	0.572	2.30	178.6
1-2	0.576	2.32	178.6
2-1	0.505	2.03	200.0
2-2	0.506	2.03	200.0
3-1	0.440	1.76	217.4
3-2	0.445	1.78	217.4

Calculated FPU/ml = 74

Glucose Std. No.	Absorbance@ 540nm	Glucose(mg/0.5ml)	
1-1	0.830	3.35	Linear regression gives r = 0.9987
1-2	0.824	3.35	
2-1	0.606	2.50	
2-2	0.648	2.50	
3-1	0.416	1.65	
3-2	0.410	1.65	
4-1	0.251	1.00	
4-2	0.248	1.00	

Simultaneous Saccharification and Fermentation of Hybrid Poplar

In this study pretreated solids from seven pretreatment reactions were used in SSF, with Cytolase CL cellulase enzyme and *S. cerevisiae* D₅A yeast. For comparison, α -cellulose (Sigma C 8002) and hybrid poplar feedstock (-40 mesh) were also used. Protocol No. 008, revision 4, Lignocellulosic Biomass Hydrolysis and Fermentation, was followed as described in Section 2.1.8. The nine samples were run in duplicate in two series; one with four pretreated solids and one with three pretreated solids plus the HP feedstock and α -cellulose.

5.2.1 Ethanol Production in SSF

Yields of ethanol from SSF, both in grams per liter and percentage of theoretical are shown in Figures 14 - 22. The theoretical ethanol is based on glucan present in the pretreated solids and 51.1% yield of ethanol based on glucose. The zero time ethanol value is defined as zero and the analytical values for ethanol in subsequent samples are reduced by the amount of ethanol added at zero time in the inoculum plus the ethanol equivalent to glucose found in added media and enzyme. Tables 33 and 34 present the same data in tabular form and show the exact sampling times.

The ethanol yields for pretreated solids varied from about 70% to 92% of theoretical. While SSF was carried out for almost 168 hours, most of the ethanol had been produced by 72 hours. As expected, the hybrid poplar feedstock (-40 mesh) yielded little ethanol, only 8% of that theoretically possible based on the glucan plus mannan content. The α -cellulose provided final ethanol yields similar to the better pretreated solids, about 85%, but showed a slower rate of ethanol production compared to them. Thus, ethanol was still being produced up to 96 hours and even later with α -cellulose. This may be related to enzyme accessibility of the α -cellulose. Although provided as a finely ground material, some fiber character was evident, and it had been dried to less than 5% moisture content which would result in some irreversible loss of surface area. The hybrid poplar pretreated solids had never been dried and were at approximately 70% moisture content.

Duplicate samples were run in all experiments and the results from both are presented in the tables and figures. Most experiments gave good replication of ethanol concentration but some anomalies were observed. During the first series of SSF experiments (reactions 1, 2, 5, 7) it was observed that water level in the fermentation lock tended to be pulled up toward the shake flask as the flask was removed from 38°C incubator shaker for sampling at ambient temperature and a vacuum formed. If water was pulled into the flask the ethanol concentration in the next and all subsequent samples would be lowered. This is the probable explanation for the lowered ethanol concentration after 96 hours in reaction 1-1. This reaction probably achieved greater than 90% yield of ethanol. Another potential problem can arise when the periodic samples are removed from the shake flask. These samples are placed in screw cap centrifuge tubes and placed in a beaker of ice and ice water while the other shake flasks are being sampled. If a tube is inadvertently pushed below the level of ice water in the beaker, and the screw cap is not completely tightened, the vacuum can draw water into the collected sample. This would result in a lower ethanol concentration for only a single sampling time and, probably, for only one of the two duplicates. This may explain the anomalies observed with reaction 5-1 at 48 hours and 5-2 and 96 hours and reaction 6-1 at 96 hours. The poor duplication of ethanol results in reaction 4 arises from differences in the production or utilization of cellobiose in the two shake flasks, possibly related to more enzyme inhibition in 4-2. The analysis of cellobiose and minor fermentation products shows marked differences between 4-1 and 4-2.

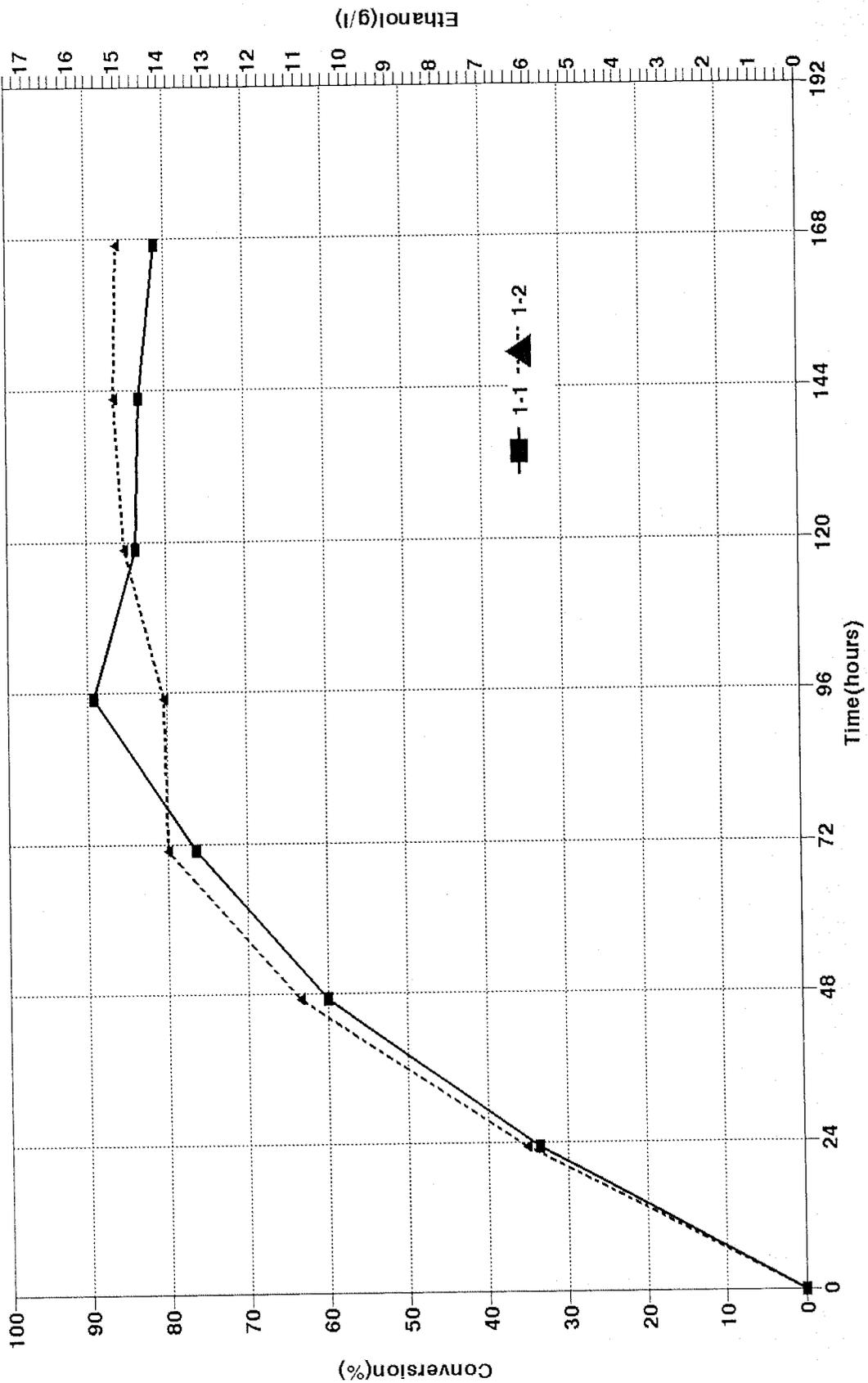


Figure 14. Ethanol Production in SSF. Hybrid Poplar Reaction 1.

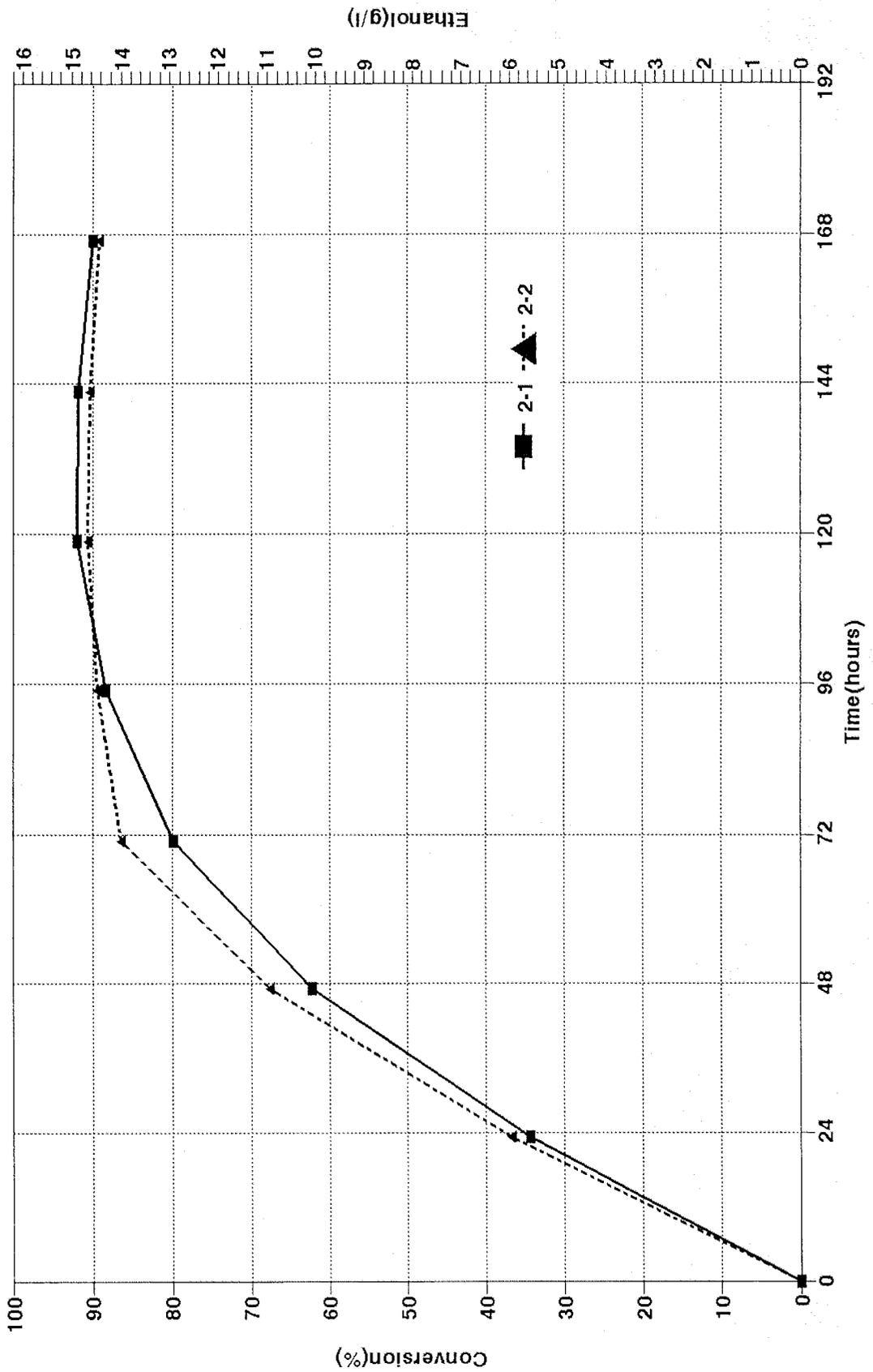


Figure 15. Ethanol Production in SSF. Hybrid Poplar Reaction 2.

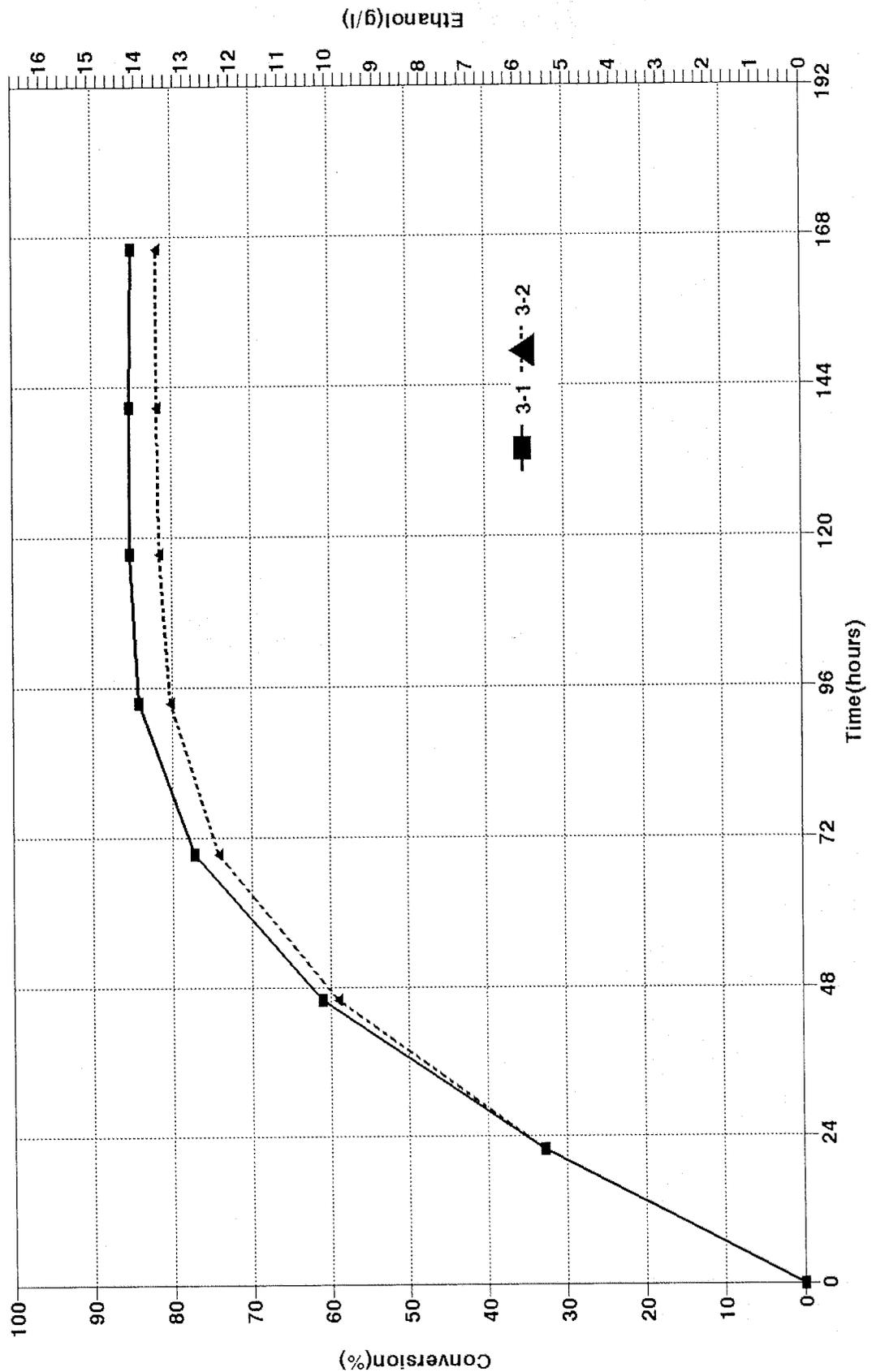


Figure 16. Ethanol Production in SSF. Hybrid Poplar Reaction 3.

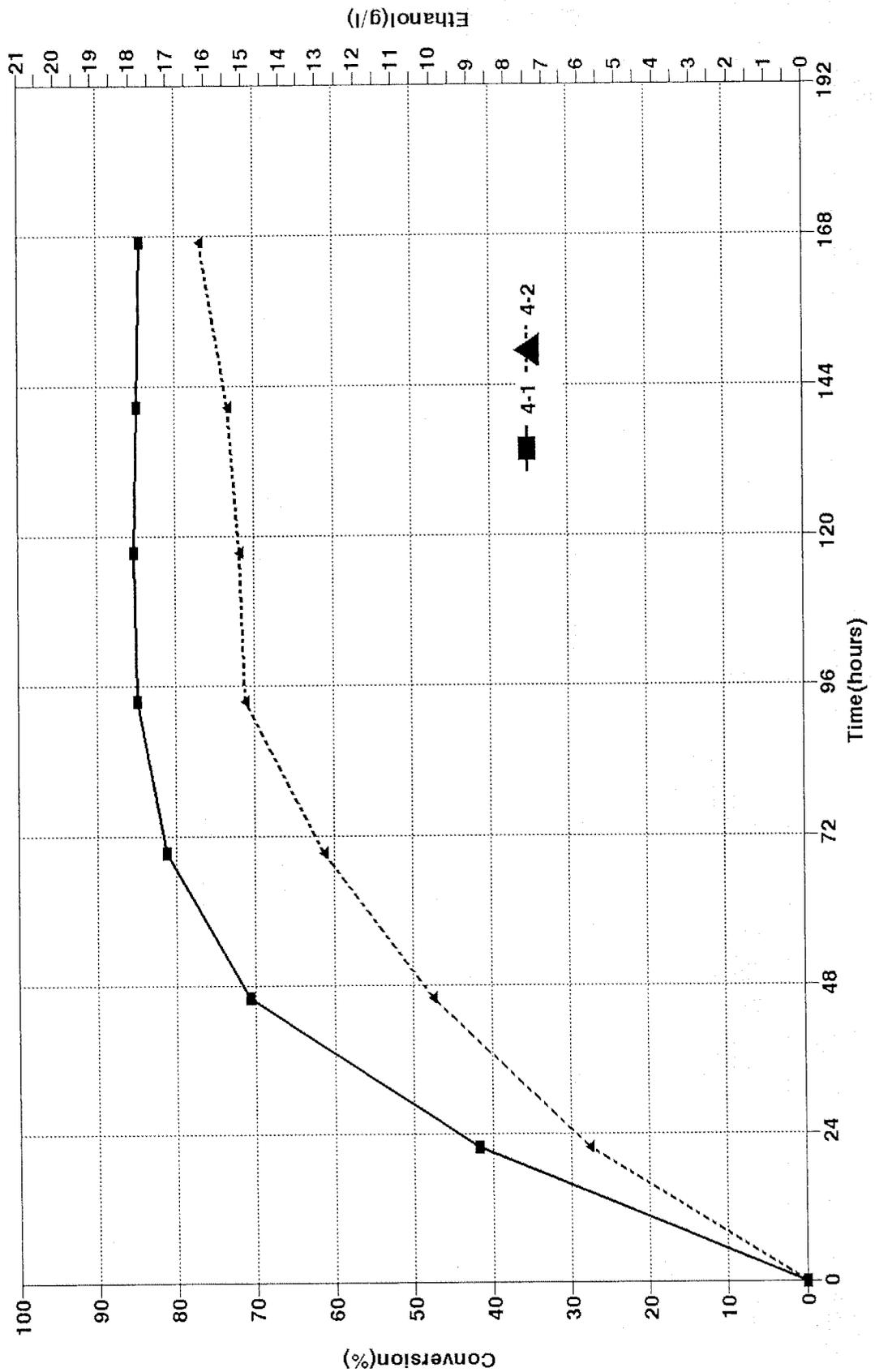


Figure 17. Ethanol Production in SSF. Hybrid Poplar Reaction 4.

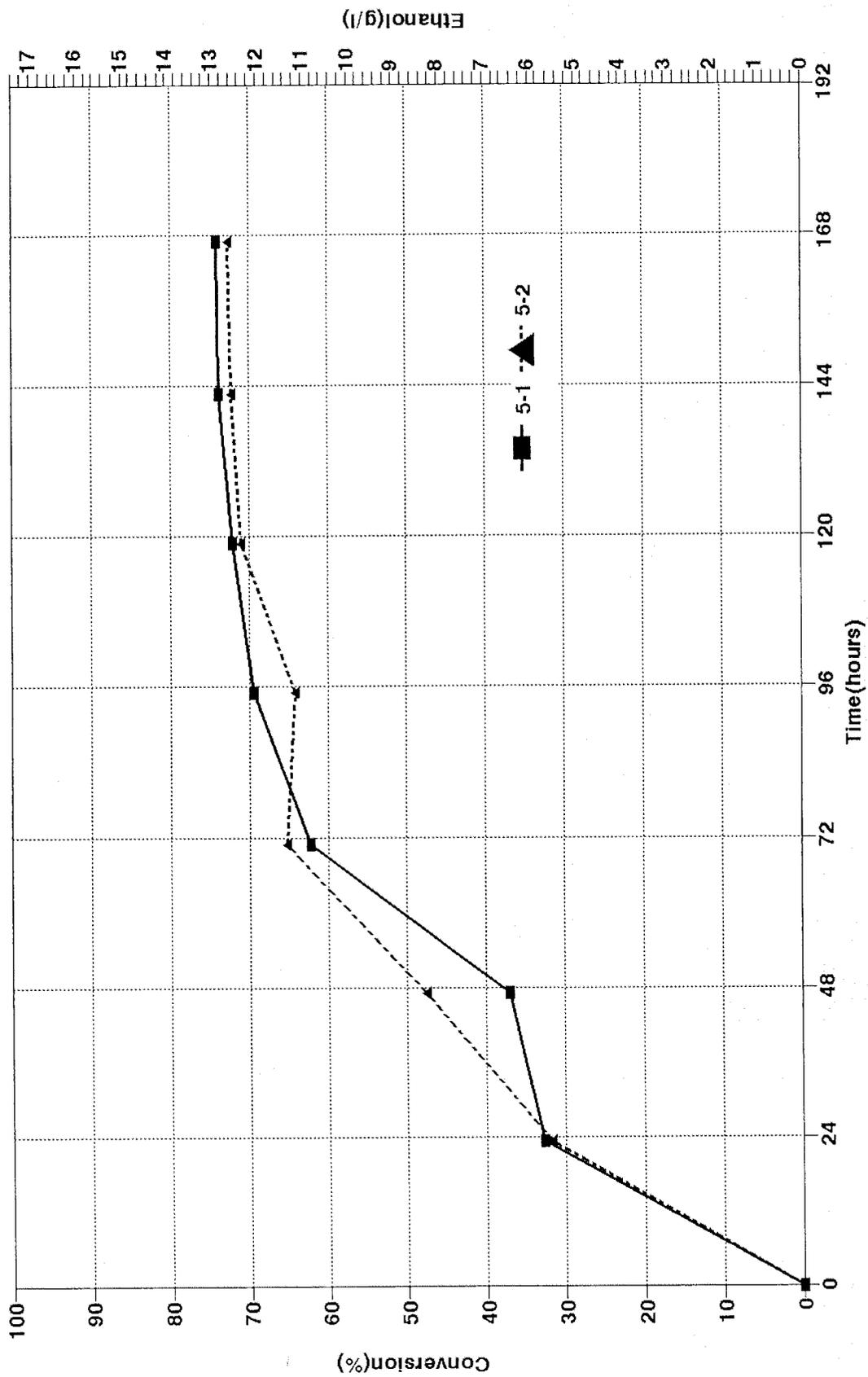


Figure 18. Ethanol Production in SSF. Hybrid Poplar Reaction 5.

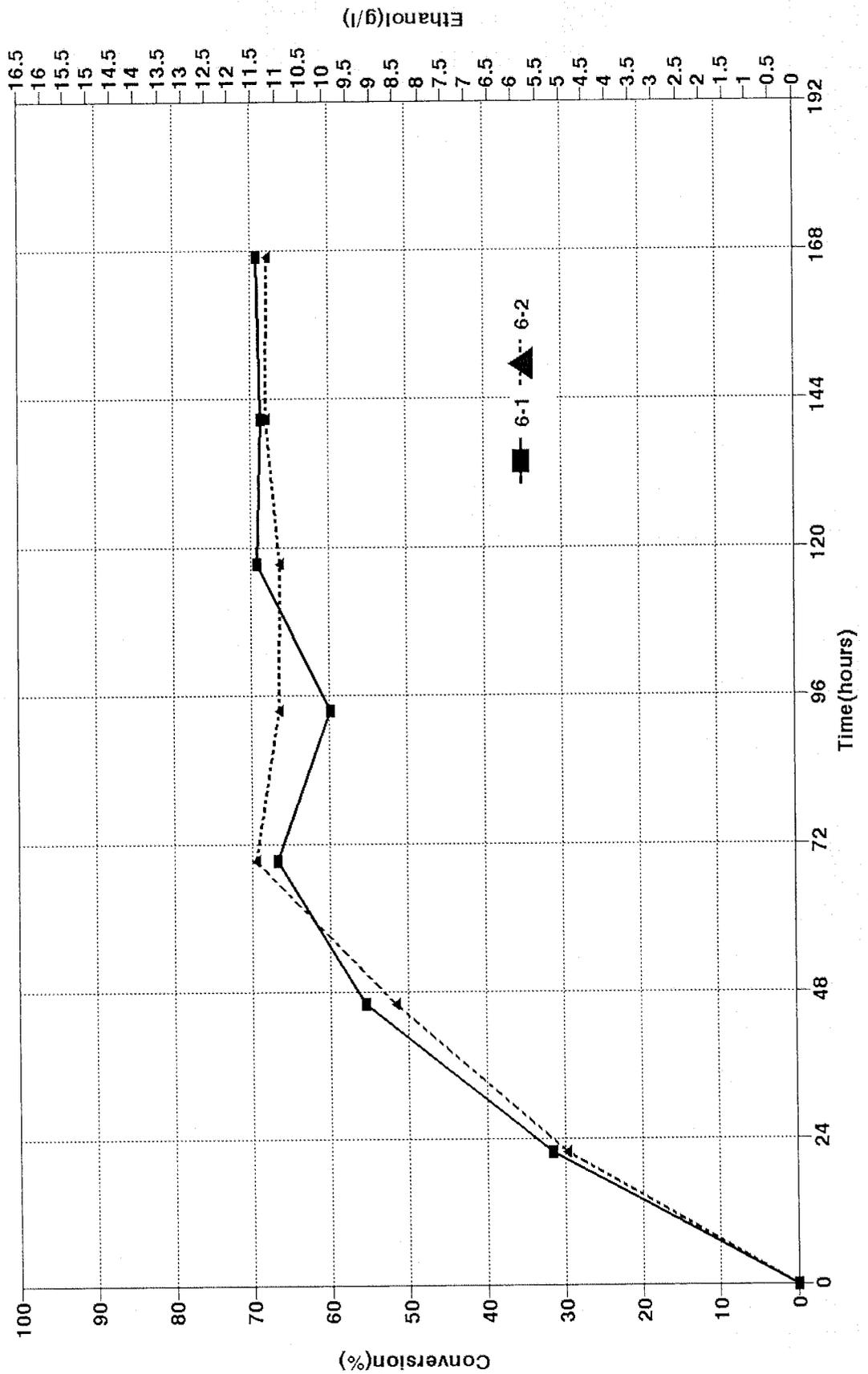


Figure 19. Ethanol Production in SSF. Hybrid Poplar Reaction 6.

