

PDU RUN REPORT

CRADA TASK #3

March, 1996

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Run number: P960122CF

Dates of run: 1/22/96 to 2/16/96

Feedstock: Blended corn fiber and cracked corn

1.0 Executive Summary

Batch fermentation experiments were conducted in the PDU to prove that the recombinant organism LNHST2 could co-ferment glucose and xylose from a pretreated corn fiber/cracked corn feedstock blend at a large scale. A total of five batch-fermentation runs were attempted; four in the 1450-L fermenters (runs 1—4) and one in the 9000-L fermenter (run 5). All runs were at a total solids concentration of approximately 20%. The 8.5 to 1 by wet weight corn fiber to cracked corn feedstock was pretreated in the Amoco Pretreatment Reactor (APR) using conditions previously identified as producing high glucose and xylose yields.

The first two runs (1 and 2) in the 1450-L fermenters were not successful (no ethanol production) because the sterilization procedure (combined sterilization of pretreated material and CSL) apparently produced fermentation inhibitors. Ethanol production was achieved when CSL was sterilized prior to addition of pretreated feed, and the pretreatment process itself was relied upon to sterilize the substrate.

Run 3 in the 1450-L fermenter achieved a final ethanol concentration of 28 g/L, but took 60 hours to consume all of the monomeric glucose. This was significantly longer than bench scale fermentations conducted at identical conditions. The lag was likely caused by inadvertent damage to the yeast inoculum. After 5 days, only 66% of the monomeric xylose was consumed.

Run 4 achieved a higher ethanol concentration of 40 g/L and consumed all of the monomeric glucose within 24 hours. However, only half of the monomeric xylose was consumed after 7 d. This may be due to inhibition by organic acids and/or ethanol. Testing underway at Purdue should determine if this is true. The same performance as run 4 was obtained with a bench scale SSCF using pretreated material taken directly from the PDU fermenter for run 4. This proves that bench scale results mimic the results of PDU runs. Thus, performance can be investigated and proven at the bench scale and the PDU can be used to examine recycles or generate material for further testing.

The best run was run 5 in the 9000-L fermenter. The ethanol concentration was 45 g/L and again all of the monomeric glucose was consumed within 24 h. After 4.5 d, 75% of the monomeric xylose was also consumed. The ethanol yield was 83% based on sugars consumed, but only 57% based on total available sugars. The difference is due to a large concentration of soluble sugars (14.3 g/L glucose primarily as oligomers and 16.3 g/L xylose, half as oligomers and the other half as monomers) left at the end of the fermentation. Xylose oligomers were not consumed or converted to monomer in any of the fermentations.

Overall carbon balance closure for pretreatment was $89.6\% \pm 14.1\%$ at a 95% confidence level based on analytical repeatability (random errors) and assumed errors for flow rate measurements. Automating APR flow rate measurements for Task 4 will provide the measurements necessary to eliminate the assumptions. Overall carbon closure for fermentation run 4 was 94.5%. Error analysis will also be done for SSCF in Task 4.

The distillation system ran successfully and solids were separated from the column bottoms stream and saved for drying and dewatering tests as a prelude to animal feed testing. Approximately 60%—65% of the total protein is in the dry residual solids remaining after fermentation.

2.0 Introduction

The primary purpose of Task 3 was to prove that a pretreated blend of corn fiber and cracked corn (corn screenings) could be successfully fermented at high solids levels in the PDU using the organism, LNHST2. This recombinant organism has been engineered to co-ferment glucose and xylose. The goal was to utilize all of the fermentable sugars and achieve high ethanol concentrations. Additionally, solids product left after fermentation was collected and sent to vendors for animal feed processing tests in drying and dewatering pilot units. Animal testing is outside the scope of Task 3 and the CRADA.

3.0 Pilot Plant Operations

Operation of the pilot plant for Task 3 began on Jan. 22 with startup of the seed train. The seed train was used to inoculate batch fermentations that were conducted in the 1450-L and 9000-L fermenters with a pretreated corn fiber/cracked corn blend produced by the APR. Ethanol was stripped from the 9000-L fermentation broth using the distillation system. The bottoms product from the column was sterilized and centrifuged to concentrate the solids.