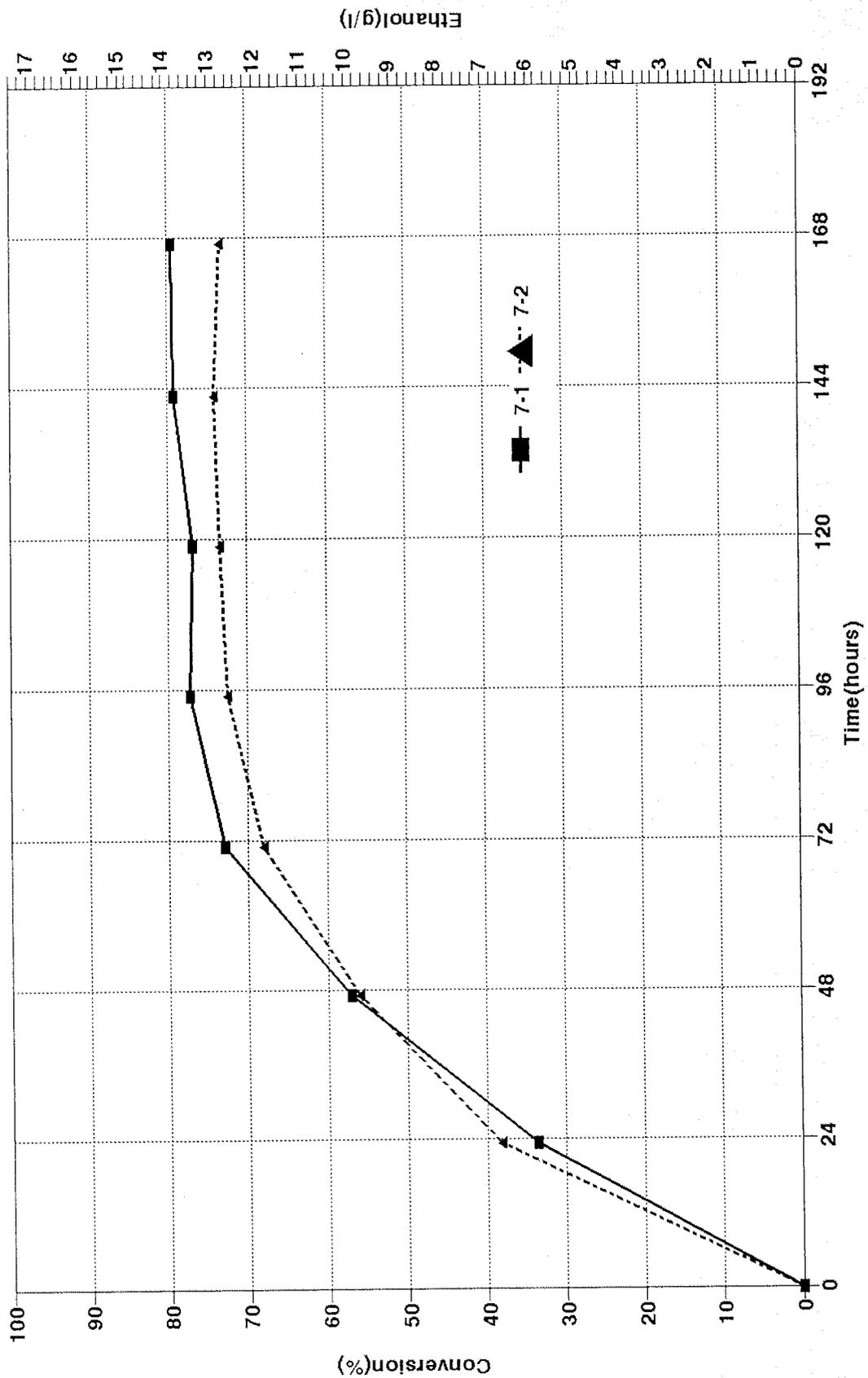


Figure 20. Ethanol Production in SSF. Hybrid Poplar Reaction 7.

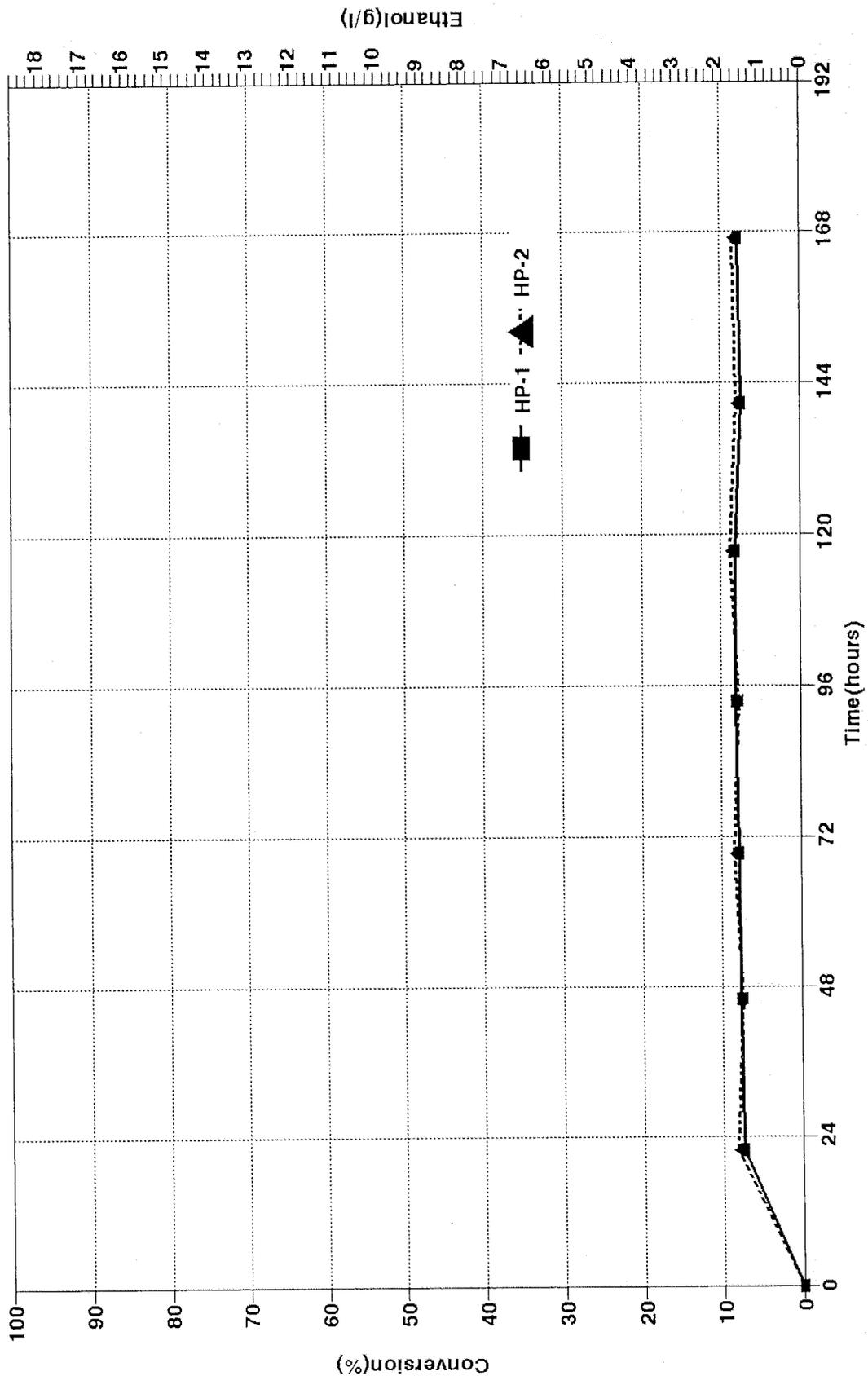


Figure 21. Ethanol Production in SSF. Hybrid Poplar Feedstock.

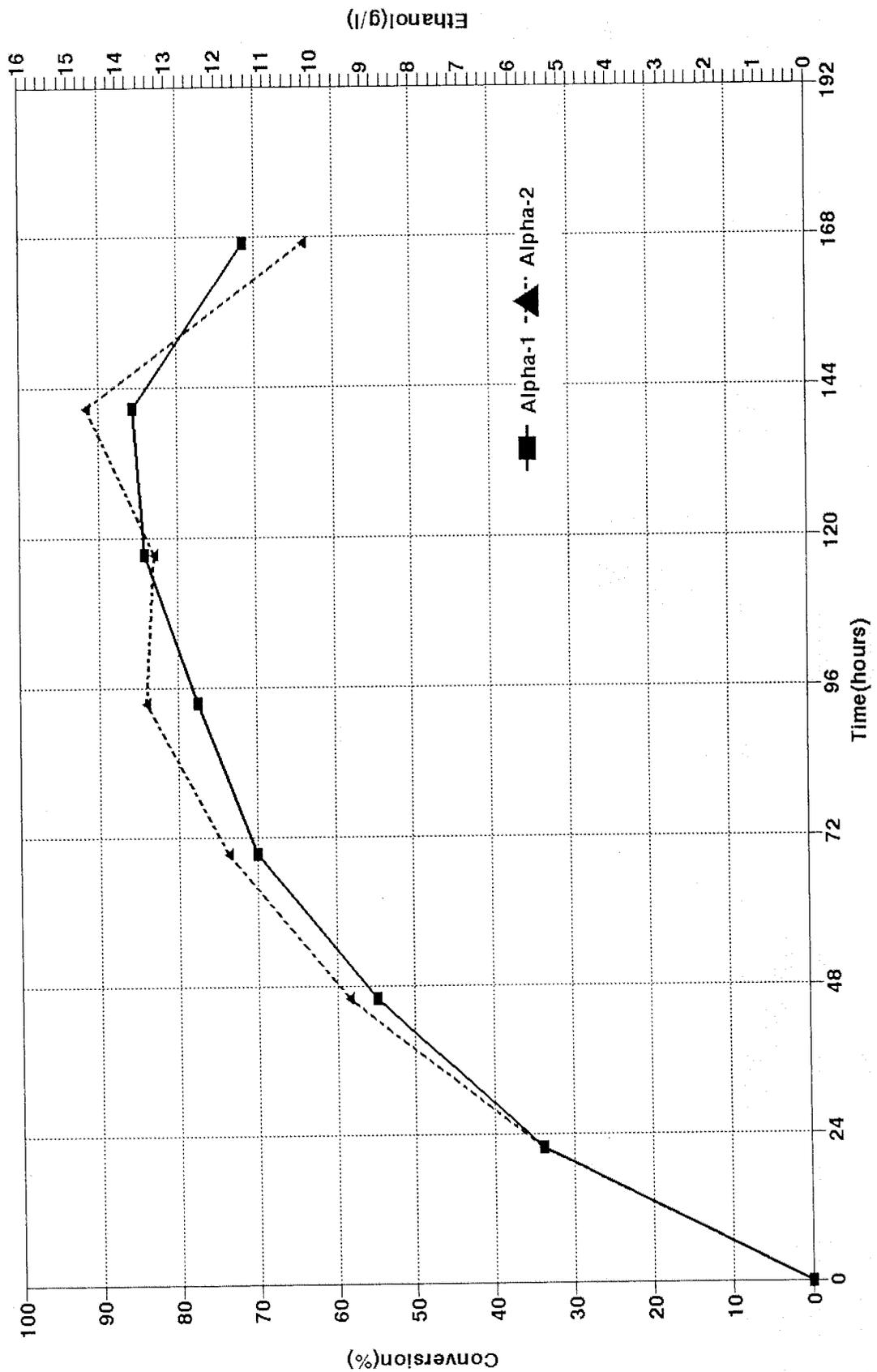


Figure 22. Ethanol Production in SSF. Alpha Cellulose.

Table 33. Ethanol Yield from SSF in Series #1.

Time,h	Reaction 1				Reaction 2			
	1-1		1-2		2-1		2-2	
	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23.3	5.8	33.7	6.1	35.2	5.6	34.4	6.0	36.9
47.3	10.4	60.0	11.0	63.4	10.1	62.1	11.0	67.5
71.1	13.2	76.3	13.9	79.9	13.0	79.7	14.1	86.4
95.1	15.5	89.2	13.9	80.4	14.4	88.4	14.5	89.4
118.8	14.5	83.7	14.8	85.1	15.0	91.9	14.7	90.5
142.9	14.4	83.1	15.0	86.4	14.9	91.8	14.7	90.4
167.2	14.1	81.1	14.9	85.9	14.6	90.0	14.5	89.1
Theor.Conv.	17.3	100.0	17.3	100.0	16.3	100.0	16.3	100.0

Time,h	Reaction 5				Reaction 7			
	5-1		5-2		7-1		7-1	
	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23.3	5.7	32.6	5.5	31.9	5.8	33.7	6.6	38.3
47.3	6.4	37.0	8.3	47.6	9.9	56.9	9.7	55.9
71.1	10.8	62.1	11.3	65.1	12.6	72.7	11.8	68.0
95.1	12.0	69.3	11.1	64.1	13.4	77.1	12.5	72.3
118.8	12.5	71.9	12.3	70.9	13.3	76.8	12.7	73.4
142.9	12.8	2.0	12.5	72.1	13.7	79.1	12.8	74.0
167.2	12.8	73.9	12.6	72.6	13.8	79.4	12.7	73.2
Theor.Conv.	17.4	100.0	17.4	100.0	17.3	100.0	17.3	100.0

Table 34. Ethanol Yield from SSF in Series #2.

Time,h	Reaction 3				Reaction 4			
	3-1		3-2		4-1		4-2	
	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21.8	5.5	32.9	5.5	32.9	8.8	41.9	5.8	27.6
46.0	10.1	61.0	9.8	58.9	14.8	70.5	10.0	47.7
69.3	12.8	77.2	12.3	74.2	17.0	81.2	12.8	61.1
93.7	14.0	84.2	13.3	80.1	17.8	84.8	14.9	71.2
117.3	14.2	85.2	13.5	81.5	17.8	85.1	15.1	71.8
140.8	14.1	85.1	13.6	81.8	17.7	84.7	15.3	73.2
166.2	14.1	84.9	13.6	81.7	17.7	84.3	16.1	76.8
Theor.Conv.	16.6	100.0	16.6	100.0	21.0	100.0	21.0	100.0

Time,h	Reaction 6				HP Feedstock (-40 mesh)			
	6-1		6-2		HP-1		HP-2	
	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21.8	5.2	31.6	4.9	29.8	1.4	7.5	1.6	8.2
46.0	9.1	55.3	8.5	51.6	1.5	7.7	1.4	7.4
69.3	11.0	66.6	4.0	69.3	1.5	7.9	1.6	8.4
93.7	9.8	59.7	10.9	66.4	1.6	8.3	1.5	7.9
117.3	11.4	69.2	10.9	66.2	1.6	8.3	1.7	8.8
140.8	11.3	68.5	11.2	67.9	1.4	7.5	1.5	8.0
166.2	11.4	69.1	11.2	67.7	1.5	7.8	1.6	8.5
Theor.Conv.	16.5	100.0	16.5	100.0	19.3	100.0	19.3	100.0

Table 34a. Ethanol Yield from SSF in Series #2 (continued).

Time,h	Alpha Cellulose			
	Alpha-1		Alpha-2	
	EtOH,g/l	Conv.,%	EtOH,g/l	Conv.,%
0.0	0.0	0.0	0.0	0.0
21.8	5.5	34.1	5.4	33.9
46.0	9.4	58.4	8.8	54.8
69.3	11.8	73.5	11.2	70.0
93.7	13.4	83.9	12.4	77.3
117.3	13.3	82.9	13.5	84.1
140.8	14.7	91.6	13.7	85.5
166.2	10.2	63.7	11.4	71.4
Theor.Conv.	16.0	100.0	16.0	100.0

Table 35. Products From SSF, Reaction 1.

1-1 Time,h	EtOH		Glucose, g/l		Cellobiose g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
	HPLC	GSC	HPLC	Sigma510A				
0.0	2.0	2.4	1.40	1.26	0	0.09	0.12	0.14
23.3	7.9	10.1	1.31	0.12	3.01	0	0.16	0.21
47.3	12.5	12.9	0.84	0.07	1.52	0	0.20	0.25
71.1	15.3	14.5	0.58	0.04	0.64	0	0.45	0.44
95.1	17.6	15.2	0.58	0.01	0	0	0.36	0.60
118.8	16.6	16.4	0.43	0.03	0	0.02	0.32	0.61
142.9	16.5	16.0	0.25	0.02	0	0	0.32	0.70
167.2	16.2	15.2	0.27	0.03	0	0	0.22	0.71
Reaction 1-2								
0.0	2.7	2.3	1.39	1.34	0	0.06	0.12	0.12
23.3	8.2	6.2	1.53	0.12	2.57	0	0.16	0.17
47.3	13.1	12.8	1.05	0.04	1.20	0	0.22	0.19
71.1	16.0	15.6	0.19	0.06	0.45	0	0.27	0.26
95.1	16.0	15.1	0.54	0.03	0.35	0	0.26	0.37
118.8	16.9	16.4	0.39	0.02	0	0	0.23	0.45
142.9	17.1	16.2	0.47	0.02	0	0	0.38	0.58
167.2	17.0	15.8	0.41	0.03	0	0	0.37	0.67

5.2.2 Other Products from SSF

The concentrations of cellobiose, lactic acid, glycerol and acetic acid at different sampling times from zero to 168 hours as determined by HPLC are shown in Tables 35 - 43. Also included is a comparison of glucose and ethanol each determined by two different methods. The concentrations of cellobiose, lactic acid, glycerol and acetic acid are plotted against time in Figures 23 - 31. The Tables compare ethanol determined by HPLC and by gas-solid chromatography (GSC). The two techniques produced similar results but the GSC values were consistently lower. The HPLC values were selected for use in calculating the adjusted ethanol concentration at zero time which subtracts the ethanol present in the inoculum added to the shake flask. The HPLC determinations used a larger number of standards and gave very good linear regression correlation coefficients. Later, a YSI analyzer was obtained and one of the sets (reaction 2-1) of samples was reanalyzed for ethanol. The YSI values were very close to those determined by HPLC as shown in Table 36. Glucose was determined by HPLC (refractive index detector) and by Sigma 510A diagnostic method. The latter utilizes glucose oxidase enzymes and color reagent which is measured spectrophotometrically. As the glucose concentration during SSF is very low at any given time (except for the zero time sample) the Sigma method with its lower level of detectability (0.01g/l) was selected for use in the SSF evaluation. For enzyme hydrolysis experiments, where glucose levels are much higher, the HPLC procedure was found to be more accurate.

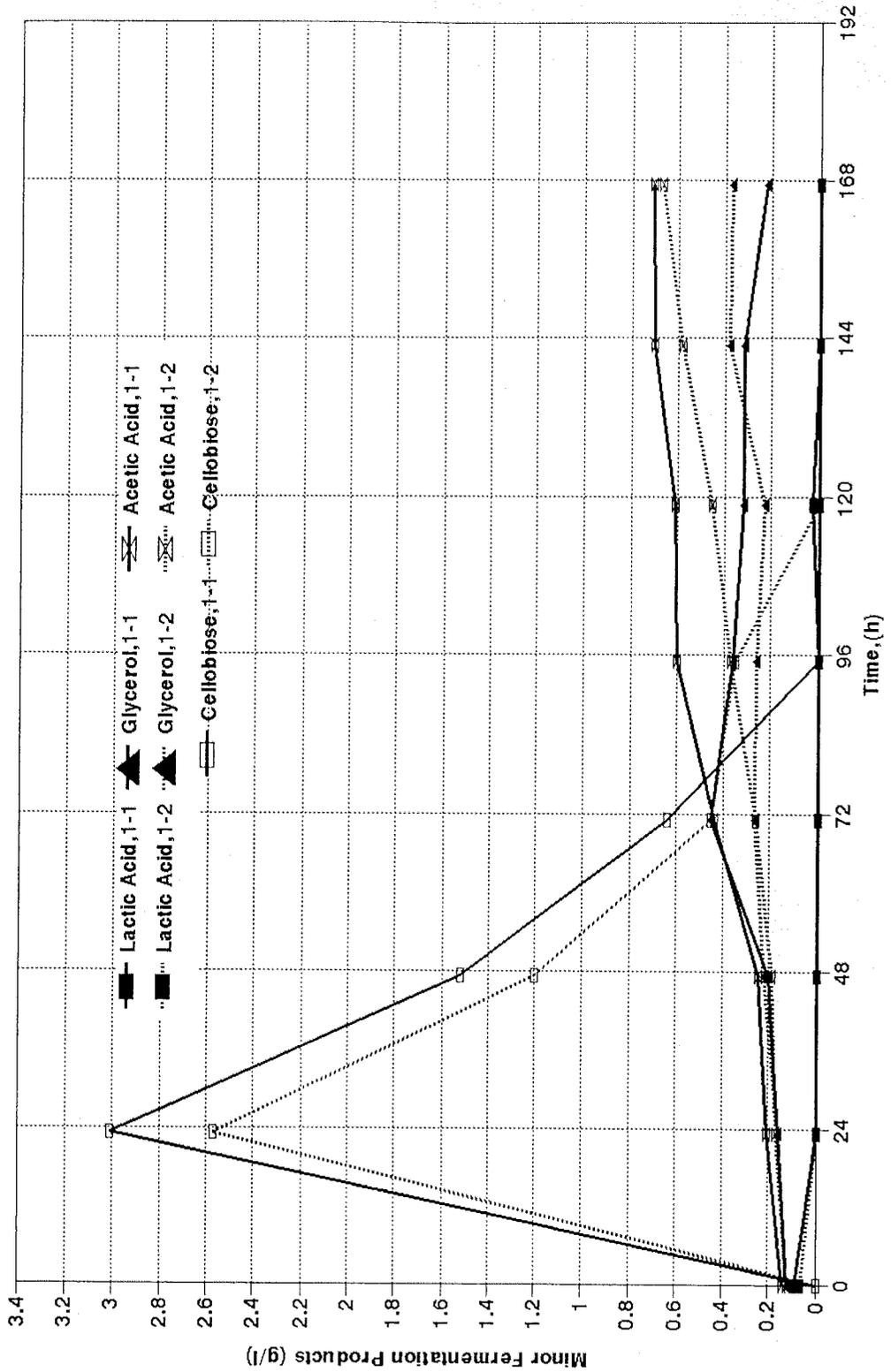


Figure 23. SSF Minor Products. Hybrid Poplar Reaction 1.

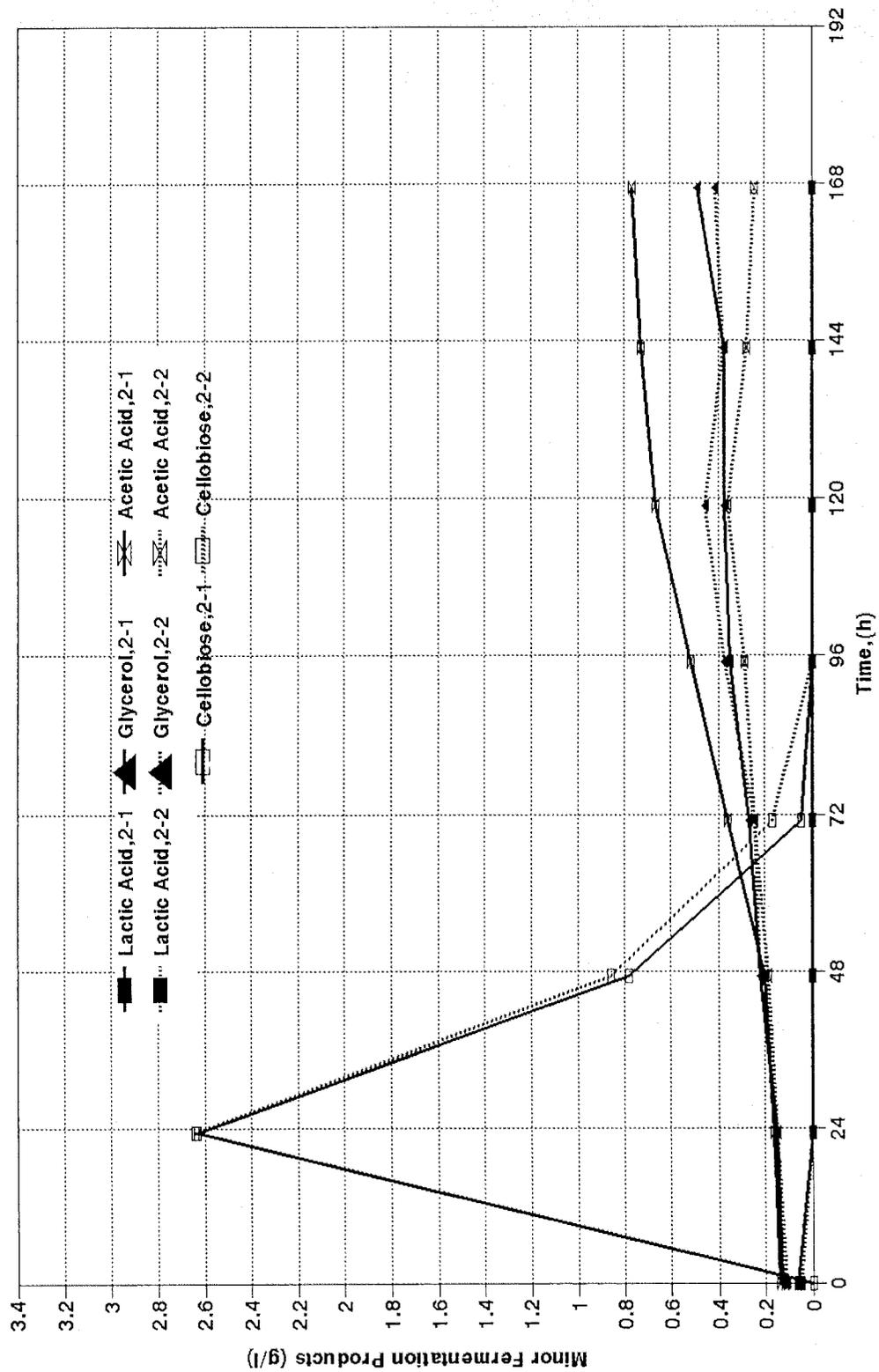


Figure 24. SSF Minor Products. Hybrid Poplar Reaction 2.

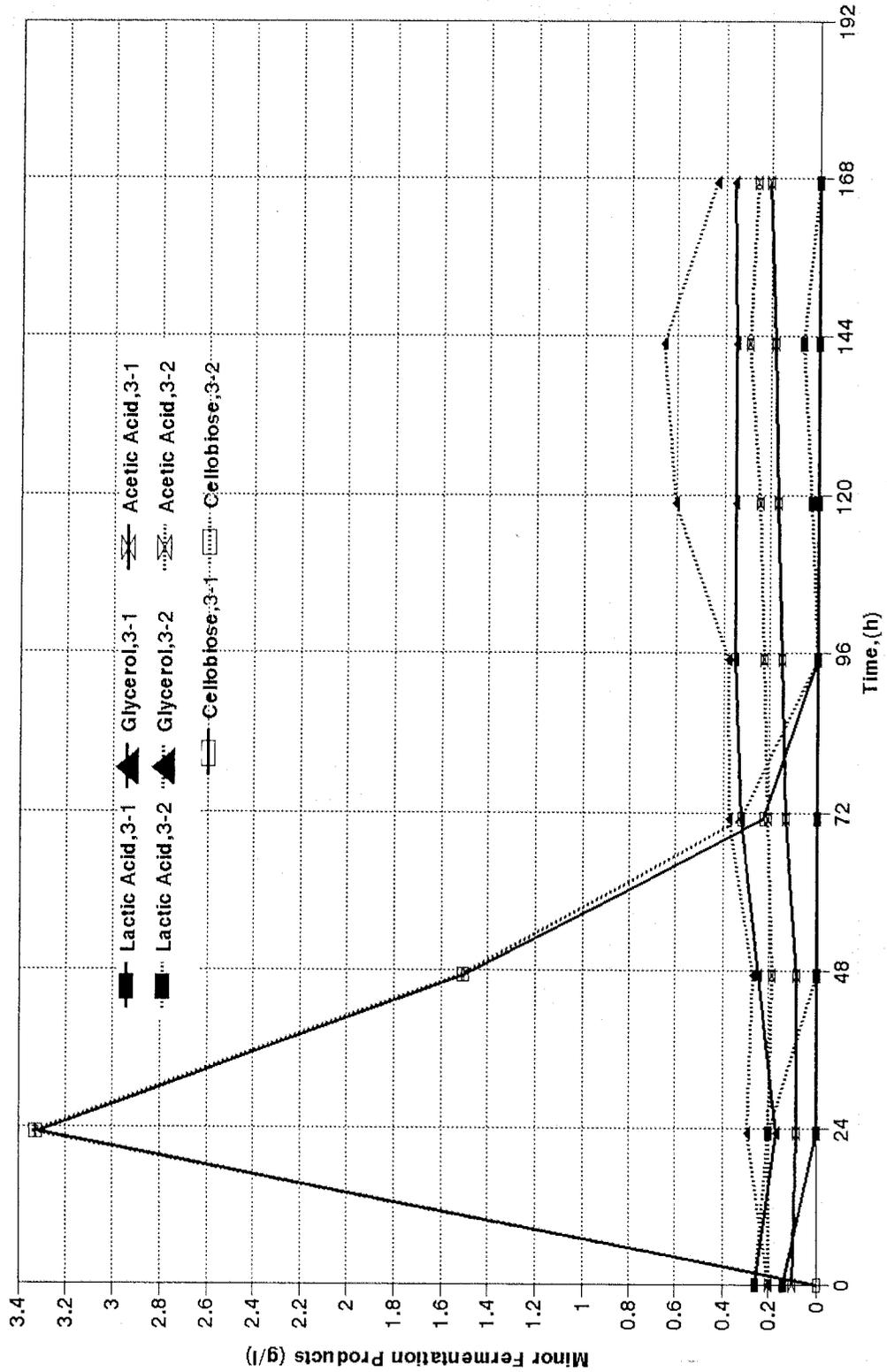


Figure 25. SSF Minor Products. Hybrid Poplar Reaction 3.

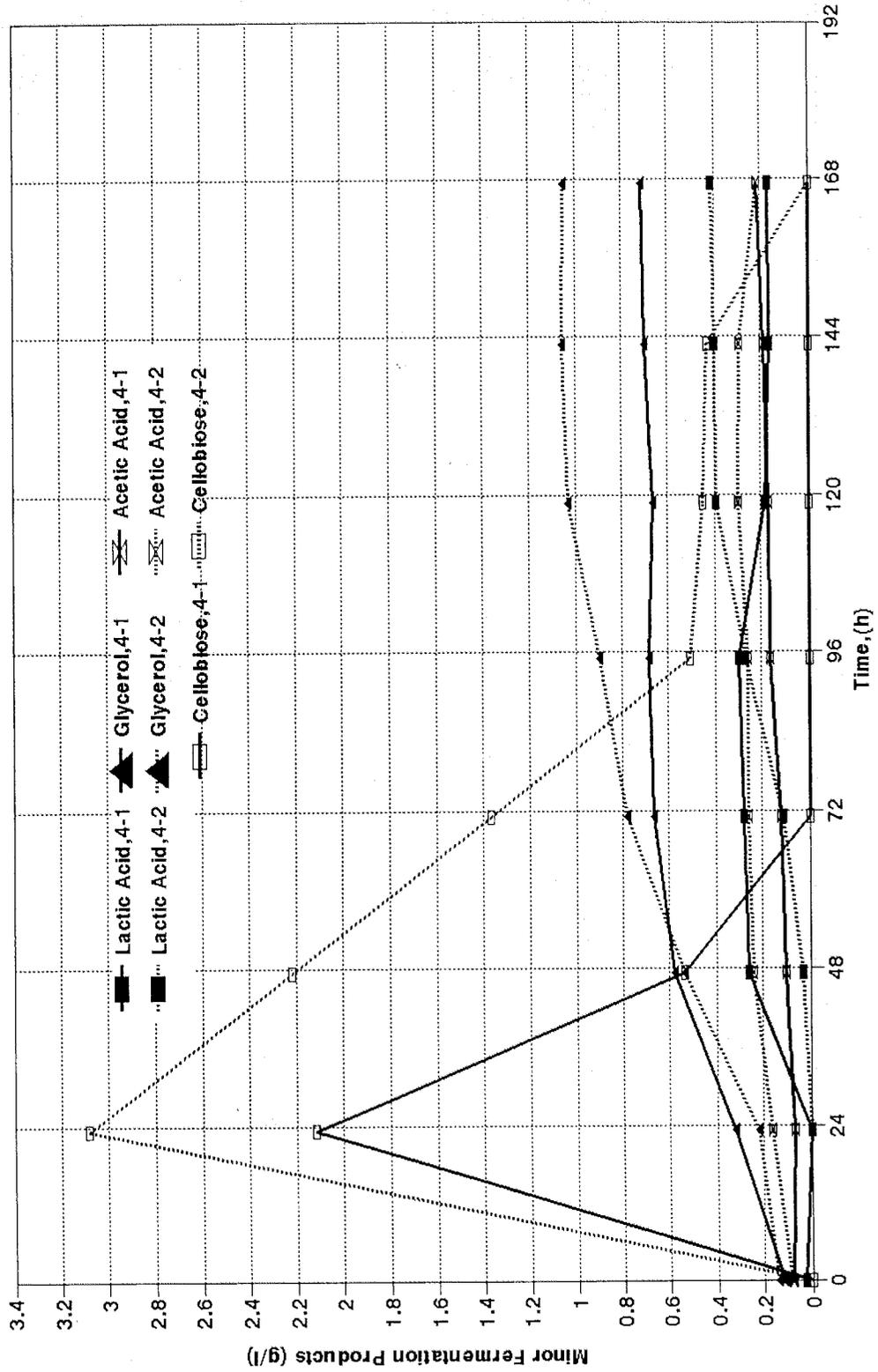


Figure 26. SSF Minor Products. Hybrid Poplar Reaction 4.

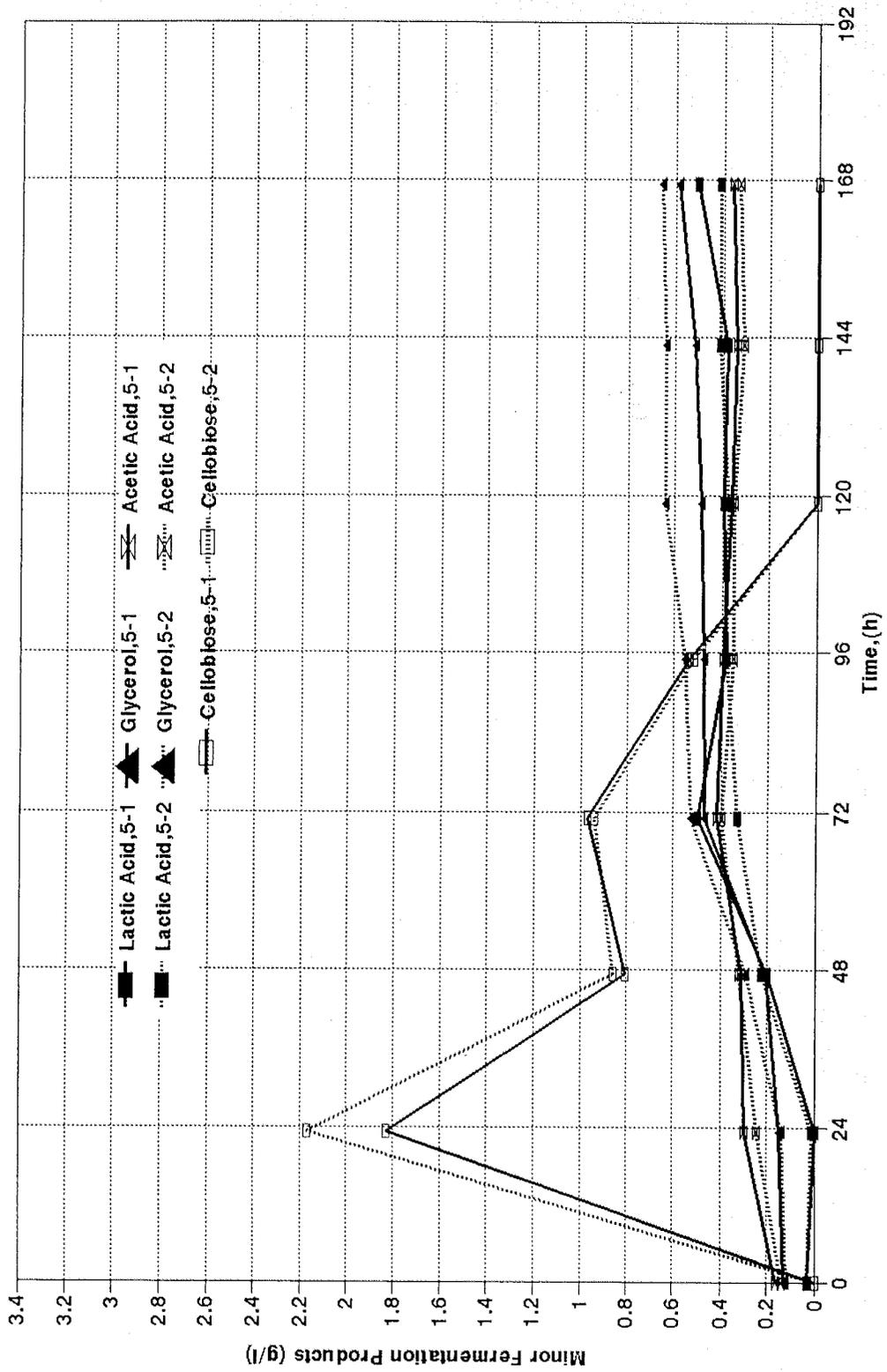


Figure 27. SSF Minor Products. Hybrid Poplar Reaction 5.

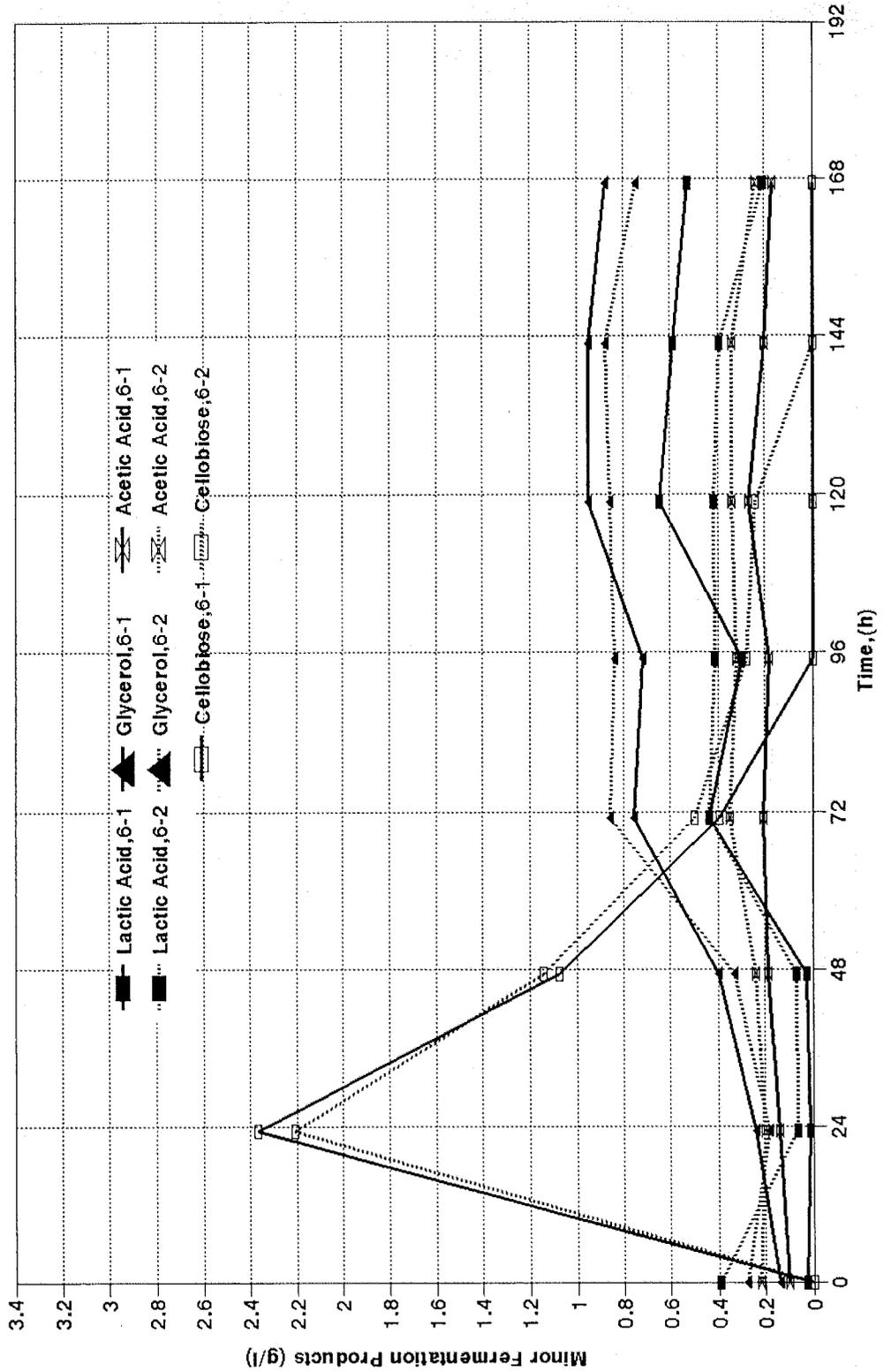


Figure 28. SSF Minor Products. Hybrid Poplar Reaction 6.

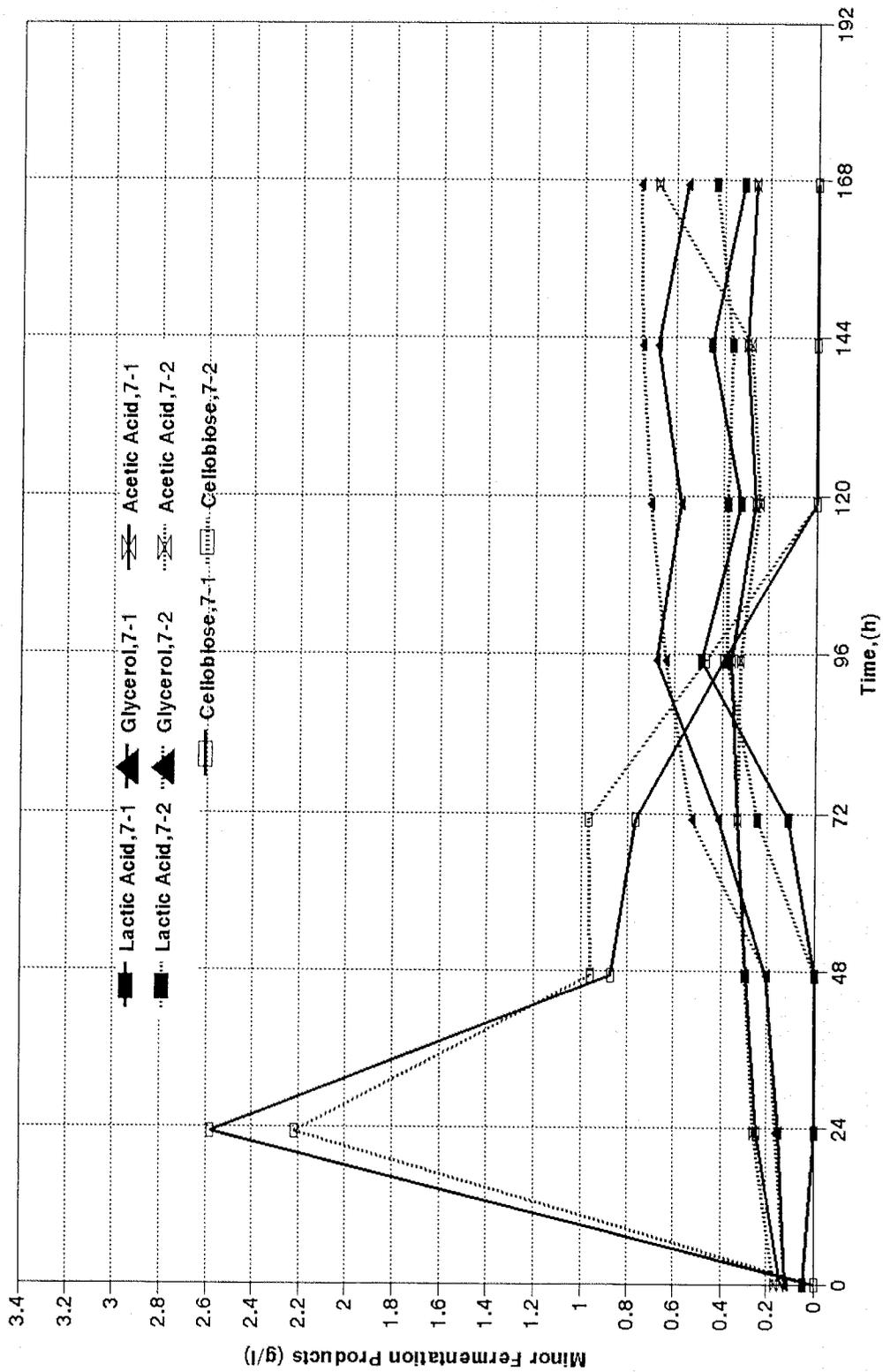


Figure 29. SSF Minor Products. Hybrid Poplar Reaction 7.

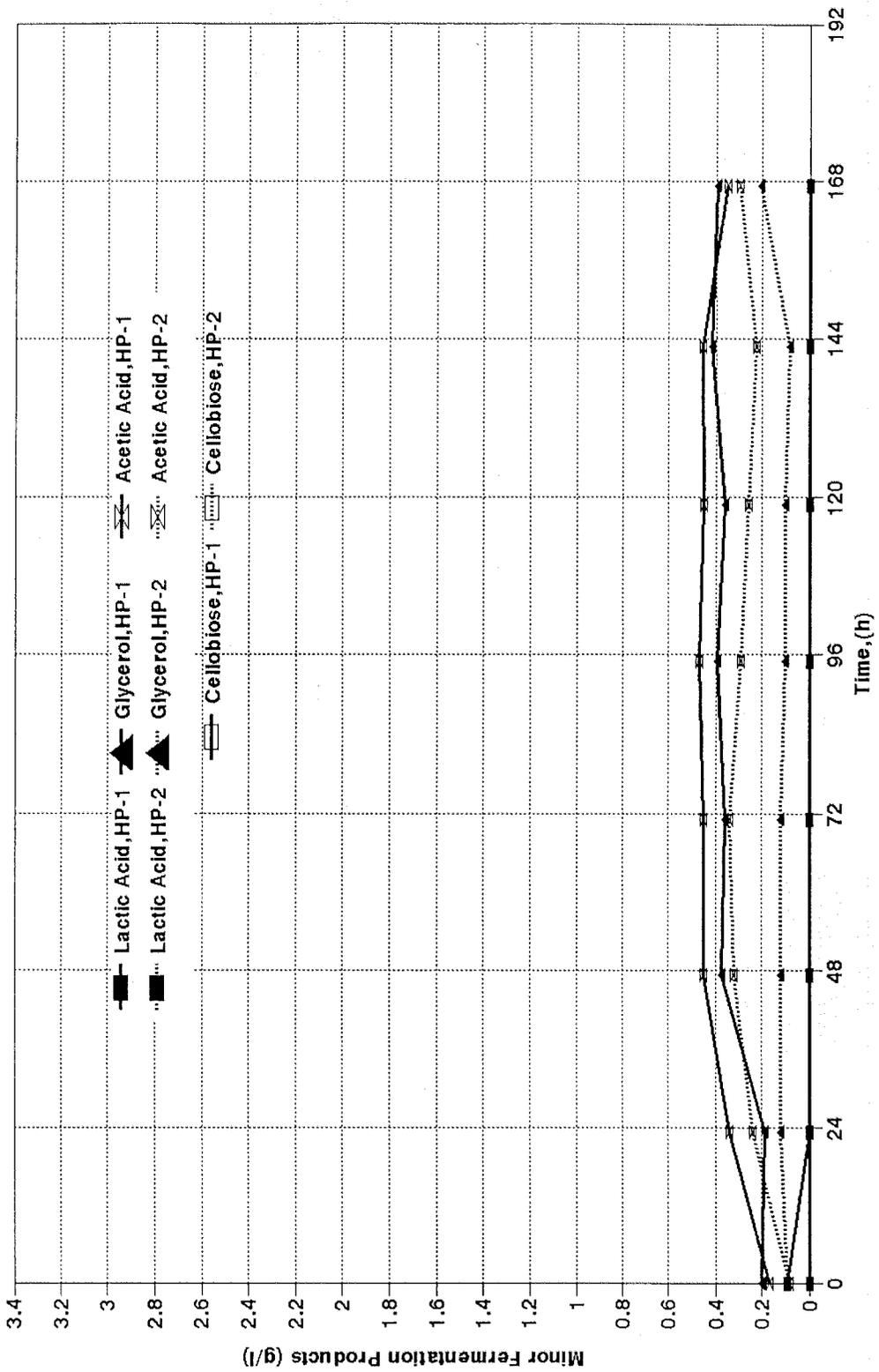


Figure 30. SSF Minor Products. Hybrid Poplar Feedstock.

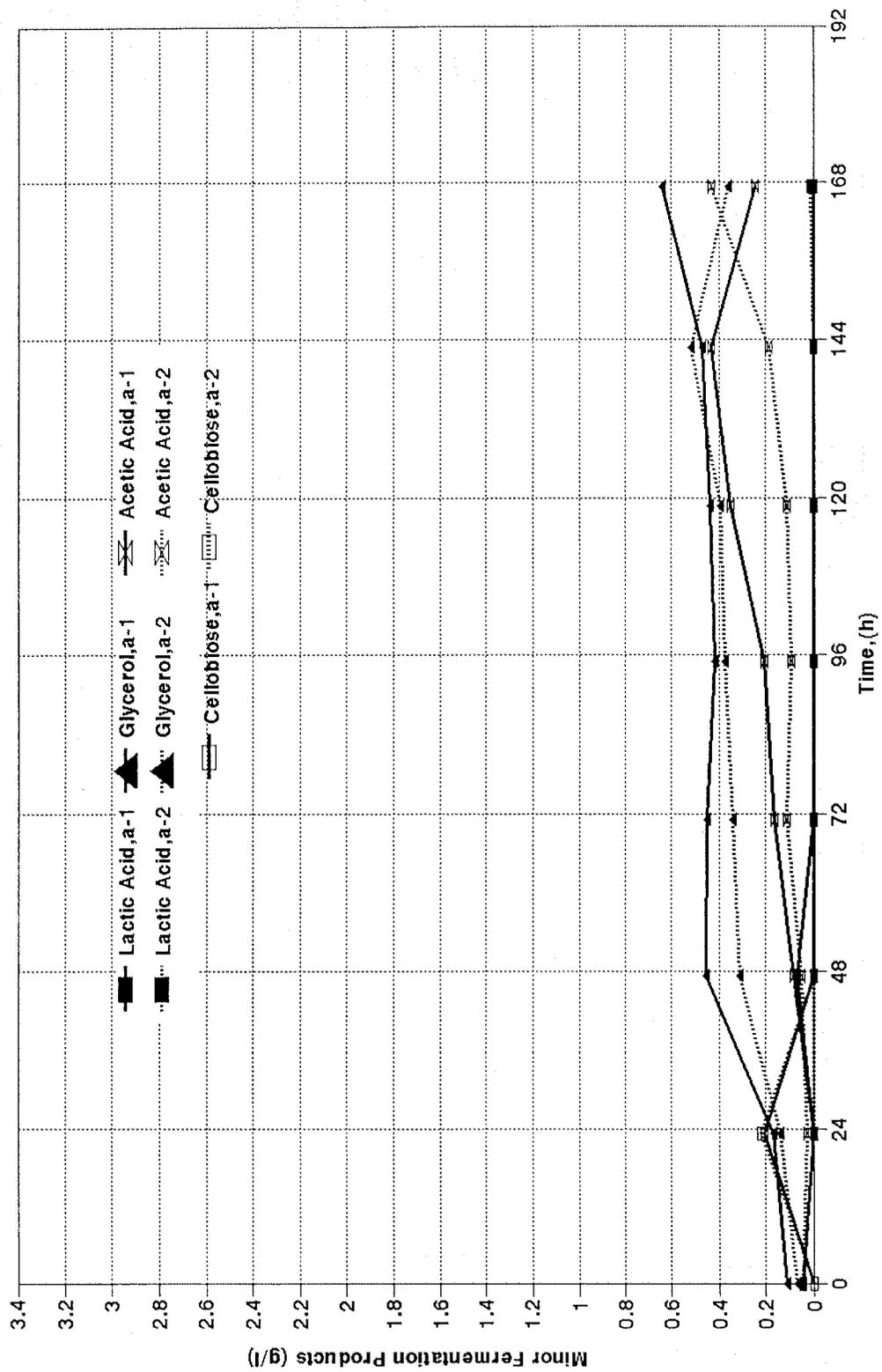


Figure 31. SSF Minor Products. Alpha Cellulose.

The cellobiose concentration was invariably highest after 24 hours of SSF and normally was not found after 72 hours. This closely parallels the completion of ethanol production from pretreated solids. The other minor products from SSF were usually present in concentrations below 0.8 g/l. In reaction 4-2 where cellobiose was present in all but the last sampling at 168 hours, the glycerol concentration was the highest of all the SSF samples at over 1 g/l. Apparently, the inhibition which resulted in decreased ethanol production allowed an increase in other metabolic products.

Table 36. Products From SSF, Reaction 2.

2-1 Time,h	EtOH g/l			Glucose, g/l		Cellobiose g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
	HPLC	GSC	YSI	HPLC	Sigma 510A				
0.0	2.0	2.8	2.0	1.47	1.20	0	0.06	0.13	0.14
23.3	7.7	8.4	-	1.57	0.09	2.63	0	0.15	0.16
47.3	12.2	12.1	11.9	1.03	0.03	0.78	0	0.22	0.20
71.1	15.1	14.4	-	0.53	0.05	0.04	0	0.27	0.36
95.1	16.5	15.9	16.3	0.52	0.04	0	0	0.35	0.52
118.8	17.1	17.4	17.1	0.39	0.03	0	0	0.37	0.67
142.9	17.0	----	17.0	0.32	0.02	0	0	0.37	0.73
167.2	16.8	16.0	16.8	0.30	0.03	0	0	0.49	0.77
Reaction 2-2									
0.0	2.0	2.5		1.41	1.00	0	0.05	0.11	0.12
23.3	8.1	8.3		1.52	0.10	2.64	0	0.15	0.15
47.3	13.1	12.9		0.91	0.02	0.86	0	0.22	0.19
71.1	16.2	15.7		0.44	0.05	0.17	0	0.25	0.25
95.1	16.7	17.3		0.34	0.03	0	0	0.37	0.29
118.8	16.8	16.0		0.14	0.03	0	0	0.46	0.36
142.9	16.8	16.1		0.12	0.03	0	0	0.38	0.28
167.2	16.6	16.0		0.11	0.03	0	0	0.41	0.25

Table 37. Products From SSF, Reaction 3.