A total of five batch fermentations were completed; four at 900 kg (in the 1450-L fermenters and identified as runs 1—4) and one at 7000 kg (in the 9000-L fermenter and identified as run 5). The first two 900 kg batches (runs 1 and 2) were sterilized prior to inoculation; neither of these produced any ethanol. It was noted that the color of the hydrolyzate darkened considerably after sterilization. Due to concerns that the sterilization was producing inhibitors, the remaining batches were not sterilized, but relied on pretreatment to sterilize the substrate. The third 900 kg batch exhibited a lag, probably because the yeast inoculum was overheated while being injected into the fermenter, but did ferment. The fourth 900 kg batch did not exhibit any lag. The 7000 kg batch also did not exhibit any appreciable lag before starting to produce ethanol.

3.1 Procedures and Operating Conditions

3.1.1 Feed Handling/Pretreatment Operating Conditions

This run used a blended feedstock of corn fiber and cracked corn in a 8.5 to 1.0 wet weight ratio (or 3.9 to 1.0 dry weight ratio assuming solids concentrations of 40% and 87% for corn fiber and cracked corn, respectively). Corn fiber and cracked corn were obtained from a corn wet-milling facility (Cardinal plant, Ontario, Canada), then blended, frozen and shipped to the PDU in 55-gal drums in a refrigerated trailer. The material was pretreated by the APR using the conditions shown in Table 1. pH was measured with a pH probe after cooling the sample to room temperature. APR sample 178 was taken during filling of the fermenter for fermentation run 1 and APR samples 179 and 180 were taken during filling of the fermenters for runs 3 and 4, respectively. APR samples 181 through 189 were taken during filling of the 9000-L fermenter. The operators attempted to maintain constant operating conditions, however, there were some variations in the pH.

3.1.2 Fermentation Operating Conditions

Operating conditions for the seed train are shown in Table 2. LNHST2 was grown by successive transfers from a small shake flask to a larger shake flask, and then to the 20-L, 160-L, and 1450-L fermenters, respectively. There was no pH control in the shake flask. pH was controlled at 5 with 3.0 molar NaOH in the 20-L and 160-L

fermenters and with 50% NaOH in the 1450-L fermenter. Initial seed batches were started with 50 g/L glucose; some of these batches exhibited slow or stalled glucose utilization. The last two seed batches used 20 g/L glucose; neither exhibited the same problem seen with 50 g/L fermentations.

Fermentation conditions in the 1450-L and 9000-L fermenters are also presented in Table 2. A 10% (w/w) inoculum was added after the feed was neutralized with NaOH and after addition of **CSL** and enzymes (cellulase and glucoamylase). The enzymes were added through a septum at the top of the tank using a peristaltic pump. pH was controlled using 50% NaOH. Solids concentrations in fermentation runs 3, 4, and 5 were 18.6%, 18.0%, and 20.9%, respectively; the target was 20% in all batches. Solids concentration of the pretreated material produced for runs 3 and 4 were lower than originally planned, which accounts for the lower solids concentration in these runs.

3.2 Run History

On January 23 and 24, the first two 1450-L fermenters (runs 1 and 2) were filled and sterilized, then inoculated the following day. On January 26, after no ethanol production was seen in the fermenters and suspecting that sterilizing the material in the fermenters was causing a problem, the third **1450-L** fermenter batch (run 3) was filled sterilely and inoculated the following day. The fourth batch (run 4) was filled on February 1; each 1450-L fermenter took approximately 6-7 hours to fill. The 9000-L fermenter was then filled in about 72 hours starting on February 2 (run 5). Several minor problems arose from conducting batch fermentations in equipment designed for continuous operation. The most significant was long transfer times of inoculum or media because of small transfer line diameters and control valves designed for low flow rates. All **1450-L** batches ran for approximately 100-144 hours; the 9000-L batch ran for 108 h. Fermentation broth from runs 4 and 5 were then combined and run through distillation in about 6 hours. The ethanol product was sent to the **PDU** ethanol storage tank. Any yeast still viable in the bottoms product was deactivated using a sterilization cycle of $>125^{\circ}\text{C}$ for 60 minutes. This stream was then centrifuged to recover the solids, which were drummed for further testing set up by SWAN. Four drums of centrate (liquid decanted) were also collected for testing. The remaining centrate was sent to disposal.

3.3 Operational Notes

The following is a discussion of significant operational notes and problems that occurred during this run.

Table 2. Fermentation operating conditions for the seed train, 1450-L and 9000-L fermenters.