3-1 Time,h	EtOH		Glucose, g/l		Cellobiose g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
	HPLC	GSC	HPLC	Sigma510A				
0	2.3	2.1	1.84	0.22	0	0.14	0.26	0.10
21.8	7.6	7.4	0.53	0.23	3.31	0	0.17	0.08
46	12.3	11.7	0.14	0.09	1.50	0	0.25	0.08
69.3	15.0	14.3	0	0.05	0.23	0	0.32	0.13
93.7	16.2	14.0	0	0.03	0	0	0.35	0.15
117.3	16.3	14.3	0	0.04	0	0	0.35	0.17
140.8	16.3	15.5	0	0.05	0	0	0.35	0.18
166.2	16.3	15.0	0	0.02	0	0	0.36	0.21

Reaction 3-2

0	1.9	2.1	1.22	0.29	0	0.26	0.20	0.20
21.8	7.6	7.6	1.15	0.25	3.34	0.2	0.29	0.20
46	12.0	12.0	0.74	0.09	1.52	0	0.27	0.19
69.3	14.5	14.6	0.44	0.06	0.32	0	0.37	0.21
93.7	15.5	15.0	0.26	0.03	0	0	0.38	0.23
117.3	15.7	15.6	0.34	0.04	0	0.02	0.61	0.25
140.8	15.8	15.5	0	0.06	0	0.06	0.66	0.29
166.2	15.7	14.4	0	0.03	0	0	0.44	0.26

Table 38. Products From SSF, Reaction 4.

4-1 Time,h	EtOH g/l		Glucose, g/l		Cellobiose g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
	HPLC	GSC	HPLC	Sigma510A				
0	2.1	2.3	1.27	0.58	0	0.03	0.12	0.08
21.8	11.0	10.7	0.71	0.35	2.12	0.00	0.32	0.07
46	16.9	16.2	0.17	0.09	0.54	0.26	0.58	0.10
69.3	19.2	17.7	0.01	0.11	0	0.28	0.67	0.12
93.7	19.9	18.6	0.00	0.04	0	0.30	0.69	0.16
117.3	20.0	18.9	0.00	0.04	0	0.18	0.67	0.17
140.8	19.9	18.2	0.00	0.04	0	0.16	0.70	0.19
166.2	19.8	18.5	0.22	0.03	0	0.17	0.72	0.22

Reaction 4-2

0	2.6	2.4	1.14	0.24	0	0.02	0.14	0.08
21.8	8.0	7.6	0.48	0.23	3.08	0.00	0.22	0.16
46	12.2	12.1	0.29	0.10	2.22	0.03	0.54	0.24
69.3	15.0	14.8	0.30	0.11	1.37	0.11	0.78	0.26
93.7	17.0	17.6	0.24	0.04	0.51	0.27	0.90	0.26
117.3	17.2	16.9	0.43	0.05	0.45	0.39	1.03	0.30
140.8	17.5	16.8	0.66	0.14	0.43	0.40	1.06	0.29
166.2	18.3	16.2	1.34	0.32	0	0.41	1.05	0.22

Table 39. Products From SSF, Reaction 5.

5-1 Time,h	g/l		Glucose, g/l		Cellobiose g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
	EtOH HPLC	GSC	HPLC	Sigma510A				
0	2.0	1.9	1.48	0.98	0	0.03	0.13	0.16
23.3	7.8	8.9	1.59	0.13	1.83	0.00	0.15	0.30
47.3	8.5	----	1.05	0.08	0.81	0.20	0.21	0.31
71.1	12.9	12.8	0.73	0.09	0.97	0.50	0.47	0.42
95.1	14.1	12.7	0.39	0.05	0.54	0.38	0.48	0.39
118.8	14.6	14.6	0.24	0.04	0	0.39	0.49	0.36
142.9	14.9	----	0.27	0.03	0	0.38	0.52	0.34
167.2	15.0	15.5	0.24	0.05	0	0.51	0.59	0.36

Reaction 5-2

0	2.1	2.2	1.26	1.24	0	0.03	0.12	0.14
23.3	7.7	7.5	1.06	0.10	2.17	0.01	0.14	0.25
47.3	10.4	11.0	0.83	0.04	0.86	0.23	0.29	0.32
71.1	13.1	12.7	0.36	0.15	0.94	0.33	0.53	0.40
95.1	13.3	11.4	1.24	0.06	0.52	0.38	0.56	0.35
118.8	14.4	14.8	0	0.04	0	0.37	0.64	0.35
142.9	14.6	14.6	0	0.03	0	0.41	0.64	0.31
167.2	14.7	14.3	0	0.05	0	0.41	0.66	0.33

Table 40. Products From SSF, Reaction 6.

6-1 Time,h	EtOH g/l		Glucose, g/l		Cellobiose g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
	HPLC	GSC	HPLC	Sigma510A				
0	1.9	2.0	0.83	0.34	0	0.02	0.14	0.10
21.8	7.4	7.3	0.31	0.28	2.37	0.01	0.24	0.14
46	11.3	11.3	0.13	0.11	1.08	0.02	0.40	0.19
69.3	13.1	13.1	0.26	0.06	0.39	0.44	0.76	0.21
93.7	12.0	13.0	0.44	0.15	0	0.3	0.72	0.18
117.3	13.6	13.3	0.34	0.06	0	0.65	0.95	0.27
140.8	13.4	12.6	0.19	0.05	0	0.59	0.95	0.20
166.2	13.5	11.9	0.12	0.03	0	0.53	0.88	0.17
Reaction 6-2								
0	2.6	2.3	1.41	0.23	0	0.39	0.28	0.22
21.8	7.1	7.3	0.39	0.28	2.21	0.06	0.18	0.20
46	10.7	10.9	0.71	0.11	1.14	0.07	0.33	0.24
69.3	12.7	12.7	1.34	0.07	0.50	0.44	0.86	0.35
93.7	13.1	13.6	1.40	0.25	0.28	0.41	0.84	0.32
117.3	13.1	13.1	1.65	0.36	0.24	0.42	0.86	0.34
140.8	13.4	12.9	0.27	0.06	0	0.39	0.88	0.34
166.2	13.3	12.7	0	0.03	0	0.21	0.75	0.24

Table 41. Products From SSF, Reaction 7.

7-1 Time,h	EtOH		Glucose, g/l		Cellobiose g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
	HPLC	GSC	HPLC	Sigma510A				
0.0	1.8	2.3	1.15	1.25	0	0.04	0.12	0.14
23.3	8.0	4.2	1.12	0.12	2.58	0	0.15	0.25
47.3	12.0	12.0	0.95	0.06	0.87	0	0.21	0.29
71.1	14.7	14.0	0.54	0.05	0.77	0.11	0.41	0.33
95.1	15.5	14.9	0.41	0.06	0.39	0.49	0.68	0.36
118.8	15.4	15.3	0.20	0.04	0	0.32	0.58	0.26
142.9	15.8	15.3	0.19	0.04	0	0.45	0.68	0.30
167.2	15.9	14.9	0.10	0.04	0	0.31	0.56	0.26

Reaction 7-2

0.0	2.1	2.3	1.27	0.88	0	0.04	0.12	0.17
23.3	7.7	9.0	1.53	0.10	2.22	0	0.16	0.26
47.3	11.8	11.5	1.11	0.05	0.96	0	0.20	0.30
71.1	13.9	13.0	0.62	0.06	0.97	0.25	0.53	0.33
95.1	14.6	14.2	0.42	0.09	0.47	0.38	0.64	0.32
118.8	14.8	14.4	0.19	0.04	0	0.38	0.71	0.24
142.9	14.9	14.7	0.20	0.04	0	0.36	0.75	0.28
167.2	14.8	16.0	0.10	0.05	0	0.43	0.76	0.68

Table 42. Products From SSF, Reaction with Feedstock.

Time,h	EtOH g/l		Glucose, g/l		Cellobiose g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
	HPLC	GSC	HPLC	Sigma510A				
0	2.1	2.1	0.9	0.40	0	0.09	0.20	0.17
21.8	3.6	3.9	0	0.02	0	0	0.19	0.34
46	3.7	3.7	0	0.07	0	0	0.37	0.45
69.3	3.7	3.9	0	0.03	0	0	0.36	0.45
93.7	3.8	3.9	0	0.00	0	0	0.39	0.47
117.3	3.8	3.9	0	0.04	0	0	0.36	0.45
140.8	3.6	3.7	0	0.01	0	0	0.42	0.46
166.2	3.7	3.7	0	0.02	0	0	0.39	0.35
Feedstock HP-2								
0	2.0	1.8	1.21	0.68	0	0	0.09	0.08
21.8	3.8	3.9	0.25	0.03	0	0	0.12	0.24
46	3.6	3.9	0.21	0.08	0	0	0.12	0.32
69.3	3.8	3.9	0.17	0.03	0	0	0.12	0.34
93.7	3.7	3.9	0.17	0.00	0	0	0.10	0.29
117.3	3.9	3.7	0.11	0.03	0	0	0.10	0.26
140.8	3.7	3.8	0.14	0.13	0	0	0.08	0.23
166.2	3.8	4.1	0.18	0.02	0	0	0.21	0.30

5.2.3 Influence of Pretreatment Conditions on SSF Results

It is of interest to compare the conditions in the pretreatment reactions with the results of SSF of the resultant pretreated solids. Selected reaction conditions from Table 4 are compared with maximum ethanol yields from SSF in Table 44. Product yields from non-hydrolyzed hybrid poplar feedstock and alpha cellulose are also included. The four reactions run at a nominal 170°C gave better ethanol yields (85%-92%) than the three nominal 160°C prehydrolysis reactions (69%-79%). It is likely that a wood residence time of about 60 minutes is more nearly optimum at 160°C than the 36 - 38 minutes used. There is an indication that at 170°C the slightly longer residence time in Reaction no. 2 (27 minutes) gave better results than in Reaction no. 1 (25 minutes). Because the ethanol yield is expressed on the basis of the glucan present in the pretreated solids, not that present in the feedstock, the recovery of glucan in the pretreated solids must also be considered to determine the economically optimum condition. This will be considered in more detail later. The better conversion of glucan to ethanol from the more severe prehydrolysis conditions is probably related to the increased accessibility to cellulose by the enzymes, which arises from solubilizing more hemicellulose and other acid-soluble components in the wood. The xylan content of the 170 °C pretreated solids was largely less than 1.0%, while it was over 2.0% in the 160 °C solids.

Table 43. Products From SSF, Reaction with α -Cellulose. (Sigma C 8002)

Time,h	EtOH		Glucose, g/l		Cellobiose g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
	HPLC	GSC	HPLC	Sigma510A				
0	2.2	2.1	1.48	0.62	0	0.04	0.11	0.04
21.8	7.6	7.8	0.65	0.23	0.21	0	0.17	0.00
46	11.0	11.0	0.41	0.10	0	0.07	0.46	0.08
69.3	13.4	12.8	0.40	0.03	0	0	0.45	0.16
93.7	14.6	15.9	0.29	0.00	0	0	0.42	0.21
117.3	15.7	14.6	0.23	0.03	0	0	0.44	0.35
140.8	15.9	14.7	0.00	0.03	0	0	0.47	0.43
166.2	13.6	14.1	0.00	0.02	0	0	0.64	0.25
Alpha-Cellulose 2								
0	1.8	2.0	0.63	1.26	0	0.04	0.07	0.04
21.8	7.6	10.0	0.31	0.25	0.22	0	0.14	0.02
46	11.5	11.6	0.31	0.11	0	0	0.31	0.05
69.3	14.0	8.0	0.26	0.04	0	0	0.34	0.11
93.7	15.6	14.8	0.19	0.01	0	0	0.37	0.09
117.3	15.5	14.5	0.13	0.04	0	0	0.39	0.11
140.8	16.8	15.2	0.16	0.03	0	0	0.52	0.19
166.2	12.4	14.0	0.00	0.02	0	0.01	0.36	0.43

5.2.4 Influence of Particle Size on SSF Results

In the prehydrolysis reactor used in this study pretreated acid and wood are introduced into the top of the reactor and flow by gravity to the bottom. At this stage the hemicellulose has been largely hydrolyzed and solubilized but the wood is still in the original chip form. Before the solids can exit the reactor they are subjected to mechanical disintegration and are reduced in size until the particles pass through a cylindrical screen made up of drilled holes on staggered centers. The disintegration takes place at operating temperatures and pressure and, since this is above the softening temperature of lignin, uses less energy than at lower temperatures.

The particle size distribution of the seven prehydrolyzed solids prepared in this study is presented in Table 22. About 50% by weight is smaller than 170 mesh (90 microns) and only 20 % is larger than 40 mesh. The size distribution is similar for all the pretreatment reactions owing to the fact that disintegrator operating parameters were not varied in this work. It is likely that the small particle size may have contributed to what seems to be relatively high levels and rapid rates of ethanol production in SSF. This could be confirmed by preparing prehydrolyzed solids prepared under similar conditions but with different screen openings or by varying disintegrator speed during a prehydrolysis reaction.

Table 44. Prehydrolysis Conditions and Ethanol/Glucose Production.

Reaction No.	Prehydrolysis Conditions			Maximum Ethanol	Max. Glucose Equivalent	
	Temperature °C	Prehydrolyzate pH	Residence Time, min.	Yield in SSF % of Theor.	38°C Hydrol. % of Theor.	50°C Hydrol. % of Theor.
1-1	170.6	2.41	25.3	90	69	97
1-2	170.6	2.41	25.3	86	66	97
2-1	171.3	2.38	27	92	80	100
2-2	171.3	2.38	27	91	79	100
3-1	171.3	2.42	24.8	85	68	83
3-2	171.3	2.42	24.8	82	73	88
4-1	171.6	2.41	25.1	85	58	85
4-2	171.6	2.41	25.1	77	53	87
5-1	161.3	2.35	36.90	74	57	73
5-2	161.3	2.35	36.9	73	55	75
6-1	160.7	2.34	37.5	69	57	81
6-2	160.7	2.34	37.5	68	61	83
7-1	161.9	2.35	36.4	79	58	79
7-2	161.9	2.35	36.4	74	59	76
Hybrid Poplar-1				8	13	6
Hybrid Poplar-2				9	11	6
α -cellulose-1				92	51	91
α -cellulose-2				85	52	91

5.3 Enzymatic Hydrolysis at 38°C

The same pretreated solids evaluated by SSF were also subjected to enzymatic hydrolysis using SSF temperature condition (38 °C) but without the presence of *S. cerevisiae* *D₅A*. The untreated hybrid poplar feedstock (-40 mesh) and an α -cellulose (Sigma C8002) were also included for comparison. The procedures outlined in CATSP No. 008, revision 4, were followed with minor variations as noted in Section 2. Cytolase CL enzyme (74 FPU/ml) was used. The enzymatic hydrolysis reactions were carried out in two series. The first was run for 48 hours and the second for 24 hours.

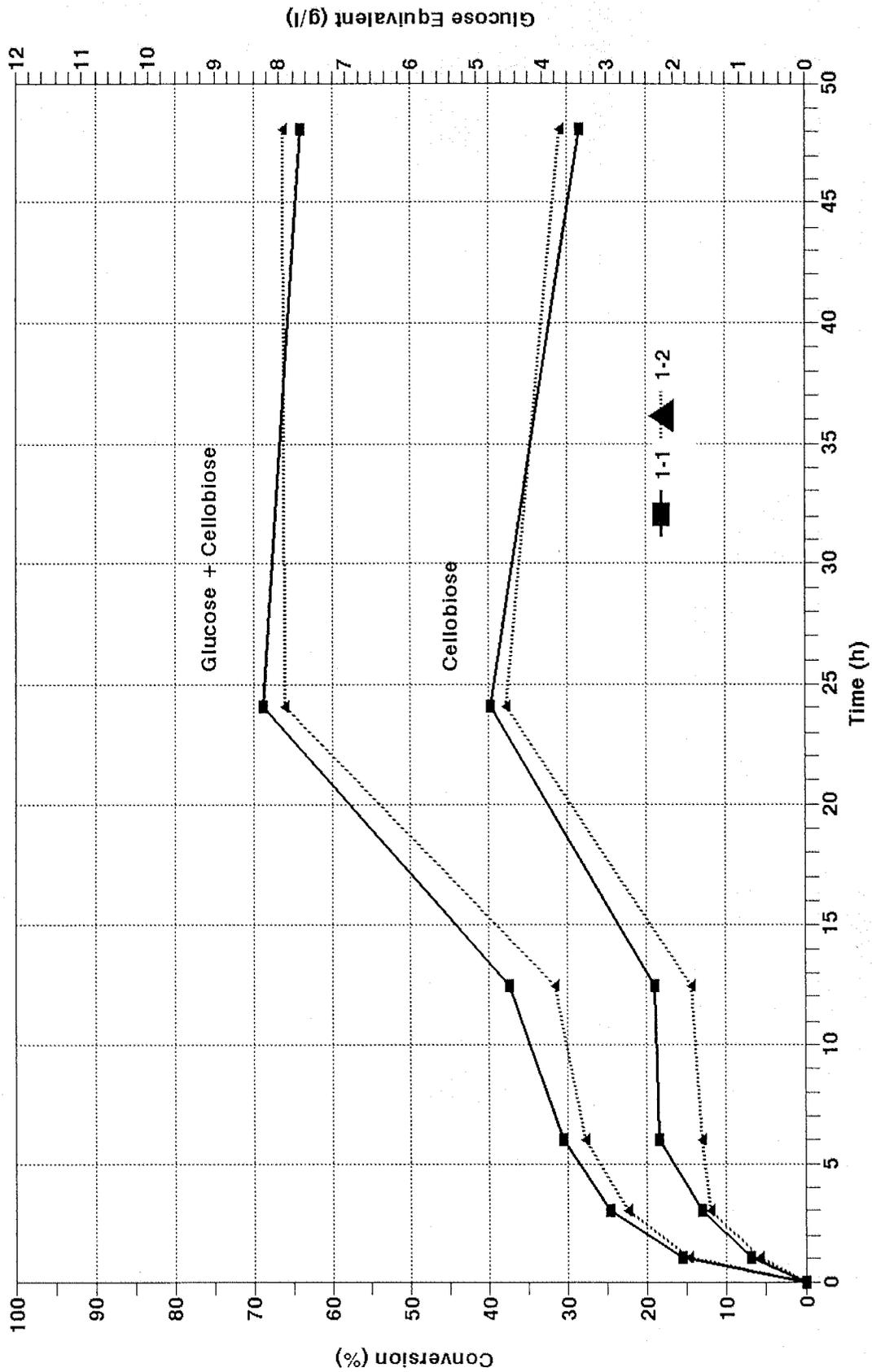


Figure 32. Enzyme Hydrolysis at 38 °C. Hybrid Poplar Reaction 1.

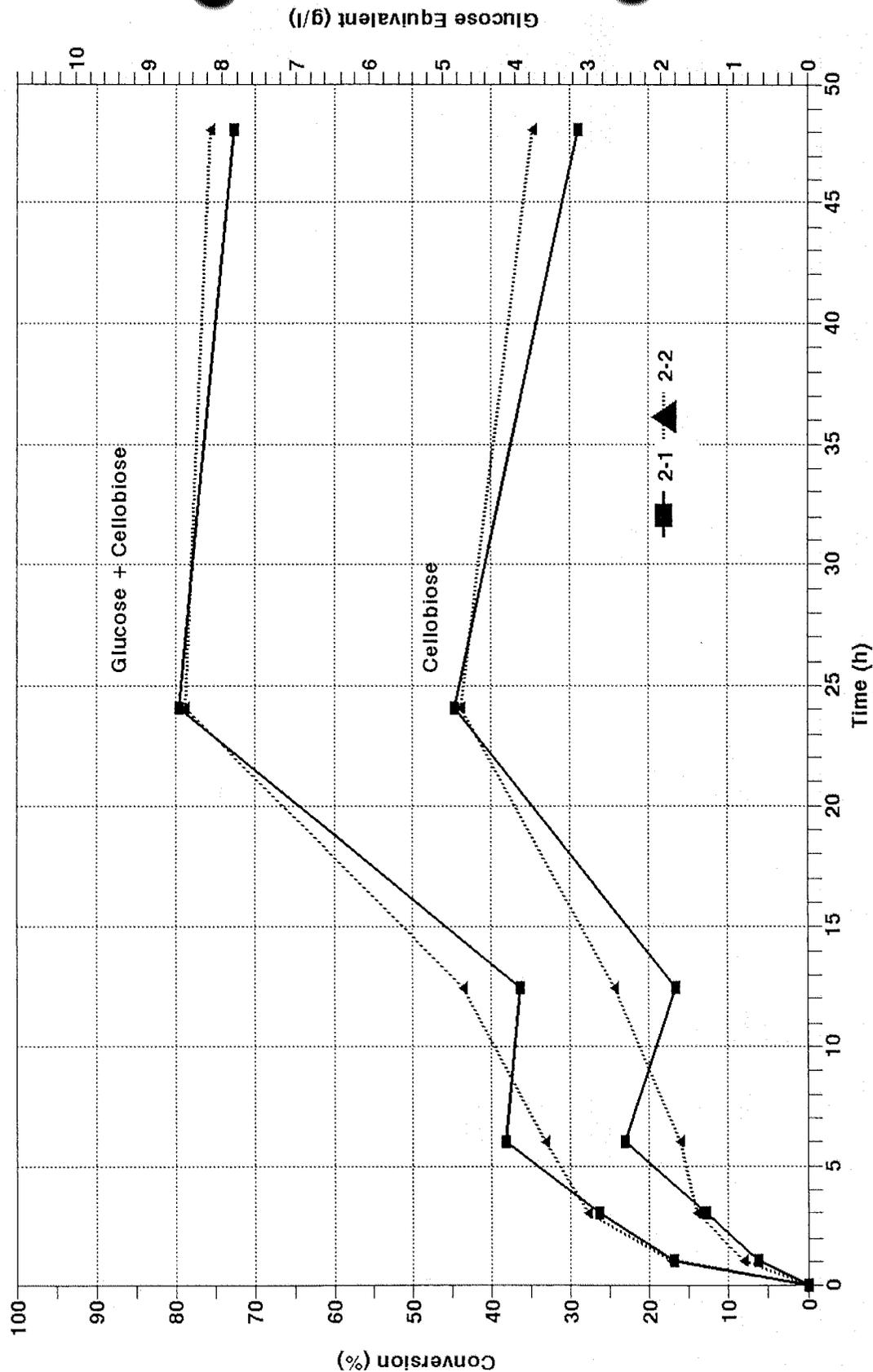


Figure 33. Enzyme Hydrolysis at 38 °C. Hybrid Poplar Reaction 2.

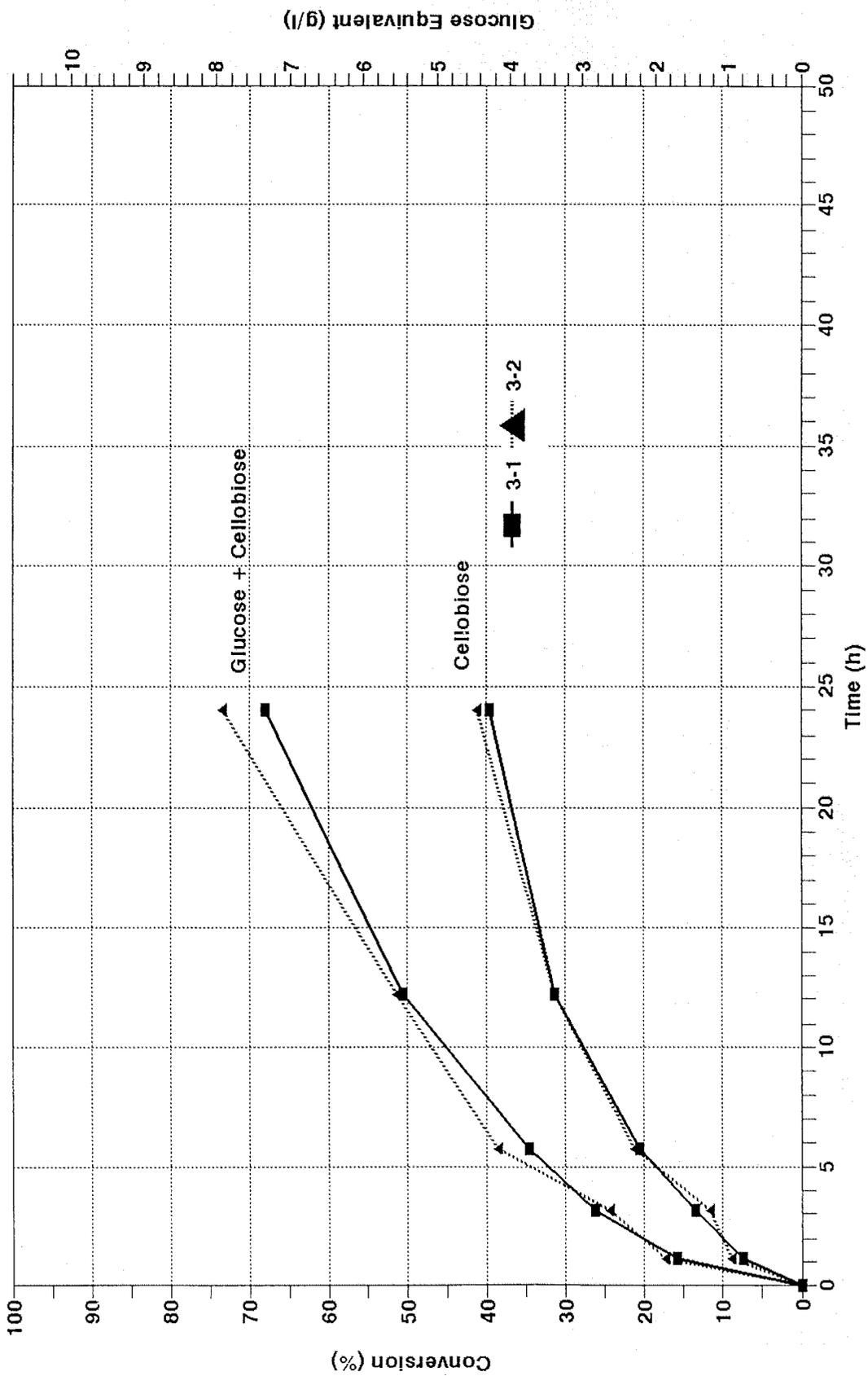


Figure 34. Enzyme Hydrolysis at 38 °C. Hybrid Poplar Reaction 3.