Operating Condition	Flask #1	Flask #2	20-L	160-L	1450-L	1450 or 9000-L
Temperature (°C)	30	30	30	30	30	30
Agitation (rpm)	150 ^a	150 ^a	150	100	75	75-125
pH	5.0	5.0	5.0	5.0	5.0	5.0
Gauge Pressure (bar)	-	-	0.33	0.33	0.33	0.33
Airflow (vvm)	-	-	0.5	0.5	0.25	0.0 ^b
Residence Time (h)	12 ^c	8 ^c	8 ^c	12 ^c	12 ^c	96-120 ^d
Media:						
Glucose (%)	2	2	2	2	2	^e
Peptone (%)	2	-	-	-	-	-
Yeast Extract (%)	1	-	-	-	-	-
CSL (%)	-	1	1	1	1	1
Antifoam (corn oil, mL/L)	-	-	0.5	0.5	0.5	0.5
Enzyme (IU/g starch)	-	-	-	-	-	2
Enzyme (IU/g cellulose)	-	-	-	-	-	10

^a laboratory shaker agitation

^b no air added to maintain a positive pressure in vessels

^c typical incubation times

^d per batch

^e substrate was pretreated corn fiber/corn screenings

3.3.1 Feed Handling/Pretreatment

Only one major problem occurred during operation of the APR during this run. During filling of the 9000-L fermenter with pretreated material, the line from the condensate receiver to the drain became plugged causing condensate to fill up the flash receiver. This liquid along with pretreated material was then pumped to the 9000-L fermenter. Once discovered the line was unplugged and normal operation resumed. This event did not significantly change component concentrations in the fermenter.

3.3.2 Fermentation

The most problematic occurrence during the fermentations were temperature spikes during fermentation run 1 attributed to faulty controls that burned out a motor on the new cooling water system. The component (a circuit board) was replaced. Neutralizing the feed before inoculation continued to be a challenge in all fermenters because of high solids levels. The pH-probes, located in the bottom of the vessels, easily fouled with solids until

the enzyme could be added to reduce the viscosity of the hydrolyzate. Increasing the agitation in the vessels and checking the pH with off-line samples is the best solution to this problem. The temperature probes have also fouled on occasion. There were a few instances of disconnection of the data acquisition and control system (DACS) system from the programmable logic controllers (PLCs) on the plant floor, caused by operators using the PLC control software to troubleshoot non-working valves or run modes. The solution to this is two-fold; make the system more reliable and provide a method of troubleshooting for the operators that does not disrupt the system.

3.3.3 Distillation

Distillation ran extremely well with no plugging, primarily because the material was well digested from pretreatment and fermentation. Ethanol concentration was approximately 40% in the product and 0.5% in the bottoms stream. Feed rate to the column was 5—6 gpm. A study was conducted during distillation to determine if the column can be used as a kill step for the recombinant yeast. Although there were live organisms in the column bottoms, it is unclear whether these are the recombinant yeast or organisms that were present in the column before the system was started. In the future, the column will be heated and sterilized before it is fed fermentation broth. The kill study will be repeated in Task 4 using this change in operating procedure. Capturing all the flush water (water used to clean out the column after operation) from the column and sending it to the kill system proved challenging. This is another reason to continue to work towards validating the column as a kill step.

3.3.4 Centrifugation

Because the solids from distillation were very fine, separation of the solids from the liquid using the Sharples centrifuge was difficult. Five drums were collected each weighing about 500 lb at a total solids concentration of 24%. The centrifuge tripped the high torque alarm several times during operation. No reason for this was identified and the centrifuge continued to operate steadily without plugging. The centrate (liquid decanted) looked very muddy.

4.0 Key Results

The following sections presents key results obtained during operation of the pilot plant.

4.1 Pretreatment

Table 3 presents compositional information on corn feedstocks including the latest materials (i.e., cracked corn and blends) used since the end of Task 2. The 1:4.5 blend was used for APR testing in Dec. 1995 and the 1:8.5 blend was used during Task 3. The last row is a calculated composition for the 1:8.5 blend. The calculated values are very close to the measured values, except for starch. An alternative assay for starch has been developed based on an enzymatic technique. The two methods will be compared during Task 4.

Figure 1 shows the concentration of monomeric sugars in the pretreated material, which are nearly equal for all samples. All data in tabular form are shown in appendix A. As previously mentioned, APR-178 was taken from pretreated material used in fermentation run 1, APR-179 in run 2, APR-180 in run 4, and APR-181 through APR-189 in run 5. Figure 2 shows the organic acids, furfural, and HMF concentrations in APR samples. The values for acetic acid and furfural are suspect for APR-187. The somewhat higher acetic acid and sugars concentrations in APR-179 would suggest that the pretreatment was more severe for this samples. But, these concentrations are not expected to be a problem after dilution of the pretreated material in fermentation, which reduces the concentration approximately 40%.