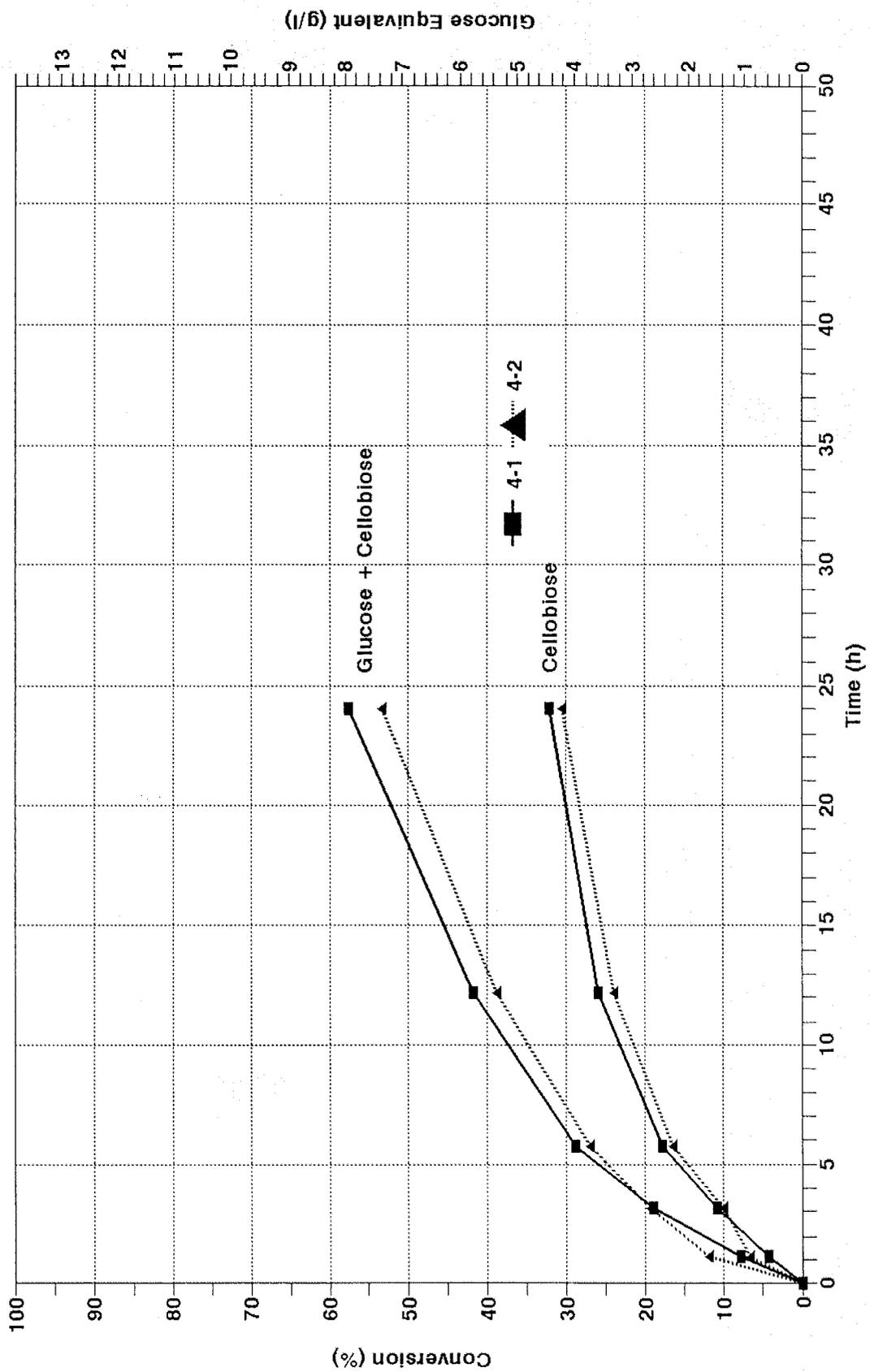


Figure 35. Enzyme Hydrolysis at 38 °C. Hybrid Poplar Reaction 4.

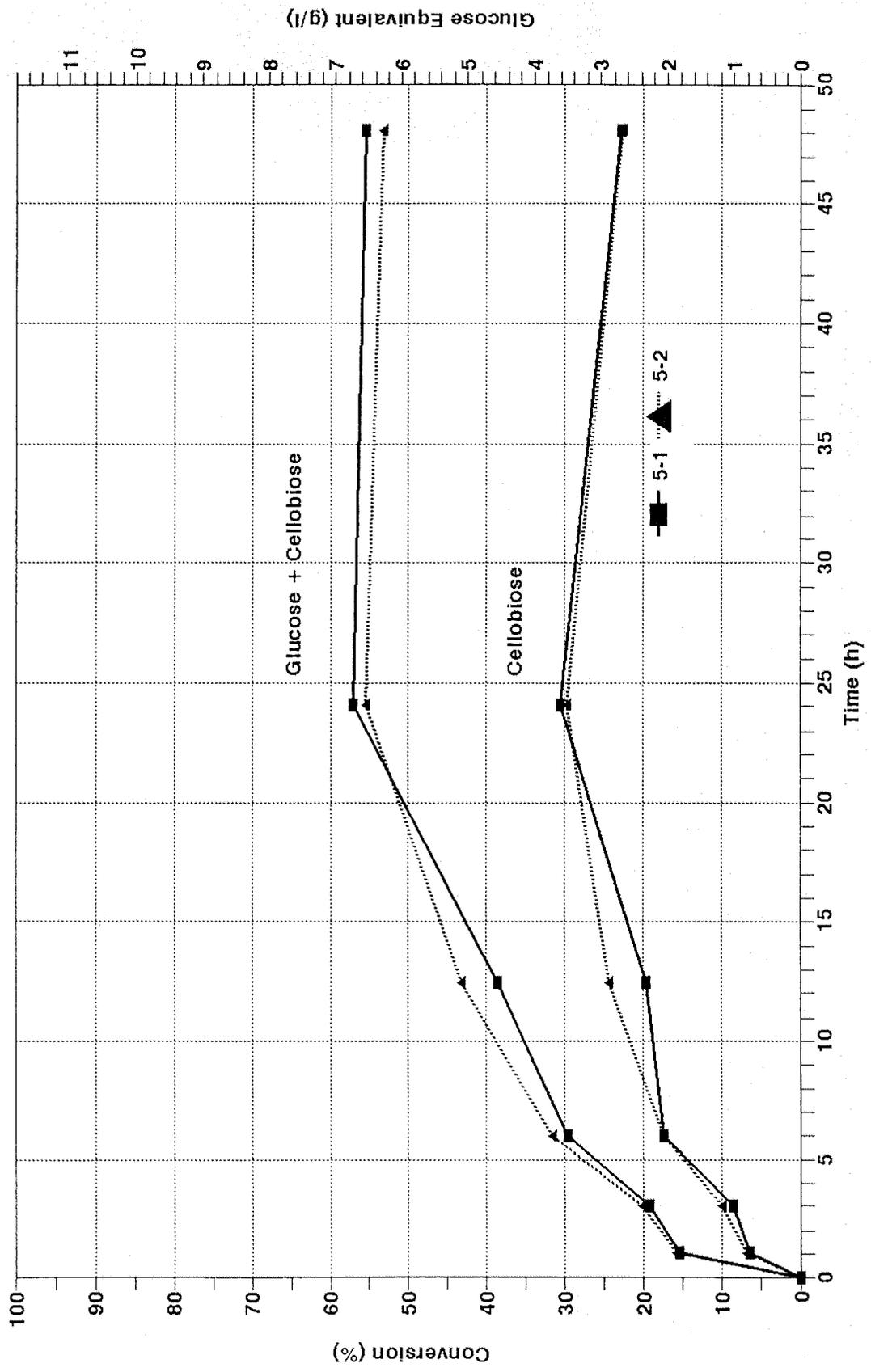


Figure 36. Enzyme Hydrolysis at 38 °C. Hybrid Poplar Reaction 5.

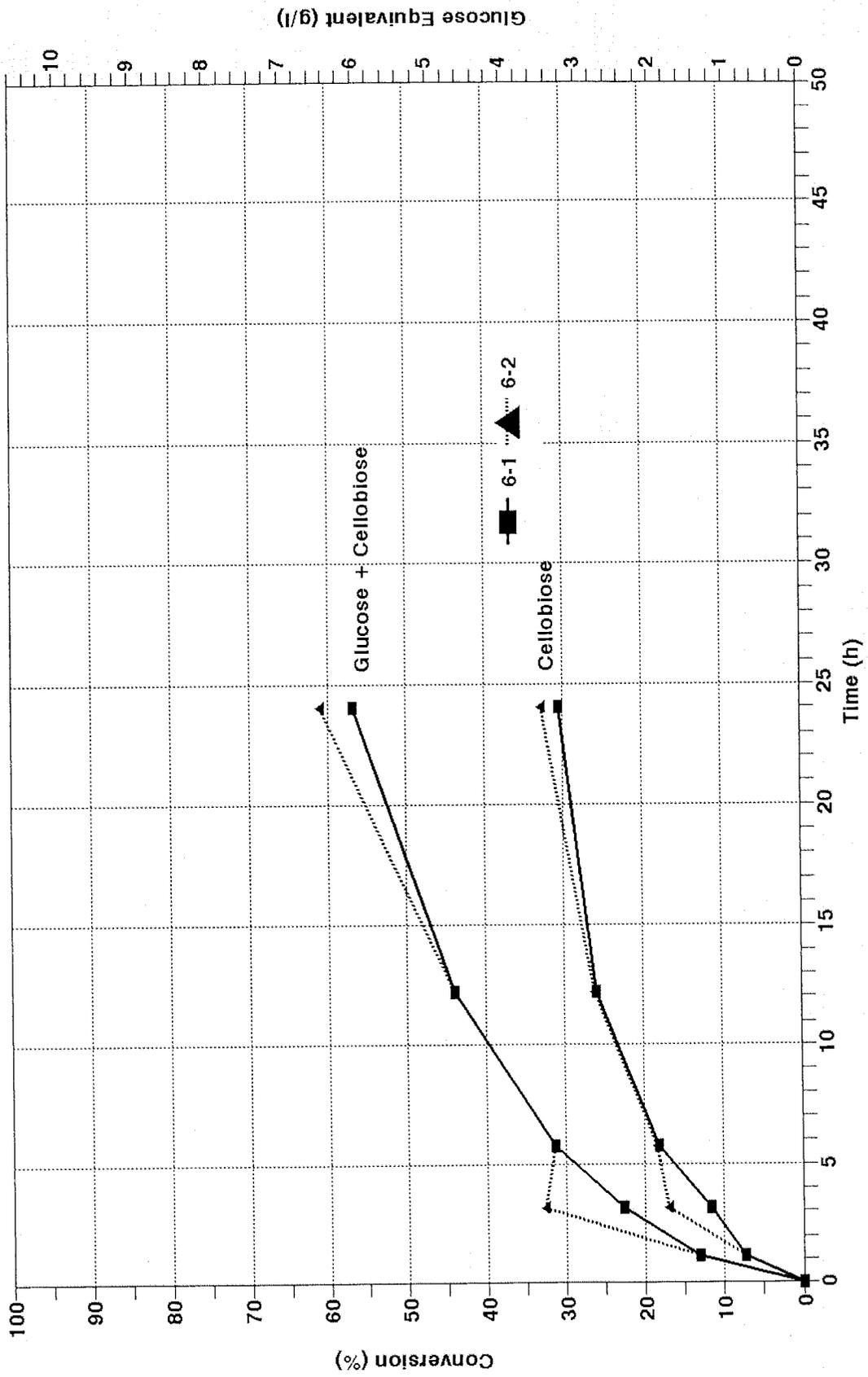


Figure 37. Enzyme Hydrolysis at 38 °C. Hybrid Poplar Reaction 6.

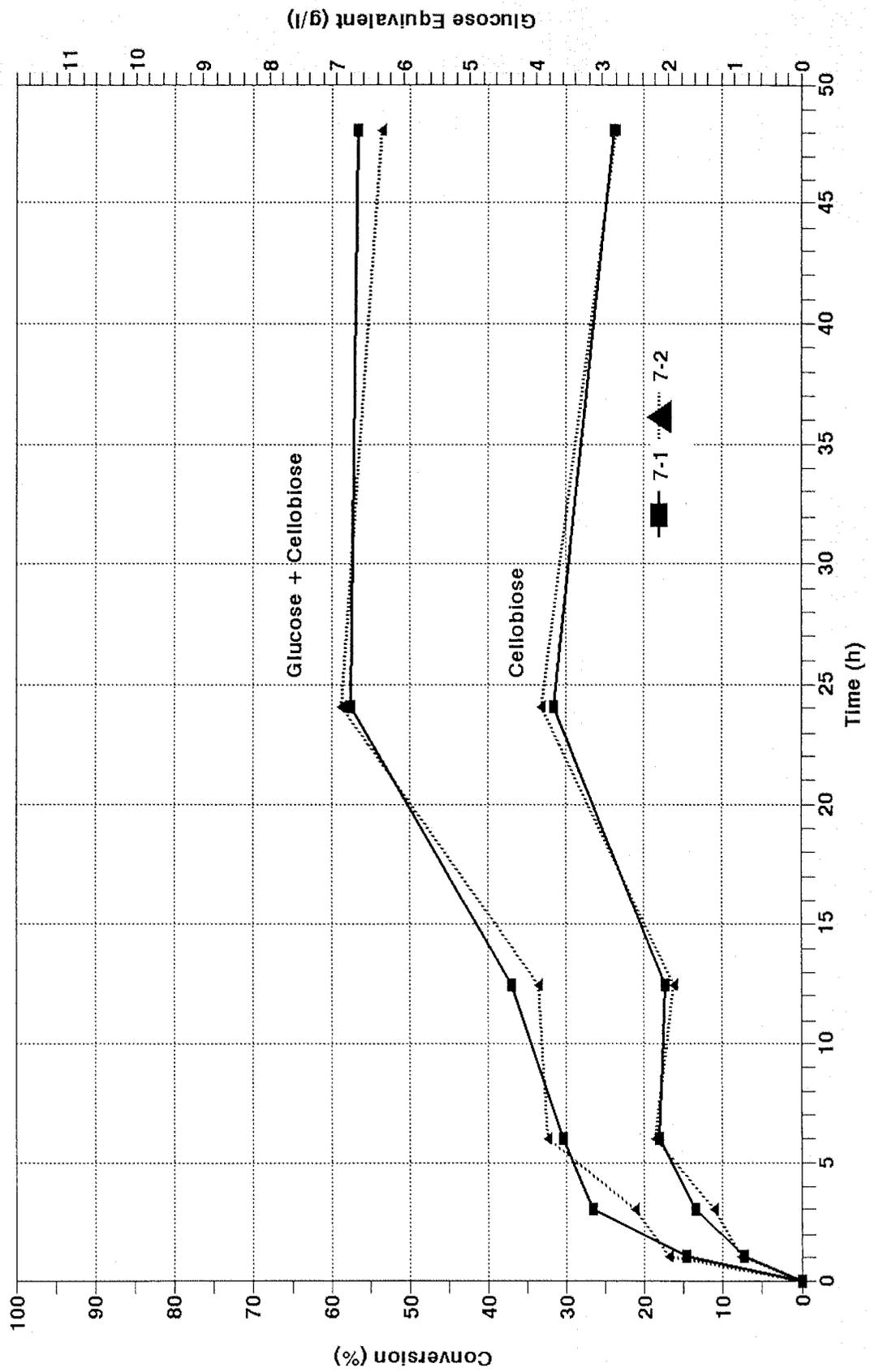


Figure 38. Enzyme Hydrolysis at 38 °C. Hybrid Poplar Reaction 7.

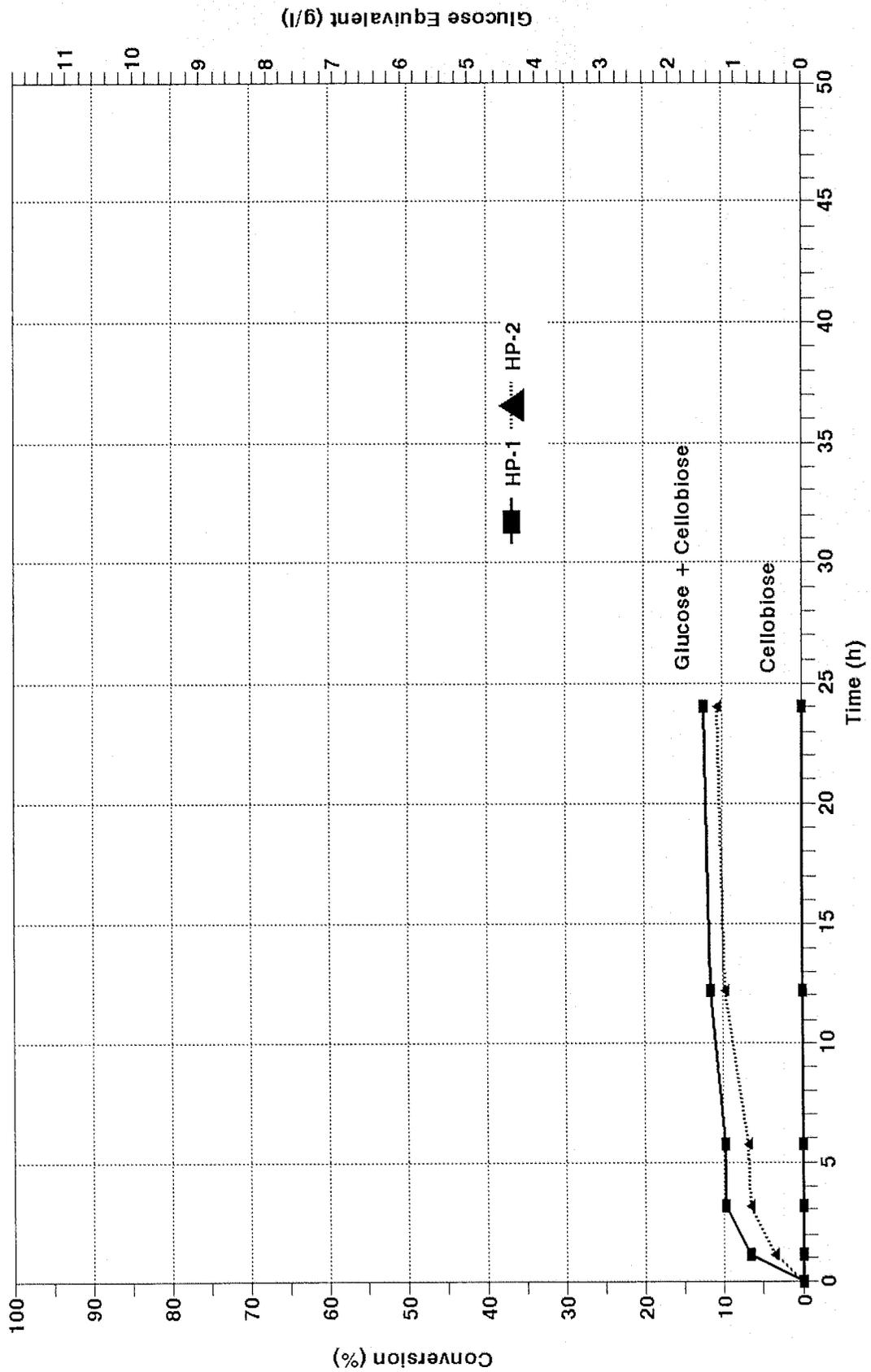


Figure 39. Enzyme Hydrolysis at 38 °C. Hybrid Poplar Feedstock.

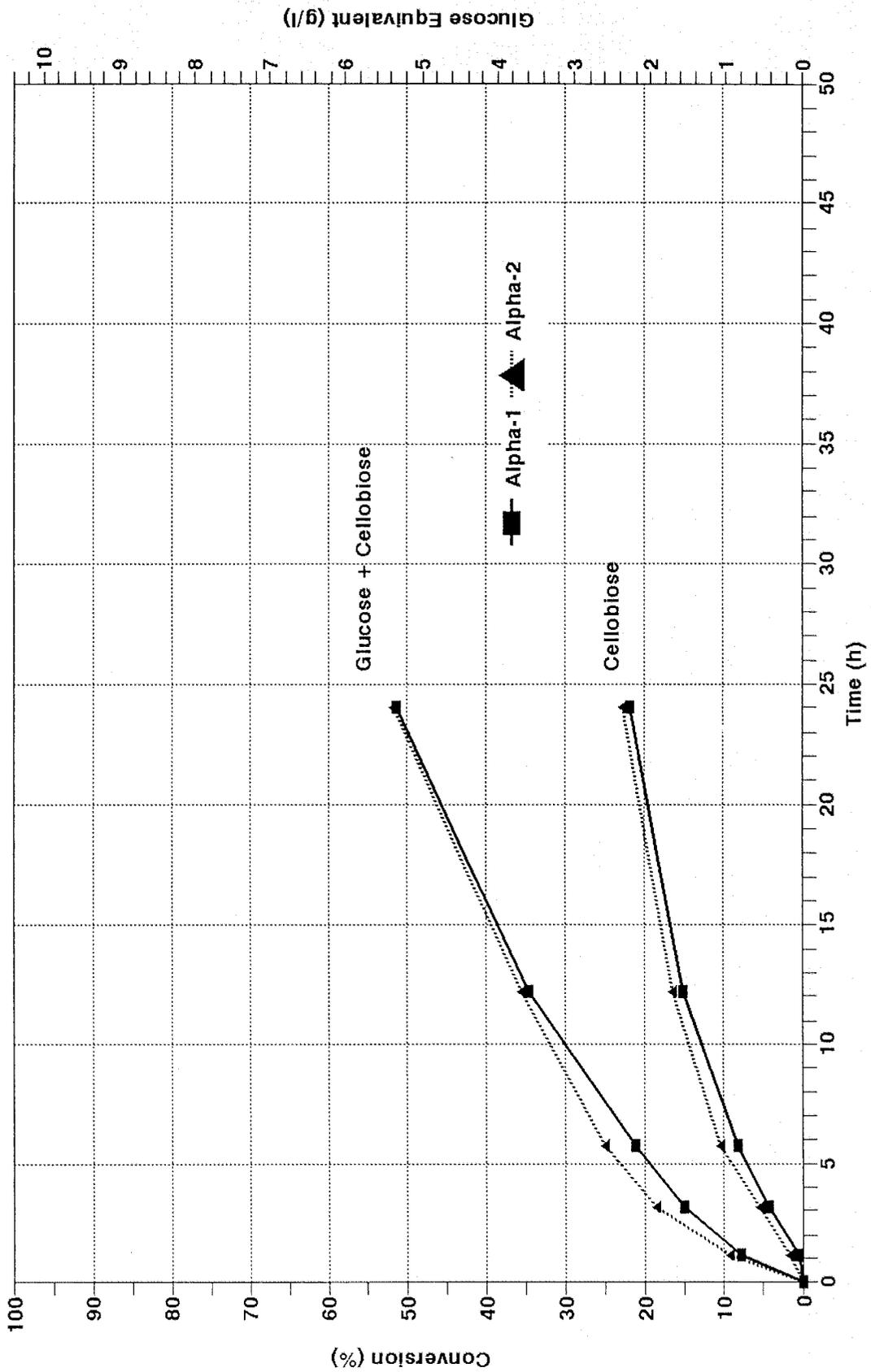


Figure 40. Enzyme Hydrolysis at 38 °C. Alpha Cellulose.

5.3.1 Production of Glucose and Cellobiose

The principal products were glucose and cellobiose as determined by HPLC. Samples for analysis were collected at zero, 1, 3, 6, 12, and 24 hours (48 hours in some cases). The results, expressed as both equivalent glucose concentration and percent of theoretical (total glucan present in the pretreated solids) are plotted in Figures 32 - 40. The analytical values used in the Figures are presented in Tables 45 - 50. The term "glucose equivalent" in these tables is used as defined in Procedure 008, Rev. #4. Although glucose values by three different analytical techniques are presented in the Tables, the HPLC values were used for the Figures for reasons discussed in Section 2.

The influence of product inhibition on enzymatic hydrolysis is prominent in all pretreated solid samples as well as with α -cellulose. This is indicated by lower levels of conversion of glucan to glucose and cellobiose than observed in SSF. For reactions 1-3 (170 °C) the theoretical yields were 65%-80% while reactions 5-7 (160 °C) gave 55%-60% conversion as did one of the 170 °C treated solids (reaction 4). α -cellulose conversion was slightly lower (52%). As shown in Table 44 the corresponding conversions of glucan to ethanol were significantly higher in SSF than 38 °C enzyme hydrolysis, to glucose plus cellobiose.

Table 45. Enzyme Hydrolysis @ 38 °C. Principal Products. Reactions 1, 2.

Time,h	Reaction 1-1						Tot.Glucose g/l	Equiv. Conv.,%
	Glucose, g/l			Cellobiose, g/l				
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.			
0	0.00	0.07	0.04	0.39	0.41	0	0.0	
1.1	1.06	0.76	0.45	0.78	0.82	1.88	15.7	
3	1.38	1.21	0.88	1.50	1.58	2.96	24.7	
6	1.45	1.52	1.11	2.10	2.21	3.66	30.6	
12.5	2.20	2.12	1.76	2.17	2.28	4.48	37.4	
24.1	3.44	3.13	2.75	4.55	4.78	8.22	68.7	
48.1	4.24	----	4.08	3.25	3.41	7.65	63.9	
Theoretical Conv.						11.97	100.0	

Time,h	Reaction 1-2						Tot.Glucose g/l	Equiv. Conv.,%
	Glucose, g/l			Cellobiose, g/l				
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.			
0	0.00	0.06	0.03	0.18	0.19	0	0.0	
1.1	1.08	0.77	0.42	0.67	0.70	1.78	14.9	
3	1.23	1.21	0.85	1.38	1.45	2.68	22.4	
6	1.76	1.52	1.06	1.50	1.58	3.34	27.9	
12.5	2.06	1.96	1.74	1.65	1.73	6.00	31.7	
24.1	3.36	3.01	2.63	4.32	4.54	7.90	66.0	
48.1	4.20	----	3.91	3.54	3.72	7.92	66.2	
Theoretical Conv.						11.97	100.0	

Time,h	Reaction 2-1						Tot.Glucose g/l	Equiv. Conv.,%
	Glucose, g/l			Cellobiose, g/l				
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.			
0	0.02	0.05	0.03	0.28	0.29	0	0.0	
1.1	1.14	0.74	0.42	0.64	0.67	1.81	16.8	
3	1.46	1.22	0.84	1.32	1.39	2.85	26.5	
6	1.62	1.51	1.19	2.37	2.49	4.11	38.2	
12.5	2.14	1.99	1.69	1.70	1.79	3.93	36.5	
24.1	3.75	3.09	2.71	4.58	4.81	8.56	79.5	
48.1	4.68	----	3.86	2.98	3.13	7.81	72.5	
Theoretical Conv.						10.77	100.0	

Time,h	Reaction 2-2						Tot.Glucose g/l	Equiv. Conv.,%
	Glucose, g/l			Cellobiose, g/l				
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.			
0	0.00	0.07	0.03	0.10	0.11	0	0.0	
1.1	0.99	0.75	0.44	0.83	0.87	1.86	17.3	
3	1.47	1.22	0.87	1.45	1.52	2.99	27.8	
6	1.85	1.62	0.82	1.65	1.73	3.58	33.2	
12.5	2.06	2.11	1.71	2.51	2.64	4.70	43.6	
24.1	3.76	3.14	2.67	4.50	4.73	8.49	78.8	
48.1	4.37	----	4.08	3.58	3.76	8.13	75.5	
Theoretical Conv.						10.77	100.0	

Table 46. Enzyme Hydrolysis @ 38 °C. Principal Products. Reactions 3, 4.

Reaction 3-1							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose g/l	Equiv. Conv.,%
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.		
0	0.01	0.07	0.05	0.35	0.37	0	0.0
1.1	0.91	0.73	0.45	0.77	0.81	1.72	15.8
3.2	1.40	1.21	0.84	1.38	1.45	2.85	26.2
8	1.53	1.44	1.06	2.13	2.24	3.77	34.7
12.2	2.11	1.99	1.61	3.23	3.39	5.50	50.6
24	3.12	2.92	2.55	4.08	4.28	7.40	68.0
Theoretical Conv.						10.88	100.0

Reaction 3-2							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose g/l	Equiv. Conv.,%
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.		
0	0.00	0.08	0.05	0.44	0.46	0.00	0.0
1.1	0.91	0.72	0.44	0.91	0.96	1.87	17.2
3.2	1.40	0.93	0.63	1.21	1.27	2.67	24.5
5.8	1.91	1.49	1.16	2.19	2.30	4.21	38.7
12.2	2.19	1.99	1.44	3.23	3.39	5.58	51.3
24	3.52	2.95	1.73	4.25	4.46	7.98	73.3
Theoretical Conv.						10.88	100.0

Reaction 4-1							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose g/l	Equiv. Conv.,%
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.		
0	0.00	0.77	0.05	0.09	0.09	0	0.0
1.1	0.50	0.71	0.46	0.56	0.59	1.09	7.9
3.2	1.11	1.26	0.84	1.44	1.51	2.62	19.0
5.8	1.50	1.53	1.10	2.35	2.47	3.97	28.8
12.2	2.16	2.16	1.74	3.42	3.59	5.75	41.8
24	3.47	3.24	2.80	4.24	4.45	7.92	57.5
Theoretical Conv.						13.77	100.0

Reaction 4-2							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose g/l	Equiv. Conv.,%
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.		
0	0.00	0.08	0.05	0.52	0.55	0	0.0
1.1	0.71	0.75	0.43	0.88	0.92	1.63	11.8
3.2	1.28	1.28	0.86	1.33	1.40	2.68	19.5
5.8	1.45	1.51	1.11	2.16	2.27	3.72	27.0
12.2	2.01	2.07	1.64	3.17	3.33	5.34	38.8
24	3.12	3.00	2.51	4.04	4.24	7.36	53.4
Theoretical Conv.						13.77	100.0

Table 47. Enzyme Hydrolysis @ 38 °C. Principal Products. Reactions 5, 6.

Reaction 5-1							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose g/l	Equiv. Conv.,%
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.		
0	0.05	0.09	0.04	0.30	0.32	0.00	0.0
1.1	1.08	0.84	0.40	0.72	0.76	1.84	15.5
3	1.27	1.21	0.83	0.97	1.02	2.29	19.3
6	1.47	1.47	0.68	1.95	2.05	3.52	29.7
12.5	2.27	2.12	0.83	2.21	2.32	4.59	38.7
24.1	3.15	2.90	2.40	3.44	3.61	6.76	57.0
48.1	3.87	----	3.67	2.57	2.70	6.57	55.4
Theoretical Conv.						11.85	100.0
Reaction 5-2							
0	0.00	0.08	0.04	0.39	0.41	0.00	0.0
1.1	1.05	0.77	0.42	0.78	0.82	1.87	15.8
3	1.20	1.19	0.81	1.13	1.19	2.39	20.2
6	1.69	1.54	0.79	1.97	2.07	3.76	31.7
12.5	2.24	2.28	0.97	2.75	2.89	5.13	43.3
24.1	3.06	2.82	2.39	3.34	3.51	6.57	55.4
48.1	3.61	----	3.70	2.55	2.68	6.29	53.1
Theoretical Conv.						11.85	100.0
Reaction 6-1							
0	0	0.69	0.05	0.38	0.40	0.00	0.0
1.1	0.62	0.43	----	0.73	0.77	1.39	13.0
3.2	1.19	1.21	0.84	1.17	1.23	2.42	22.7
5.8	1.42	1.43	1.09	1.84	1.93	3.35	31.4
12.2	1.93	1.96	1.59	2.64	2.77	4.70	44.0
24	2.81	2.79	2.42	3.09	3.24	6.05	56.7
Theoretical Conv.						10.67	100.0
Reaction 6-2							
0	0	0.07	0.06	0.23	0.24	0.00	0.0
1.1	0.62	0.76	0.47	0.73	0.77	1.39	13.0
3.2	1.68	1.51	0.63	1.71	1.80	3.48	32.6
5.8	1.39	1.47	1.20	1.87	1.96	3.35	31.4
12.2	1.93	2.03	1.64	2.65	2.78	4.71	44.1
24	3.02	2.93	2.45	3.31	3.48	6.50	60.9
Theoretical Conv.						10.67	100.0

Another indication of product inhibition is the significant decline in the rate of cellobiose production observed between one and 12 hours of enzymatic hydrolysis. All the pretreated solids show at least a brief period of reduced cellobiose generation, and those from reactions 1 and 2, the best substrates for SSF, were the most affected. After 12 hours the rate of cellobiose production increased for these solids as did glucose concentration. The maximum cellobiose concentration in all reactions occurred at 24 hours. In SSF, the cellobiose concentration was also at a maximum at this time. Hybrid poplar feedstock (-40 mesh), not subjected to dilute acid pretreatment, did not produce any cellobiose and only low levels (10%) of glucose. The α -cellulose gave about 52% theoretical conversion after 24 hours as compared to 55%-80% with pretreated solids. This lower degree of conversion is probably related to the particle size of the α -cellulose. This commercial product was designed for use as a filter aid and has a median fiber length of 100-150 μ m.

Table 48. Enzyme Hydrolysis @ 38 °C. Principal Products. Reactions 7, Feedstock.

Reaction 7-1							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose g/l	Equiv. Conv.,%
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.		
0	0.07	0.11	0.05	0.46	0.48	0	0.0
1.1	0.86	0.80	0.41	0.83	0.87	1.73	14.6
3	1.57	1.21	0.82	1.51	1.59	3.16	26.6
6	1.50	1.47	1.09	2.02	2.12	3.62	30.5
12.5	2.37	2.18	1.58	1.94	2.04	4.41	37.1
24.1	3.09	2.85	2.38	3.56	3.74	6.83	57.5
48.1	3.90	----	3.68	2.68	2.81	6.71	56.5
Theoretical Conv.						11.88	100.0

Reaction 7-2							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose g/l	Equiv. Conv.,%
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.		
0	0.01	0.09	0.04	0.37	0.39	0	0.0
1.1	1.18	0.78	0.42	0.86	0.90	2.08	16.9
3	1.22	1.20	0.82	1.25	1.31	2.53	21.3
6	1.64	1.59	1.12	2.10	2.21	3.85	32.4
12.5	2.09	1.97	1.60	1.82	1.91	4.00	33.7
24.1	3.08	2.88	2.41	3.72	3.91	6.99	58.8
48.1	3.56	----	3.43	2.67	2.80	6.37	53.5
Theoretical Conv.						11.88	100.0

Hybrid Poplar Feedstock; HP-1							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose g/l	Equiv. Conv.,%
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.		
0	0.19	0.06	0.03	0.36	0.38	0	0.0
1.1	0.79	0.61	0.49	0	0	0.79	6.7
3.2	1.15	1.02	0.87	0	0	1.15	9.8
5.8	1.16	1.10	0.96	0	0	1.16	9.9
12.2	1.38	1.26	0.77	0	0	1.38	11.7
24	1.47	1.39	1.01	0	0	1.47	12.5
Theoretical Conv.						11.75	100.0

Hybrid Poplar Feedstock; HP-2							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose g/l	Equiv. Conv.,%
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.		
0	0	0.05	0.03	0.2	0.21	0	0.0
1.1	0.42	0.63	0.53	0	0	0.42	3.6
3.2	0.78	1.00	0.81	0	0	0.78	6.6
5.8	0.80	1.04	0.90	0	0	0.80	6.8
12.2	1.15	1.18	0.77	0	0	1.15	9.8
24	1.24	1.34	0.80	0	0	1.24	10.6
Theoretical Conv.						11.75	100.0

Table 49. Enzyme Hydrolysis @ 38 °C. Principal Products. α -Cellulose.

Alpha-Cellulose; α -1							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose Equiv.	
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0	0.01	0.07	0.04	0.32	0.34	0.00	0.0
1.1	0.77	0.79	0.69	0.05	0.05	0.82	7.9
3.2	1.13	1.13	0.90	0.42	0.44	1.57	15.1
5.8	1.34	1.31	1.15	0.83	0.87	2.21	21.3
12.2	2.00	1.80	1.66	1.51	1.59	3.59	34.6
24	3.06	2.73	1.23	2.17	2.28	5.34	51.4
Theoretical Conv.						10.38	100.0

Alpha-Cellulose; α -2							
Time,h	Glucose, g/l			Cellobiose, g/l		Tot.Glucose Equiv.	
	HPLC	YSI	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0	0.12	0.07	0.03	0.29	0.30	0.00	0.0
1.1	0.79	0.73	0.64	0.16	0.17	0.96	9.2
3.2	1.36	1.20	0.43	0.55	0.58	1.94	18.7
5.8	1.51	1.27	1.17	1.05	1.10	2.61	25.1
12.2	1.99	1.75	1.62	1.63	1.71	3.70	35.6
24	2.99	2.63	1.63	2.27	2.38	5.37	51.7
Theoretical Conv.						10.38	100.0

The inhibition of cellulase enzymes by glucose and cellobiose products is to be expected and can be minimized by the use of SSF, as was demonstrated in this study. The use of enzymes at 50 °C, a more nearly optimum temperature for enzymatic hydrolysis, was also investigated and is discussed in a following section.

5.3.2 Minor Products from Enzymatic Hydrolysis at 38 °C

During the HPLC analysis of enzymatic hydrolysis products, other components besides glucose and cellobiose were monitored; namely lactic and acetic acids, and glycerol. These analytical results are presented in Tables 50 and 51. In most cases these components were either absent or present in trace amounts. There is some indication of increased levels of all three products, particularly lactic acid, during periods of abrupt changes in rate of glucose and cellobiose production, i.e., during inhibition. At present the identification of these minor compounds has not been confirmed and the validity of these small concentration variations and their significance will require further evaluation.

Table 50. Minor Fermentation Products from Hybrid Poplar. Enzyme Hydrolysis @ 38 °C.

Time;h	Reaction 1-1			Reaction 1-2		
	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
0.0	0.09	0.05	0.00	0.00	0.00	0.00
1.1	0.28	0.06	0.07	0.32	0.06	0.11
3.0	0.14	0.09	0.00	0.00	0.00	0.00
6.0	0.07	0.02	0.02	0.25	0.15	0.13
12.5	0.00	0.00	0.00	0.00	0.02	0.04
24.1	0.00	0.00	0.00	0.00	0.00	0.00
48.1	0.43	0.18	0.04	0.08	0.00	0.06
Time;h	Reaction 2-1			Reaction 2-2		
	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
0.0	0.80	0.04	0.00	0.01	0.00	0.00
1.1	0.32	0.06	0.09	0.00	0.00	0.12
3.0	0.10	0.04	0.12	0.00	0.00	0.00
6.0	0.08	0.06	0.00	0.29	0.18	0.12
12.5	0.26	0.15	0.15	0.38	0.16	0.05
24.1	0.00	0.00	0.00	0.00	0.00	0.00
48.1	0.17	0.05	0.00	0.09	0.00	0.05
Time;h	Reaction 3-1			Reaction 3-2		
	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
0.0	0.04	0.11	0.03	0.01	0.01	0.02
1.1	0.00	0.00	0.01	0.02	0.02	0.03
3.2	0.00	0.00	0.02	0.01	0.01	0.03
5.8	0.00	0.00	0.02	0.02	0.01	0.03
12.2	0.00	0.00	0.02	0.04	0.03	0.03
24.0	0.00	0.01	0.02	0.03	0.03	0.05
Time;h	Reaction 4-1			Reaction 4-2		
	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
0.0	0.00	0.00	0.00	0.00	0.00	0.00
1.1	0.00	0.00	0.00	0.00	0.00	0.01
3.2	0.00	0.00	0.00	0.00	0.00	0.01
5.8	0.00	0.00	0.01	0.00	0.00	0.02
12.2	0.00	0.00	0.02	0.00	0.00	0.03
24.0	0.00	0.00	0.01	0.00	0.00	0.02

Table 51. Minor Fermentation Products from Hybrid Poplar. Enzyme Hydrolysis @ 38 °C.

Time;h	Reaction 5-1			Reaction 5-2		
	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)	Lactic Acid g/l(HPLC)	Glycerol g/l(HPLC)	Acetic Acid g/l(HPLC)
0.0	0.09	0.03	0.00	0.02	0.00	0.00
1.1	0.38	0.08	0.12	0.03	0.00	0.13
3.0	0.25	0.05	0.07	0.00	0.00	0.00
6.0	0.07	0.04	0.00	0.00	0.00	0.00
12.5	0.27	0.16	0.13	0.42	0.17	0.12
24.1	0.00	0.00	0.02	0.00	0.00	0.01
48.1	0.41	0.18	0.11	0.09	0.00	0.09
	Reaction 6-1			Reaction 6-2		
0.0	0.01	0.01	0.02	0.01	0.01	0.02
1.1	0.01	0.01	0.02	0.01	0.01	0.02
3.2	0.01	0.01	0.02	0.07	0.06	0.08
5.8	0.02	0.01	0.03	0.01	0.01	0.03
12.2	0.02	0.01	0.03	0.01	0.01	0.04
24.0	0.01	0.01	0.04	0.01	0.01	0.04
	Reaction 7-1			Reaction 7-2		
0.0	0.09	0.05	0.00	0.00	0.00	0.00
1.1	0.08	0.05	0.08	0.43	0.07	0.17
3.0	0.47	0.07	0.15	0.00	0.00	0.00
6.0	0.00	0.00	0.00	0.00	0.00	0.00
12.5	0.28	0.19	0.18	0.00	0.00	0.13
24.1	0.00	0.00	0.01	0.00	0.00	0.00
48.1	0.40	0.16	0.08	0.14	0.01	0.03
	Hybrid Poplar Feedstock HP-1			Hybrid Poplar Feedstock HP-2		
0.0	0.03	0.03	0.04	0.00	0.00	0.01
1.1	0.00	0.01	0.03	0.01	0.00	0.03
3.2	0.03	0.02	0.05	0.00	0.01	0.03
5.8	0.03	0.02	0.06	0.00	0.00	0.03
12.2	0.03	0.03	0.08	0.00	0.01	0.06
24.0	0.03	0.02	0.07	0.01	0.02	0.06
	Alpha-Cellulose Alpha-1			Alpha-Cellulose Alpha-2		
0.0	0.01	0.00	0.02	0.02	0.02	0.02
1.1	0.00	0.00	0.01	0.01	0.01	0.01
3.2	0.00	0.00	0.02	0.03	0.02	0.03
5.8	0.00	0.00	0.02	0.01	0.01	0.02
12.2	0.00	0.00	0.02	0.00	0.00	0.02
24.0	0.02	0.02	0.04	0.02	0.02	0.03

5.4 Enzymatic Hydrolysis at 50°C

The same hybrid poplar pretreated solids, feedstock, and α -cellulose used for SSF and 38 °C enzyme hydrolysis were subjected to enzymatic hydrolysis at 50 °C. The same Cytolase CL cellulase enzyme (74 FPU/ml) was used. The procedure described in CATSP No. 009, revision #1, was followed with some minor changes in apparatus and the incorporation of a boiling water step immediately after sampling, to terminate enzyme activity. These procedural changes are discussed in Section 2. The reactions were carried out in two series. In the first series samples were taken at 24 hour intervals: at 0, 24, 48, 72, 96, 144, and 168 hours. In the second series samples were collected for only 96 hours, because the initial series indicated maximum glucose production was reached by this time.

5.4.1 Products from Enzymatic Hydrolysis at 50 °C

Results of the 50 °C enzymatic hydrolysis of the 7 pretreated solids, hybrid poplar feedstock and α -cellulose are shown in Figures 41 - 49. Both glucose and cellobiose concentrations and theoretical conversion (based on available glucan) are plotted against hydrolysis time for each pair of duplicate samples. The analytical values are presented in Tables 52-55. Values obtained by HPLC analysis were generally used for the Figures. The Sigma 510 analyses of glucose have reasonably good comparisons with HPLC and were used where HPLC data was not available. Conversions of glucan to glucose plus cellobiose from the 7 pretreated solids ranged from 73% to over 100% of theoretical. The concentration of glucose was always much higher than that of cellobiose, in contrast to 38 °C hydrolysis where cellobiose concentration was more nearly equal or greater than glucose for much of the hydrolysis reaction. The most notable result was that with all pretreated substrates almost all the conversion to glucose plus cellobiose that could be achieved was completed in 24 hours. In the case of the best reactions, which includes reactions 1 and 2, this conversion was over 90% of theoretical. The maximum production of glucose in most of these reactions was achieved by 72 hours. The cellobiose concentration was highest at 24 hours, as it was in SSF and 38 °C enzymatic hydrolysis, at 10%-15% of available glucan. In comparison, the α -cellulose was eventually converted in 91% yield but required 96 hours to do so; glucose plus cellobiose yields at 24 hours were 71%. Untreated hybrid poplar feedstock was not significantly hydrolyzed under these conditions. Glucose conversion increased from 4% at 24 hours to 6% at 96 hours; no cellobiose was detected.

The HPLC analyses of enzyme hydrolyzates did not show the presence of any lactic or acetic acids or glycerol. A small amount of xylose and one small unknown peak were noted.

The ability to completely solubilize cellulose to glucose and cellobiose in about 24 hours opens up the possibility of separating the ligneous residue and fermenting the aqueous phase separately. This would use an organism, preferably one which can ferment cellobiose directly, to produce ethanol and a clean cell mass which would be free of contamination. The fermentation could be carried out at the optimum conditions for the organism and provide an additional product as single-cell protein.

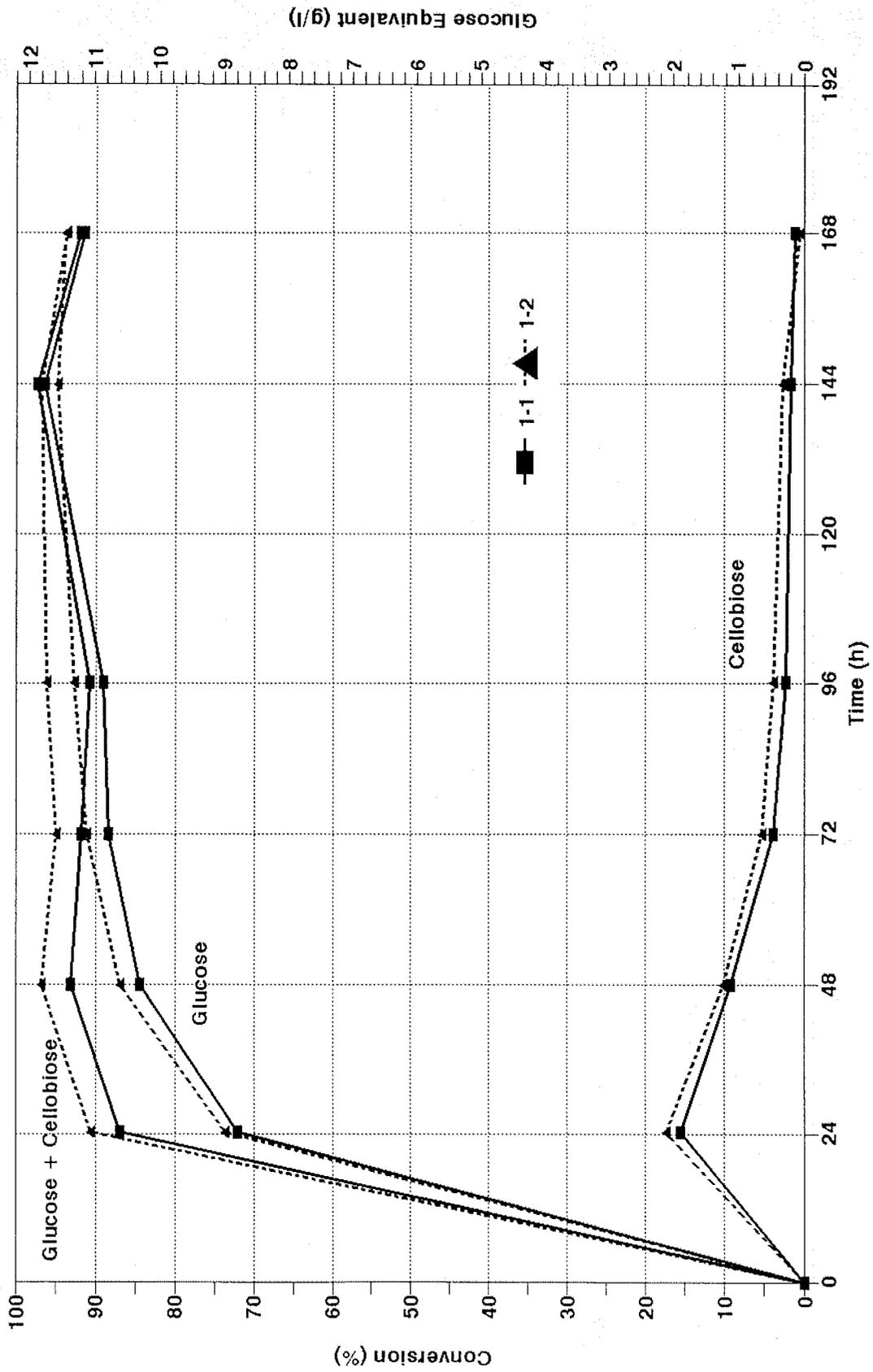


Figure 41. Enzyme Hydrolysis @ 50 °C. Hybrid Poplar Reaction 1.

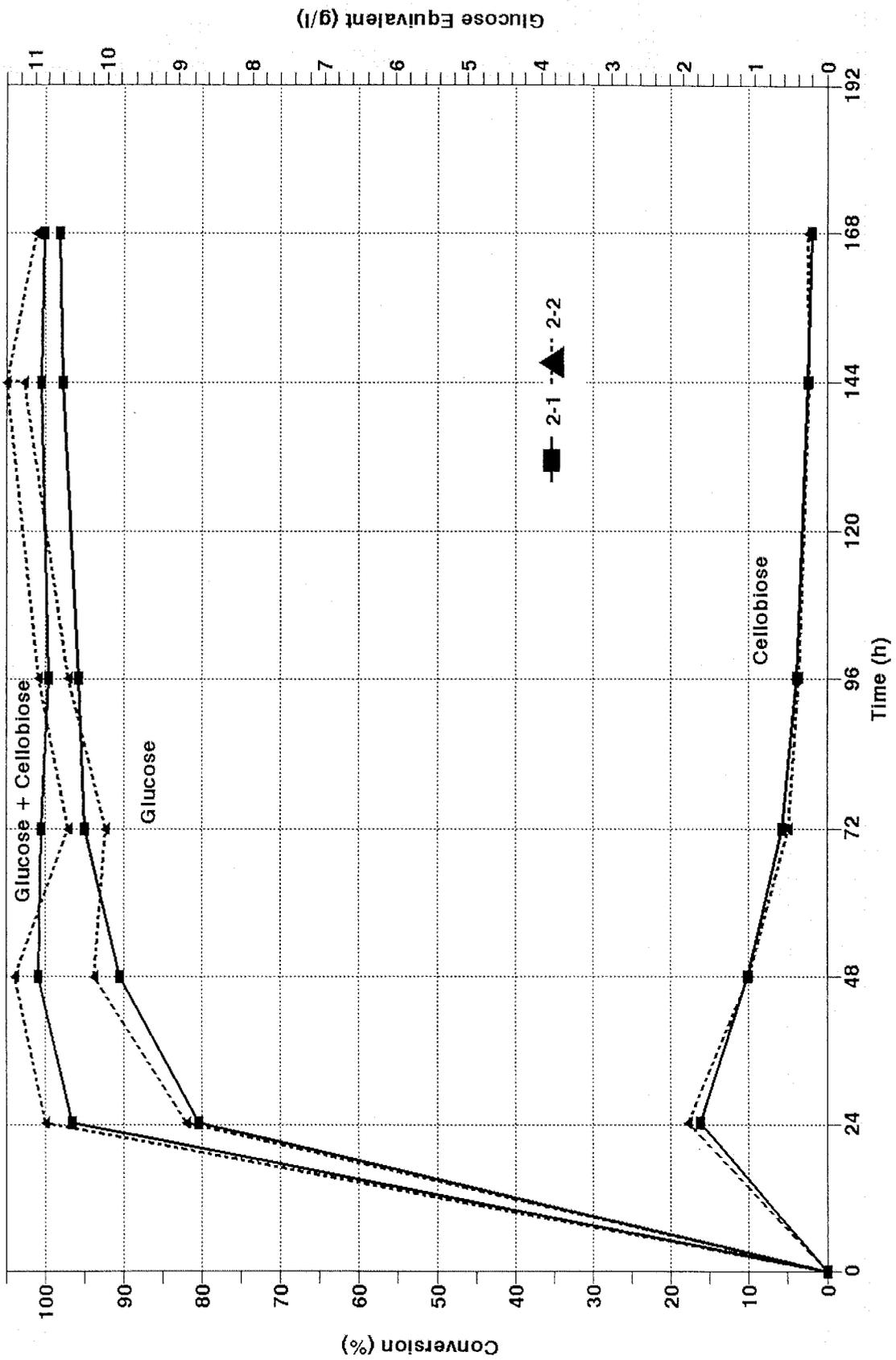


Figure 42. Enzyme Hydrolysis @ 50°C. Hybrid Poplar Reaction 2.

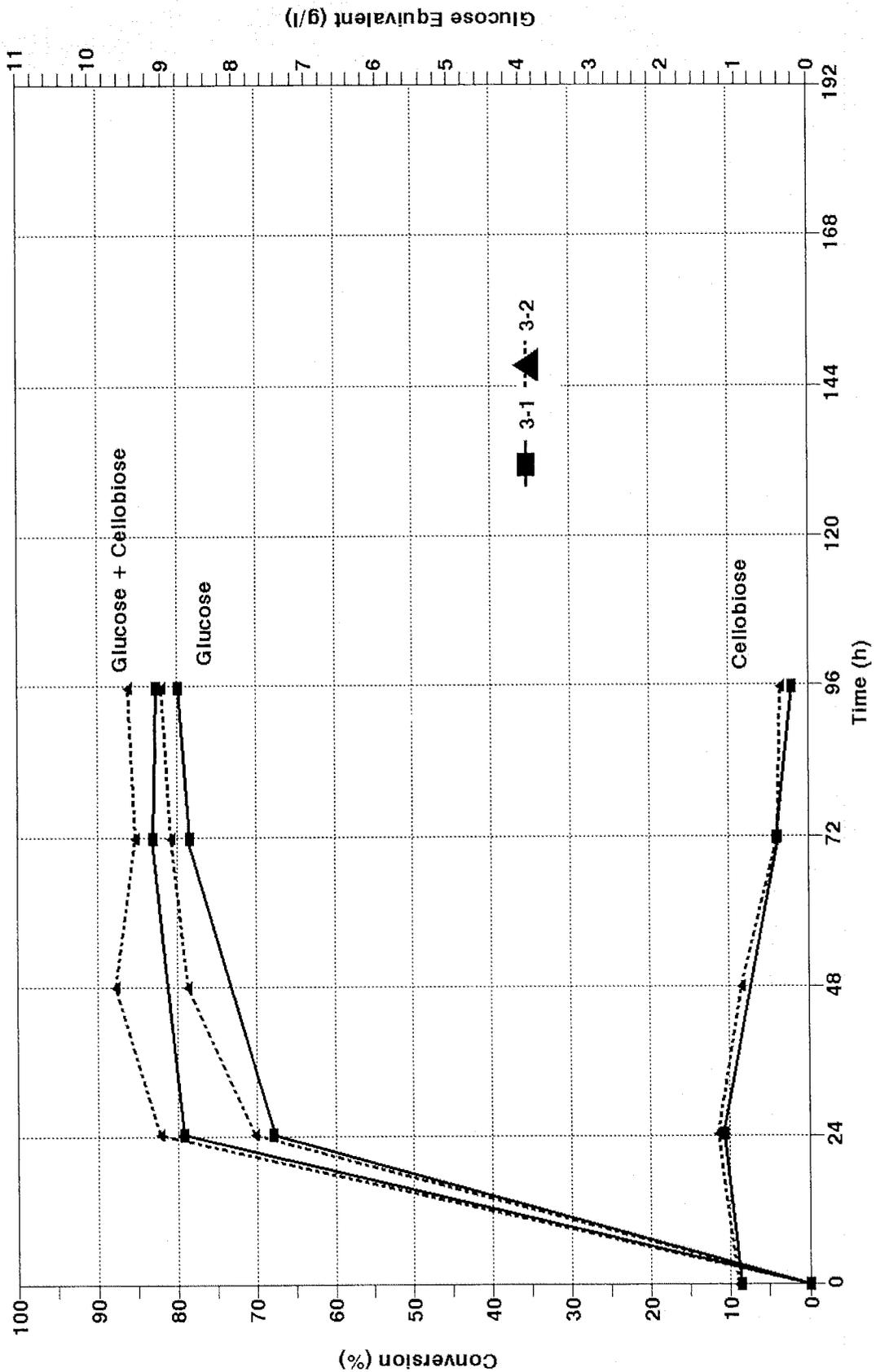


Figure 43. Enzyme Hydrolysis @ 50°C. Hybrid Poplar Reaction 3.