Table 3. Corn Fiber Composition

Lot #	Used In	Source	Moisture (%)	Glucose (%)	Xylose (%)	Gal. (%)	Ara. (%)	Mannose (%)	Lignin (%)	ASL (1) (%)	Ash (%)	Ext. (2) (%)	Starch (3) (%)	Protein (4) (%)	C (%)	H (%)	N (%)
	P950310CF	Casco	60	33.4	23.7	3.9	15.5	0.1	6.5	3.4	1	-	15.7	-	-	-	-
1	P950425CF	GTC	57.6	41.6	21.2	7.7	12.8	0	7.8	7.8	0.9	-	24.9	10.4	-	-	-
2	P950425CF	GTC	54.1	39.9	21.5	7.6	12.2	0	8.5	8.1	0.9	-	25.6	11.6	-	-	-
1	P951101CF	Casco	61	44.7	13.3	3	12.5	0.3	4.5	2.4	1	12.4	18.4	9.1	48.2	6.8	1.8
2	P951101CF	Casco	58	35.5	19.8	4	16.1	0	6.2	5	0.9	9.8	12	10	48.8	6.7	1.9
3	P951101CF	Casco	57	37.4	19.6	4	15.8	0	6.1	5	0.7	9.1	14.5	9.2	48.8	6.8	1.8
	Cracked Corn	Casco	13.3	93.1	2.2	0.5	2	0	2	2.2	0.1	6.7	75.7	4.65	41.57	6.04	0.75
	Blend 1:4.5	Casco	44.5	62.3	13.7	2.4	10.7	0	2.7	4.2	0.9	11.3	41.3	-	-	-	-
	Blend 1:8.5	Casco	54	48.3	15.1	3.2	11.1	0	5.5	4.7	0.7	9.1	41.7	-	-	-	-
	Calc. 1:8.5 Blend	Casco	55.1	47.6	16.1	3.3	13.2	0	5.3	4.4	0.8		28.3				

(1) Acid Soluble Lignin

(2) 95% ethanol extraction, extractives include solubilized protein included in the protein number

(3) starch is also included in the glucose number

(4) Protein calculated from nitrogen content

The severity of the pretreatment appears nearly equal for APR-178 and the APR -181 through APR-189. Thus, pretreatment severity does not explain the lack of ethanol production in the first two fermentation runs. A successful bench scale fermentation was completed on APR-178 pretreated feedstock. The only difference between the PDU fermentation run 1 and the bench scale experiment was that in the PDU CSL was sterilized together with pretreated material. Whereas, in bench scale experiments, CSL and pretreated material were sterilized separately. Even the cells used to inoculate the bench scale fermenter were the same used to inoculated the 1450-L fermenter. Some inhibitors were possibly generated by sterilization of combined pretreated material and CSL. Additional bench-scale work also showed that combining media during sterilization produced significant lags (2 days) in ethanol production in shake flasks. Pretreated material and CSL were not sterilized together for fermentation runs 3, 4, and 5.

Figure 3 shows total soluble (oligomeric and monomeric) glucose and xylose yields for the APR samples. The results show complete hydrolysis of the xylans. Glucose yields were nearly constant at approximately 60%, which is approximately the ratio of starch to total glucans based on a blend composition calculated from the cracked corn and corn fiber components.

Table 4 presents carbon closure data for APR-178 using liquid composition data generated by our normal HPLC method (Biorad columns) and by the HPAEC-PAD (High Performance Anion Exchange Chromatography - Pulsed Amphoteric Detection) system. The PAD analysis is expected to be more accurate because this method does not suffer from the baseline problems seen with the Biorad columns. The carbon closure data is better with the PAD numbers because the consistently high numbers for carbon closure on five-carbon sugars (over 100%) were not seen, and the overall balance is more reasonable at less than 100%. A closure less than 100% would be more likely because it is difficult to account for all the products because of the many reactions that occur during pretreatment. In the future, PAD analysis will be used for all pretreatment and fermentation material balance data.

As in the past, lignin closure continues to be a difficult problem. Lignin closure in Task 2 runs ranged from 100%—150%. There may be some correlation with the extent of the pretreatment as Task 2 runs were pretreated at a lower severity than APR-178. Perhaps a lignin condensation or reaction product initially produced by the reaction may be degrading at greater pretreatment severity.