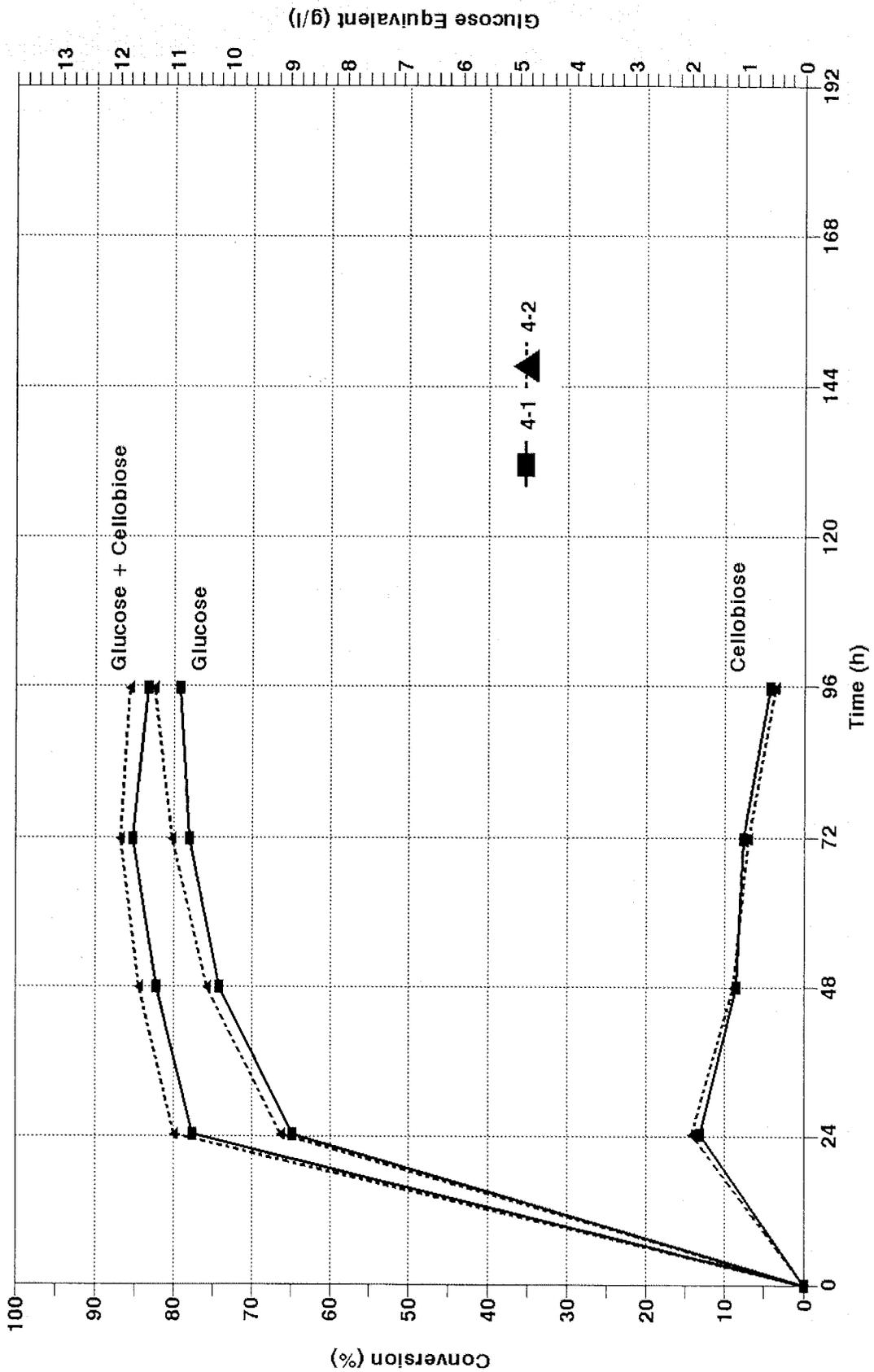


Figure 44. Enzyme Hydrolysis @ 50°C. Hybrid Poplar Reaction 4.

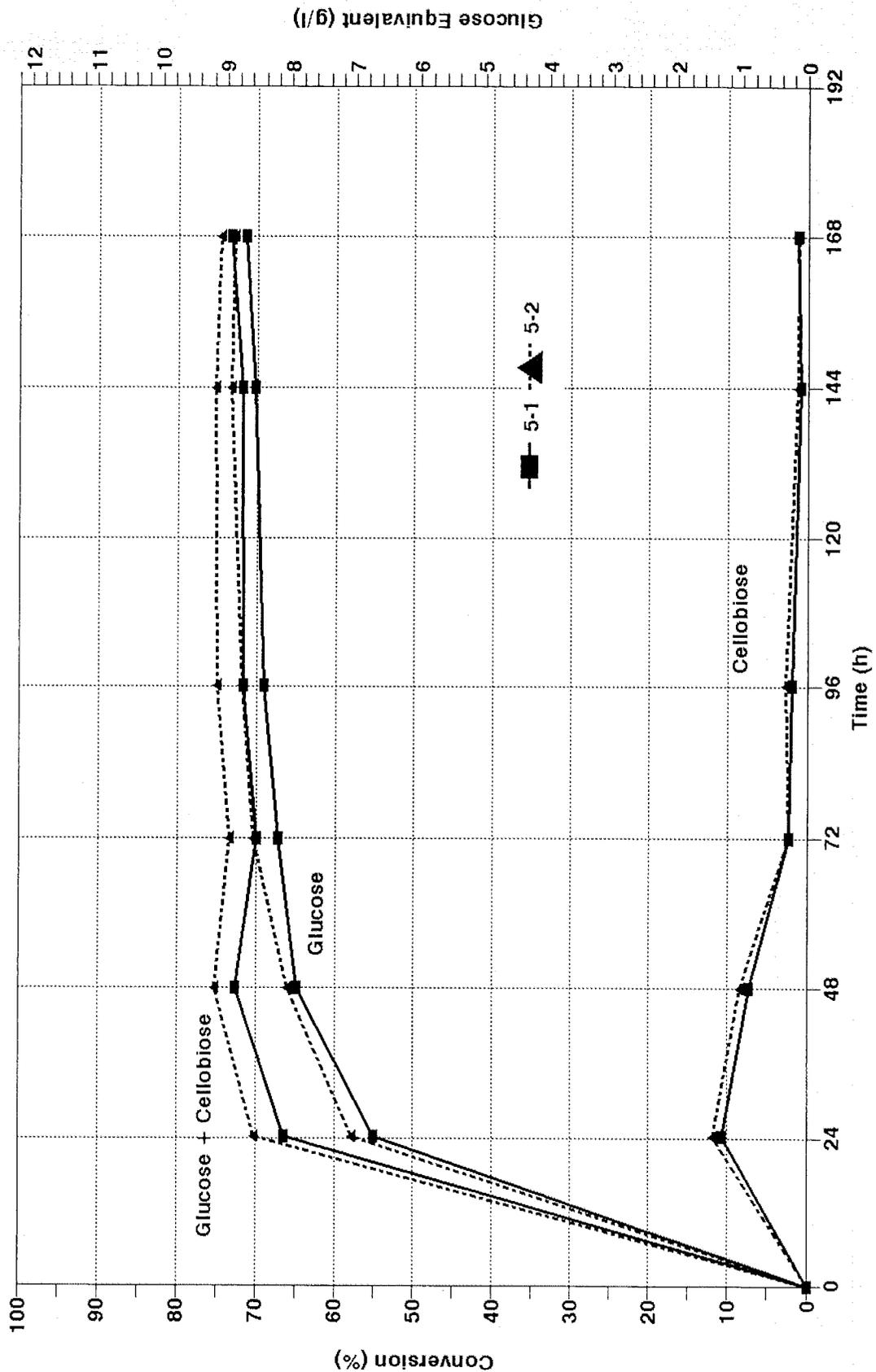


Figure 45. Enzyme Hydrolysis @ 50°C. Hybrid Poplar Reaction 5.

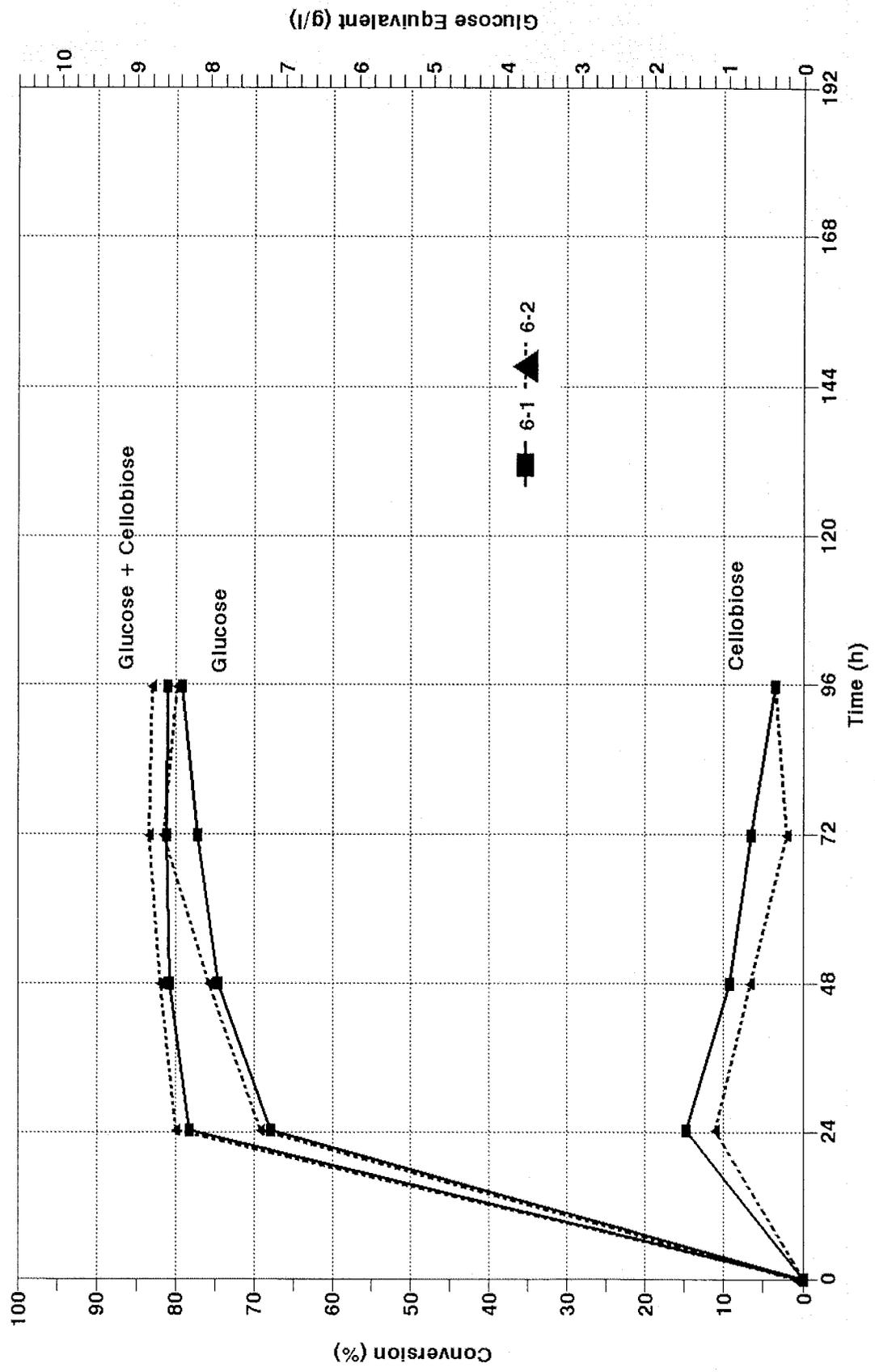


Figure 46. Enzyme Hydrolysis @ 50°C. Hybrid Poplar Reaction 6.

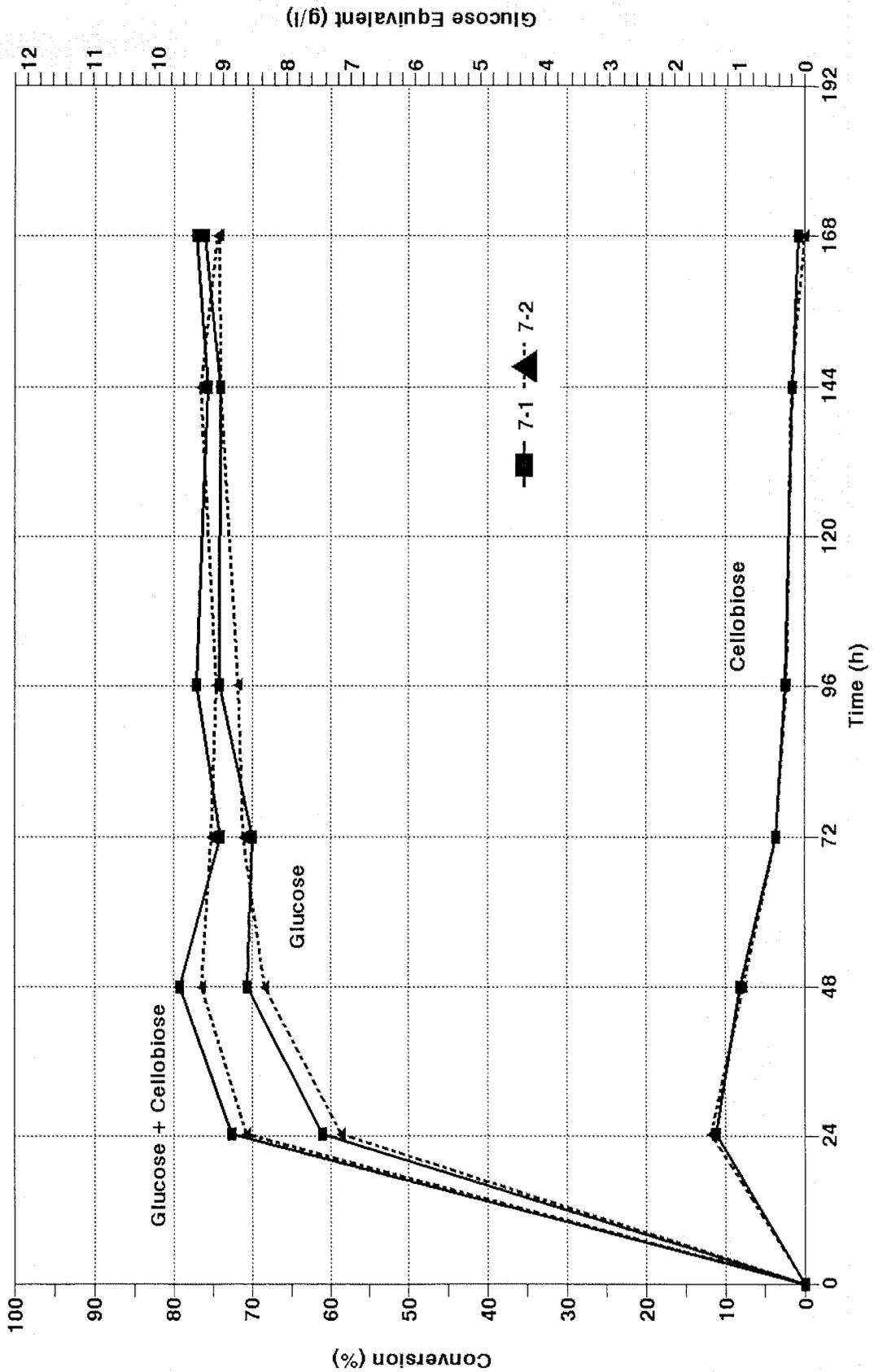


Figure 47. Enzyme Hydrolysis @ 50°C. Hybrid Poplar Reaction 7.

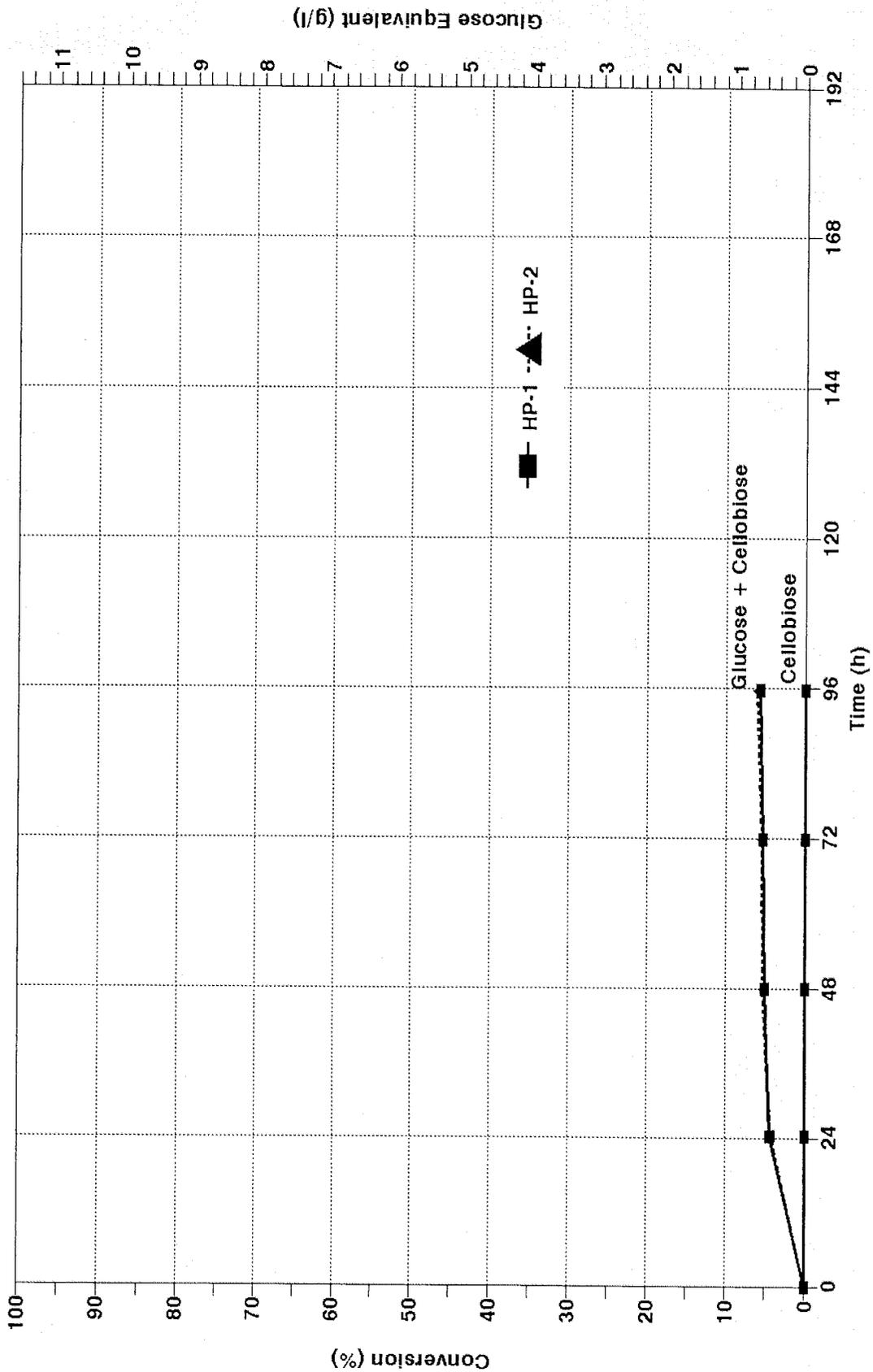


Figure 48. Enzyme Hydrolysis @ 50°C. Hybrid Poplar Feedstock.

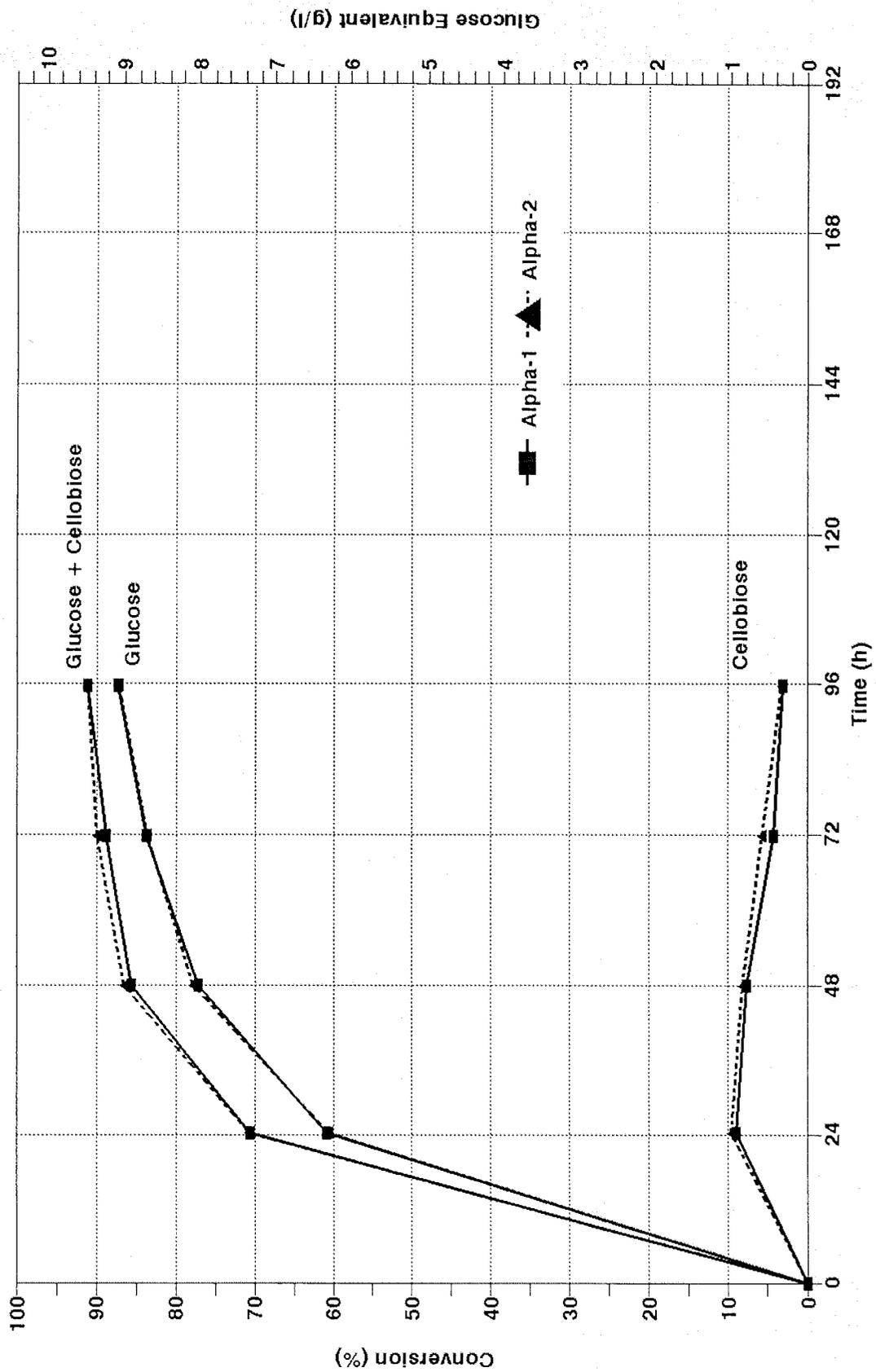


Figure 49. Enzyme Hydrolysis @ 50 °C. Alpha Cellulose.

Table 52. Enzyme Hydrolysis @ 50 °C. Series #1: Reactions 1, 2.

Time,h	Reaction 1-1					
	Glucose,g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	Sigma510A		HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.03	0.00	0.00	0.00	0.0
24.1	8.77	8.53	1.81	1.91	10.68	87.0
48.0	8.00	10.22	1.08	1.14	11.42	93.1
72.0	10.78	10.53	0.46	0.48	11.26	91.8
96.5	10.84	10.42	0.28	0.29	11.13	90.7
144.1	11.74	10.45	0.19	0.20	11.94	97.3
168.0	11.14	10.92	0.12	0.13	11.27	91.9
Theor.Conv.					12.27	100.0

Time,h	Reaction 1-2					
	Glucose,g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	Sigma510A		HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.00	0.00	0.00	0.00	0.0
24.1	8.97	9.03	2.01	2.12	11.10	90.5
48.0	10.60	10.52	1.20	1.26	11.86	96.7
72.0	11.11	10.46	0.51	0.64	11.65	94.9
96.5	11.32	10.63	0.46	0.48	11.80	96.1
144.1	11.55	11.46	0.30	0.32	11.87	96.7
168.0	-	11.44	0.05	0.05	11.49	93.6
Theor.Conv.					12.27	100.0

Time,h	Reaction 2-1					
	Glucose,g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	Sigma510A		HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.03	0.00	0.00	0.00	0.0
24.1	8.72	8.61	1.67	1.76	10.48	96.6
48.0	9.83	9.77	1.06	1.12	10.95	100.9
72.0	10.30	10.16	0.59	0.62	10.92	100.6
96.5	10.39	9.90	0.40	0.42	10.81	99.6
144.1	10.62	10.15	0.27	0.28	10.90	100.5
168.0	10.66	11.08	0.20	0.21	10.87	100.2
Theor.Conv.					10.85	100.0

Time,h	reaction 2-2					
	Glucose,g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	Sigma510A		HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.03	0.00	0.00	0.00	0.0
24.1	8.90	8.64	1.84	1.94	10.84	99.9
48.0	10.18	10.07	1.04	1.10	11.28	104.0
72.0	10.02	9.55	0.49	0.52	10.54	97.1
96.5	10.55	10.10	0.38	0.40	10.95	100.9
144.1	11.15	10.77	0.23	0.24	11.39	105.0
168.0	10.71	10.60	0.26	0.27	10.98	101.2
Theor.Conv.					10.85	100.0

Table 53. Enzyme Hydrolysis @ 50 °C. Series #1: Reactions 5, 7.

Time,h	Reaction 5-1					
	Glucose ,g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.04	0.00	0.00	0.00	0.0
24.1	6.72	6.74	1.24	1.31	8.03	66.4
48.0	7.89	7.86	0.85	0.89	8.78	72.6
72.0	8.20	7.98	0.27	0.28	8.48	70.0
96.5	8.41	7.95	0.23	0.24	8.65	71.5
144.1	8.57	8.06	0.10	0.11	8.68	71.7
168.0	8.70	8.56	0.14	0.16	8.86	73.2
Theor.Conv.					12.10	100.0

Time,h	Reaction 5-2					
	Glucose ,g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.04	0.00	0.00	0.00	0.0
24.1	7.04	6.99	1.39	1.46	8.50	70.2
48.0	8.06	8.16	0.99	1.04	9.10	75.2
72.0	8.58	8.31	0.28	0.29	8.87	73.3
96.5	8.75	8.29	0.30	0.32	9.07	75.0
144.1	8.93	8.59	0.15	0.16	9.09	75.1
168.0	8.88	8.92	0.13	0.14	9.02	74.5
Theor.Conv.					12.10	100.0

Time,h	Reaction 7-1					
	Glucose ,g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.03	0.00	0.00	0.00	0.0
24.1	7.46	7.24	1.31	1.38	8.83	72.6
48.0	8.62	8.27	0.95	1.00	9.62	79.1
72.0	8.54	8.54	0.43	0.45	8.99	73.9
96.5	9.04	8.41	0.29	0.31	9.35	76.9
144.1	9.01	9.22	0.17	0.18	9.18	75.5
168.0	9.27	9.08	0.08	0.08	9.35	76.9
Theor.Conv.					12.16	100.0

Time,h	Reaction 7-2					
	Glucose ,g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.04	0.00	0.00	0.00	0.0
24.1	7.15	7.28	1.39	1.46	8.61	70.8
48.0	8.33	8.42	0.90	0.95	9.28	76.3
72.0	8.69	8.31	0.43	0.45	9.14	75.2
96.5	8.75	8.37	0.27	0.28	9.07	74.6
144.1	9.01	9.10	0.19	0.20	9.30	76.5
168.0	9.04	8.84	0.00	0.00	9.04	74.3
Theor.Conv.					12.16	100.0

Table 54. Enzyme Hydrolysis @ 50 °C. Series #2: Reactions 3, 4.

Time,h	Reaction 3-1					
	Glucose, g/l		Cellobios ,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.04	0.90	0.95	0.00	0.0
24.1	7.46	8.77	1.11	1.17	8.63	79.1
48.0	-	-	-	-	-	-
71.9	8.61	8.40	0.42	0.44	9.05	83.0
95.8	8.78	8.56	0.22	0.23	9.01	82.6
Theor.Conv.					10.91	100.0

Time,h	Reaction 3-2					
	Glucose, g/l		Cellobios ,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.04	0.94	0.99	0.00	0.0
24.1	7.71	7.96	1.20	1.26	8.97	82.2
48.0	8.65	8.54	0.88	0.93	9.58	87.8
71.9	8.88	8.68	0.40	0.42	9.30	85.2
95.8	9.01	8.58	0.37	0.39	9.40	86.2
Theor.Conv.					10.91	100.0

Time,h	Reaction 4-1					
	Glucose, g/l		Cellobios ,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.04	0.00	0.00	0.00	0.0
24.1	8.93	8.88	1.75	1.84	10.77	77.6
48.0	10.23	10.06	1.15	1.21	11.44	82.2
71.9	10.75	10.55	1.01	1.06	11.81	85.1
95.8	10.92	10.35	0.57	0.60	11.52	83.1
Theor.Conv.					13.86	100.0

Time,h	Reaction 4-2					
	Glucose, g/l		Cellobios ,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.04	0.00	0.00	0.00	0.0
24.1	9.14	9.08	1.86	1.96	11.10	80.0
48.0	10.45	10.06	1.20	1.26	11.71	84.4
71.9	11.06	10.37	0.92	0.97	12.03	86.8
95.8	11.35	10.51	0.48	0.51	11.86	85.5
Theor.Conv.					13.86	100.0

Table 55. Enzyme Hydrolysis @ 50 °C. Series #2: Reactions 6, Feedstock, α -Cellulose.

Time,h	Reaction 6-1					
	Glucose, g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.04	0.00	0.00	0.00	0.0
24.1	7.19	6.91	1.08	1.13	8.32	78.1
48.0	7.91	7.42	0.66	0.69	8.60	80.8
71.9	8.17	7.80	0.45	0.47	8.64	81.2
95.8	8.38	7.56	0.22	0.23	8.61	80.9
Theor.Conv.					10.65	100.0
Time,h	Reaction 6-2					
	Glucose, g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.09	0.04	0.00	0.00	0.00	0.0
24.1	7.34	6.77	1.12	1.18	8.52	80.0
48.0	8.02	7.61	0.67	0.71	8.73	82.0
71.9	8.65	7.91	0.21	0.22	8.87	83.3
95.8	8.46	7.66	0.35	0.37	8.83	82.9
Theor.Conv.					10.65	100.0
Time,h	Feedstock HP-1					
	Glucose, g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.03	0.00	0.00	0.00	0.0
24.1	0.51	0.90	0.00	0.00	0.51	4.4
48.0	0.58	0.77	0.00	0.00	0.58	5.0
71.9	0.61	0.96	0.00	0.00	0.61	5.2
95.8	0.66	0.93	0.00	0.00	0.66	5.6
Theor.Conv.					11.69	100.0
Time,h	Feedstock HP-2					
	Glucose, g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.04	2.00	0.00	0.00	0.0
24.1	0.50	0.65	0.00	0.00	0.50	4.3
48.0	0.61	0.77	0.00	0.00	0.61	5.2
71.9	0.63	1.02	0.00	0.00	0.63	5.4
95.8	0.70	0.92	0.00	0.00	0.70	6.0
Theor.Conv.					11.69	100.0
Time,h	Alpha Cellulose; Alpha-1					
	Glucose, g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.03	0.00	0.00	0.00	0.0
24.1	6.33	6.14	0.90	0.95	7.28	70.5
48.0	8.02	7.72	0.76	0.80	8.82	85.5
71.9	8.71	8.20	0.42	0.44	9.15	88.7
95.8	9.08	8.32	0.31	0.33	9.41	91.2
Theor.Co					10.32	
Theor.Conv.						100.0
Time,h	Alpha Cellulose; Alpha-2					
	Glucose, g/l		Cellobiose,g/l		Tot.Glu.Equiv.	
	HPLC	Sigma510A	HPLC	Glu.Equiv.	g/l	Conv.,%
0.0	0.00	0.04	0.00	0.00	0.00	0.0
24.1	6.29	6.02	0.96	1.01	7.30	70.7
48.0	8.08	7.50	0.82	0.86	8.94	86.6
71.9	8.69	8.18	0.57	0.60	9.29	90.0
95.8	9.07	7.84	0.32	0.34	9.41	91.2
Theor.Conv.					10.32	100.0

5.4.2 Influence of Pretreatment Conditions on 50 °C Enzymatic Hydrolysis Results.

The maximum glucose plus cellobiose yields from 50 °C and 38 °C enzyme hydrolysis and maximum ethanol yields are compared with selected prehydrolysis reaction conditions in Table 44. The 170 °C reactions (1-4) gave better results than the 160 °C reactions (5-7) in 50 °C enzyme hydrolysis, just as they did in SSF and 38 °C enzyme hydrolysis.

Reaction 2 gave the best results of all the pretreated solids. For a given pretreated solid, the yields of ethanol from SSF were similar to, or lower than, those of glucose plus cellobiose from 50 °C enzymatic hydrolysis. In the case of Reaction 6, a 160 °C reaction, the yield from 50 °C enzyme hydrolysis was 12%-15% greater than that of ethanol from SSF.

Conclusions

Overall the results achieved in this project have met the major objectives. The problems encountered have either been solved or the method of solution has been suggested.

The CFHR simulates a practical design that can be scaled up to a commercial size. The continuous operation of the CFHR unit for pretreatment of lignocellulosic materials was successfully carried out using dilute nitric acid to hydrolyze the hemicelluloses present in Hybrid Poplar. These results were obtained in a minimal number of steady-state intervals. Only a limited time could be devoted to the pretreatment task of this project. It became imperative to proceed with the analysis of the steady-state reaction products that had been prepared in order to undertake the critical evaluation of the pretreated residues by SSF to produce ethanol and enzymatic hydrolysis under Task 5. There was simply insufficient time to undertake both an optimization of the operation of the CFHR and the work under Task 5. The work under Task 5 was selected and the attention of all members of the team were directed to this effort.

Adaptation of NREL's CATSP protocols and work to obtain approval of these were initiated simultaneously with the work on SSF. Protocol modifications, submitted in the QA/QC report September, 1994, were not approved. Adaptations of certain protocols were worked out in conferences with NREL in January and February, 1994. Laboratory demonstration gained approval of the modifications in mid-March and the formal SSF evaluations were initiated using the NREL approved protocols.

6.1 Pretreatment

The best conditions that were used in operation of the CFHR in this study, based on consideration of overall results, were carried out at a nominal temperature of 170°C, using a calculated initial acid concentration of 0.35% in the reactor and a residence time of 27 minutes (Reaction 2). These results were essentially duplicated using an acid concentration of 0.25%, and a residence time of 25 minutes (Reaction 1).

The unconverted xylan contents of the residues obtained in Reaction 2, 3, and 4 were about 0.9%; that of reaction 1 was 1.55%. This indicates that optimum conditions were being approached using 170°C for maximum hydrolysis of the hemicellulosic content of the pretreatment residues produced. The removal of hemicelluloses and, perhaps, as much of the soluble lignin as possible, is a prerequisite for obtaining highest yields of xylose; highest yields and highest rates of ethanol production by SSF; and highest yields of glucose and cellobiose in 38°C and of glucose in 50°C enzymatic hydrolysis.

Results of Steady-state Reactions

In the steady-state reactions, in the order given, Reactions 1 through 7, steady improvement was made in the techniques used in operating the CFHR. These reactions may be considered as shakedown runs in the operation of a plant using a green crew of 6 members and two experienced operators. A considerable amount of physical work and monitoring are required in the operation. As experience was gained substantial improvements were made. A critical product property showing improvement in the operation of the prehydrolysis reactor was the increase in the percentages of the soluble(hydrolyzate) and the pretreated residue in the slurry. Thus, the percentages of pretreated residues in the slurry increased from about 3% to

about 6% as experience was gained. The objective was about 9%-10% that had been obtained in previous work. This is considered entirely feasible using the present feedstock.

The relatively low consistency of solids produced in the slurry can be attributed, in part, to the use of a new feedstock. The hybrid poplar feedstock being evaluated had one critical property that had not been examined previously in making runs with the CFHR; namely, the moisture content of the feedstock. The high moisture content, i.e., 56-58 % was not considered a problem and no steps were taken to assure penetration of the feedstock with acid in the lockhopper. To avoid slow acid penetration, mechanical impregnation of the feedstock with dilute acid prior to introduction into the lockhopper should be undertaken. This would have greatly improved the results obtained, especially assuring the retention of acid in the wood slurry being processed in the reactor.

Sensing the wood level in the operation of the CFHR is an important control which we have addressed without a successful resolution. A gamma-ray gage would overcome this problem. We are seeking a more inexpensive but effective solution to this problem.

One of the reasons that higher material balance closures have not been achieved may involve production of dilute slurries as discussed above. As the consistency of this slurry is increased it is less difficult to obtain a representative sample. The problem of accurate sampling was addressed but it is not certain that it was solved. The high percentage of feedstock solubilized is a strong indication that inadequate sampling was carried out in the runs made. If this was the case it can be corrected and steps will be taken to insure that samples of the uniformly mixed slurries are taken.

The disintegration of the prehydrolysis residues at the reaction temperatures used appears to have facilitated enzymatic hydrolysis. No discernable untoward effects of this disintegration have been observed.

Energy requirements for disintegration appear to be higher than those found earlier. This is attributed to a lower than expected solids concentration in the prehydrolysis reactor. Also, the disintegrator was used at a fairly uniform set of conditions. It was not investigated as a reaction parameter. With optimization of both pretreatment reaction parameters and the parameters associated with disintegration it is anticipated that the power requirements will be substantially reduced.

Evaluation of Hydrolyzates

The evaluation of hydrolyzates was given lowest priority in the work carried out from mid January to mid April, 1994 after the steady-state reactions had been carried out. This was an unfortunate decision as during the three months of elapsed time the furfural content of the hydrolyzates was lost and the concentration of xylose was decreased. A further loss of xylose in the refrigerated hydrolyzates was observed in analyses carried out between April of 1994 and January of 1995. Based on these results it is imperative that analysis and fermentation of the hydrolyzates be carried out as soon after production as possible.

6.2 SSF and Enzymatic Hydrolysis

Results obtained under the reaction conditions were very positive with respect to producing ethanol using NREL's SSF protocol CATSP 008 and glucose using 50°C enzymatic protocol CATSP 009. The evaluation by the SSF protocol was given top priority in this study to provide critical information relative

to the direction to be taken in the development of the process involving dilute acid pretreatment followed by cellulose conversion to ethanol or glucose and then ethanol.

Better SSF results (higher ethanol production) were found from pretreated residues prepared at 170°C (Reactions 1 - 4) than at 160°C (Reactions 5 - 7) due to more complete hydrolysis of hemicelluloses and other acid-soluble components. This may be attributed to the more optimum combinations of temperature and residence times used in the reactions at 170°C than used at 160°C. The greater hydrolysis and dissolution of the hemicellulose increased the cellulose surface area available for binding with the enzymes.

The SSF reactions gave relatively high conversions to ethanol, as compared with NREL's 1992 results (Wyman et al. 1992). All the solids from 170°C reactions gave 85% to 92% conversions to ethanol.

The overall results obtained in Reaction 2 were the best obtained with very similar results obtained in Reaction 1. These were better than those obtained in Reactions 3 and 4 which were similar. Finally those obtained in Reactions 3 and 4 were generally better than those obtained in Reactions 5, 6 and 7. It may be concluded that this order is directly correlated with the low hemicellulosic content (xylan).

The pretreated residues produced under the best conditions used (Reaction No 2) provided a substrate for SSF which in 96 hours gave yields of ethanol of 88% and 89% of the theoretical yield based on the glucan content of the substrate. Maximum yields of ethanol were 91% and 92% of the theoretical.

In all seven pretreatment residues evaluated the rates of ethanol production in SSF were high. Virtually all of the ethanol was produced in 96 hours and about 90% of that was converted in 72 hours.

These yields of ethanol production were similar to those obtained for alpha cellulose and the rate of production was higher than that obtained for alpha cellulose. This may be attributed to use of dried alpha cellulose with accompanying loss of readily available surface for enzymatic hydrolysis. The presence of residual xylan also may be a factor.

The relatively high rates of conversion of cellulose to ethanol in SSF reactions is attributed to the small particle size produced by the mechanical action of the disintegrator and the production of a large surface available for enzymatic hydrolysis of the cellulose.

In the evaluation of the pretreated reaction solids by 50°C enzymatic hydrolysis, the duplicate evaluations made of the substrate from Reaction 2 gave yields of glucose plus cellobiose of 96.6% and 99.9% in 24 hrs. The results from Reaction No. 1 gave the next highest yields and rates of conversion of any of the other five evaluations carried out.

The high yields and rates of ethanol production in SSF indicates that there is no inhibitory effect in the use of dilute nitric acid as the catalyst used in hydrolysis.

Enzymatic hydrolysis at 50°C was fast, with over 90% of the cellulose being solubilized to glucose plus cellobiose in 24 hours. This is attributed to the small particle size produced in the course of the pretreatment reactions. At 38°C the rapid production of glucose and cellobiose leads to serious end-product inhibition and lower overall conversions to glucose.

The use of dilute nitric acid as the prehydrolysis catalyst does not appear to introduce any special problems of toxicity to enzyme or yeast. However, well washed pretreatment residues were used which excludes the effects that dilute solutions of nitric might have.

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Abstract

Pretreatment of a hybrid Poplar chipped feedstock was performed in a series of steady-state reactions, using dilute nitric acid and temperatures from 160 °C to 170 °C. Feed rates from 0.143 to 0.202 kilograms per minute were maintained.

Simultaneous saccharification and fermentation (SSF) treatments were applied to the pretreated solids, to produce ethanol in high yields. Enzymatic hydrolysis studies at 38 °C and 50 °C were also applied to the pretreated solids, to investigate possible optimization and inhibition factors in the enzymatic process.

The pretreated residues produced under the best conditions used (Reaction No 2) provided a substrate for SSF which in 96 hours gave yields of ethanol of 88% to 89% of the theoretical yield based on the glucan content of the substrate. Maximum yields of ethanol were 91% and 92% of theoretical.

The relatively high rates of conversion of cellulose to ethanol in SSF reactions is attributed to the small particle size produced by the mechanical action of the disintegrator and the production of a large surface available for enzymatic hydrolysis of the cellulose. The high yields and rates of ethanol production in SSF indicate that there is no inhibitory effect attributable to the use of nitric acid rather than sulfuric acid as the catalyst in hydrolysis.

As Analyzed Tables Appendix.

These tables compliment the Pretreated Solids, Feedstock, and Conversion Tables of the report. They contain, in the same format, results that have not had correction factors based on experimental sugar degradations applied. They are supplied without comment, for reference and as a basis for technical discussion.

Pretreated Solids Compositions

Table A1. Reaction 1, as Analyzed.

				%	Lb	Kg
Pretreated Solids				8.62	3.91	
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	54.88	4.73	2.15	Glucan	49.4	4.26 1.93
Mannose	0	0	0	Mannan	0.0	0 0
Galactose	0	0	0	Galactan	0.0	0 0
Subtotal	54.88	4.73	2.15	Subtotal	49.4	4.26 1.93
Pentose				Pentosan		
Xylose	1.40	0.121	0.055	Xylan	1.23	0.106 0.048
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	1.40	0.121	0.055	Subtotal	1.232	0.106 0.048
Total Sugars	56.3	4.85	2.20	Total Glycan	50.6	4.36 1.98
Acid Insoluble Residue				40.4	3.48	1.58
Ash	1.46	0.126	0.057			

Table A2. Reaction 2, as Analyzed.

				%	Lb	Kg
Pretreated Solids					3.97	1.80
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	51.7	2.05	0.931	Glucan	46.6	1.85 0.839
Mannose	0	0	0	Mannan	0.0	0 0
Galactose	0	0	0	Galactan	0.0	0 0
Subtotal	51.7	2.05	0.931	Subtotal	46.6	1.85 0.839
Pentose				Pentosan		
Xylose	0.87	0.035	0.016	Xylan	0.77	0.031 0.014
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	0.87	0.035	0.016	Subtotal	0.766	0.031 0.014
Total Sugars	52.6	2.09	0.947	Total Glycan	47.3	1.88 0.853
Acid Insoluble Residue					39.9	1.55 0.70
Ash					1.35	0.054 0.024

Table A3. Reaction 3, as Analyzed.

				%	Lb	Kg
Pretreated Solids					10.3	4.66
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	54.4	5.61	2.54	Glucan	49.0	5.05 2.28
Mannose	0	0	0	Mannan	0.0	0 0
Galactose	0	0	0	Galactan	0.0	0 0
Subtotal	54.4	5.61	2.54	Subtotal	49.0	5.05 2.28
Pentose				Pentosan		
Xylose	0.85	0.088	0.04	Xylan	0.75	0.077 0.035
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	0.85	0.088	0.04	Subtotal	0.748	0.077 0.035
Total Sugars	55.3	5.70	2.58	Total Glycan	49.7	5.12 2.318
Acid Insoluble Residue					40.5	4.17 1.89
Ash					2.46	0.253 0.115

Table A4. Reaction 4, as Analyzed.

				%	Lb	Kg
Pretreated Solids					6.32	2.87
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	53.9	3.41	1.55	Glucan	48.5	3.06 1.39
Mannose	0	0	0	Mannan	0.0	0 0
Galactose	0	0	0	Galactan	0.0	0 0
Subtotal	53.9	3.41	1.55	Subtotal	48.5	3.06 1.39
Pentose				Pentosan		
Xylose	0.78	0.049	0.022	Xylan	0.686	0.043 0.020
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	0.78	0.049	0.022	Subtotal	0.686	0.043 0.020
Total Sugars	54.7	3.46	1.57	Total Glycan	49.2	3.10 1.41
Acid Insoluble Residue					41.2	2.60 1.18
Ash					2.29	0.145 0.066

Table A5. Reaction 5, as Analyzed.

				%	Lb	Kg
Pretreated Solids					8.90	4.04
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	50.6	4.51	2.05	Glucan	45.6	4.05 1.84
Mannose	0	0	0	Mannan	0.0	0 0
Galactose	0	0	0	Galactan	0.0	0 0
Subtotal	50.6	4.51	2.05	Subtotal	45.6	4.05 1.84
Pentose				Pentosan		
Xylose	2.51	0.223	0.101	Xylan	2.21	0.197 0.089
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	2.51	0.223	0.101	Subtotal	2.21	0.197 0.089
Total Sugars	53.1	4.73	2.15	Total Glycan	47.8	4.25 1.93
Acid Insoluble Residue					39.8	3.54 1.61
Acid Soluble Lignin					1.07	0.1 0.04
Ash					2.01	0.179 0.081

Table A6. Reaction 6, as Analyzed.

				%	Lb	Kg
Pretreated Solids					9.94	4.51
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	53.6	5.33	2.42	Glucan	48.2	4.80 2.18
Mannose	0	0	0	Mannan	0.0	0 0
Galactose	0	0	0	Galactan	0.0	0 0
Subtotal	53.6	5.33	2.42	Subtotal	48.2	4.80 2.18
Pentose				Pentosan		
Xylose	1.87	0.186	0.084	Xylan	1.65	0.164 0.074
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	1.87	0.186	0.084	Subtotal	1.646	0.164 0.074
Total Sugars	55.5	5.515	2.502	Total Glycan	49.9	4.96 2.25
Acid Insoluble Residue					41.2	4.1 1.86
Ash	2.30	0.229	0.104			

Table A7. Reaction 7, as Analyzed.

				%	Lb	Kg
Pretreated Solids					9.60	4.36
Sugars						
As Monosaccharides	%	Lb	Kg	As Polyglycan		
Hexose				Hexosan		
Glucose	53.1	5.10	2.31	Glucan	47.8	4.59 2.08
Mannose	0	0	0	Mannan	0.0	0 0
Galactose	0	0	0	Galactan	0.0	0 0
Subtotal	53.1	5.10	2.31	Subtotal	47.8	4.59 2.08
Pentose				Pentosan		
Xylose	2.01	0.193	0.088	Xylan	1.77	0.17 0.077
Arabinose	0	0	0	Arabinan	0	0 0
Subtotal	2.01	0.193	0.088	Subtotal	1.769	0.17 0.077
Total Sugars	55.1	5.29	2.40	Total Glycan	49.5	4.76 2.16
Acid Insoluble Residue					38.9	3.74 1.7
Ash	2.09	0.201	0.091			

Table A10. Hexosan Conversion, Yield, Unconverted, Recovery (as Analyzed).

Reaction	Hexosan	Feedstock Kg	Hydrolyzate Kg	Solids Kg	Converted %	Yield %	Unconverted %	Recovery %
1	glucan	3.30	0.20	1.93	41.6	6.05	58.4	64.5
	mannan	0.211	0.13	0	100	61.6	0.0	61.6
	galactan	0.116	0.11	0	100	94.9	0.0	94.9
	total	3.63	0.44	1.93	46.8	12.1	53.2	65.3
2	glucan	1.73	0.07	0.82	52.6	4.04	47.4	51.4
	mannan	0.111	0.06	0	100	54.3	0.0	54.3
	galactan	0.061	0.04	0	100	65.8	0.0	65.8
	total	1.90	0.17	0.82	56.9	8.94	43.1	52.0
3	glucan	3.71	0.20	2.28	38.5	5.40	61.5	66.9
	mannan	0.237	0.20	0	100	84.5	0.0	84.5
	galactan	0.13	0.12	0	100	92.3	0.0	92.3
	total	4.07	0.52	2.28	44.0	12.8	56.0	68.8
4	glucan	2.71	0.13	1.39	48.8	4.79	51.2	56.0
	mannan	0.173	0.12	0	100	69.2	0.0	69.2
	galactan	0.095	0.08	0	100	84.0	0.0	84.0
	total	2.98	0.33	1.39	53.4	11.1	46.6	57.7
5	glucan	3.63	0.14	1.84	49.3	3.86	50.7	54.6
	mannan	0.232	0.11	0	100	47.5	0.0	47.5
	galactan	0.127	0.11	0	100	86.4	0.0	86.4
	total	3.99	0.36	1.84	53.9	9.03	46.1	55.2
6	glucan	3.57	0.20	2.18	39.0	5.60	61.0	66.6
	mannan	0.228	0.15	0	100	65.7	0.0	65.7
	galactan	0.125	0.12	0	100	95.7	0.0	95.7
	total	3.93	0.47	2.18	44.5	12.0	55.5	67.5
7	glucan	2.86	0.15	2.08	27.3	5.25	72.7	78.0
	mannan	0.183	0.11	0	100	60.2	0.0	60.2
	galactan	0.10	0.08	0	100	79.7	0.0	79.7
	total	3.14	0.34	2.08	33.8	10.8	66.2	77.0

Table A11. Pentosan Conversion, Yield, Unconverted, Recovery (as Analyzed).

React- ion	Pentosan	Feedstock	Hydrolysate	Solids	Converted	Yield	Unconverted	Recovery
		Kg	Kg	Kg	as pentosan %	%	%	as pentosan %
1	Xylan	1.112	0.55	0.048	95.7	49.5	4.32	53.8
	Arabinan	0.138	0.06		100	43.4	0	43.4
	total	1.25	0.61	0.048	96.2	48.8	3.84	52.6
2	Xylan	0.582	0.28	0.013	97.8	48.1	2.23	50.3
	Arabinan	0.072	0.03		100	41.4	0	41.4
	total	0.655	0.31	0.013	98.0	47.3	1.99	49.3
3	Xylan	1.247	0.68	0.035	97.2	54.5	2.81	57.3
	Arabinan	0.155	0.09		100	58.0	0	58.0
	total	1.402	0.77	0.035	97.5	54.9	2.50	57.4
4	Xylan	0.913	0.49	0.020	97.8	53.7	2.19	55.9
	Arabinan	0.114	0.05		100	44.0	0	44.0
	total	1.027	0.54	0.020	98.1	52.6	1.95	54.5
5	Xylan	1.221	0.52	0.089	92.7	42.6	7.29	49.9
	Arabinan	0.152	0.08		100	52.6	0	52.6
	total	1.373	0.60	0.089	93.5	43.7	6.48	50.2
6	Xylan	1.202	0.71	0.074	93.8	59.1	6.16	65.2
	Arabinan	0.15	0.10		100	66.8	0	66.8
	total	1.352	0.81	0.074	94.5	59.9	5.47	65.4
7	Xylan	0.962	0.44	0.077	92.0	45.7	8.00	53.7
	Arabinan	0.12	0.07		100	58.5	0	58.5
	total	1.082	0.51	0.077	92.9	47.1	7.12	54.3