Table 4. Pretreatment carbon closure (APR-178) with standard deviations using composition data generated by HPLC and PAD.

Component	% Closure (HPLC numbers)	% Closure (PAD numbers)	Standard Deviation (PAD numbers)
Glucose	102.6	87.9	10.4
Galactose	128.3	73.5	9.1
Xylose	130.3	106.6	14.4
Arabinose	119.2	89.5	14.9
Lignin	71.4	71.4	21.9
Overall	106.6	89.6	7.1

Standard deviations (SD) associated with random errors (shown in Table 4) were calculated from SD of component concentrations and flow rate measurements. Component SD were calculated from numerous determinations of the raw corn fiber compositions (data supplied by Bob Lumpkin) and repeat analysis of APR-178 solid and liquid composition. Assumed values for SD of flow rates and feedstock solids concentration are shown in Table 5. Some of the larger relative errors reflect more uncertainty in the estimate. Measured values will be used once the APR measurement systems are automated and additional analytical data is collected. Errors were combined using the propagation of error formula, which is based on a Taylor Series approximation (methodology defined in standard ANSI/ASME PTC 19.1-1985).

Ignoring bias errors and random instrument errors and thus assuming that process variation is the dominate error term, then a 95% confidence interval on the closure numbers shown in Table 3 is approximated by two times the standard deviation. A 95% confidence interval on the overall balance is 89.6% \pm 14.2%. Error in the glucose number is the dominate error term in the overall closure calculations. But, the glucose term also has three nearly equal contributions from flow rate, composition, and feedstock solids concentration errors. Thus, no single factor is responsible for the large confidence interval. Automated APR measurements will be used to determine flow rate errors in Task 4, as well as extending error analysis to SSF material balances.

Table 5. Measurements used in carbon balance closure calculations and assumed relative errors (standard deviation / measured value).

Measurement	Relative Error (%)
Feed flow rate	5.0
Steam flow rate	2.0
Acid flow rate	2.0
Valve water flow rate	2.0
Flash vapor flow rate (calculated)	10.0
Feed solids concentration	5.0
Hydrolyzate insoluble solids	10.0

4.2 Fermentations

4.2.1 Seed Train

During Task 3, several changes were made to the inoculum protocol to improve the viability of the yeast. First, cultures were transferred before all the glucose was consumed. This change keeps the yeast out of stationary phase and avoids any lag in the next stage of the fermentation. When cell viability was checked at transfer, the cells were nearly 100% viable when residual glucose was present. When cells were glucose limited for 6 hours, the viability dropped to 64%. This drop in viability shows that holding the cells for 48 hours after the glucose is consumed, as has been done in the past, could lead to a drop in viability and subsequent lag in the fermentation.

In an attempt to increase yeast cell mass, the air flow rate was increased and the glucose concentration in the seed fermenters was increased from 20 g/L to 50 g/L. Figure 4 shows the glucose consumption in the 20-L fermenter and 160-L fermenter for four batches with aerobic conditions. Glucose consumption in batches 3 and 4 in the 160-L fermenter leveled off. When the cells were transferred to the 1450-L fermenter to begin anaerobic fermentation, there was a significant lag before the cells started metabolizing glucose and producing ethanol.

The slow glucose consumption could be caused by a process perturbation, such as a temperature increase or pH problem. A toxin introduced to the fermenter or a nutrient limitation could also keep the cells from consuming all the glucose. Since no known toxin was introduced into the fermenter and there were no problems controlling temperature or pH, a nutrient problem seemed likely. To test for a nutrient limitation, broth was taken from the 160-L fermenter while there was still glucose available (approximately 20 g/L) and put into a shake flask spiked with additional CSL. A flask without CSL was included for comparison. Once the CSL was added, the cells immediately began metabolizing the glucose at a much faster rate compared to the unspiked flask (Figure 5). The flask without additional corn steep continued consuming glucose at an extremely slow rate. Additional corn steep liquor was added to one of the 160-L fermenters during aerobic cell growth, this also produced faster glucose consumption and a drop in dissolved oxygen. This clearly indicates that there was a nutrient limitation with 1% CSL and 50 g/L glucose. When glucose concentration in the seed train was returned to 20 g/L (for fermentation run 4), no more problems were encountered with the seed growth. There is a concern that nutrient limitation may be a problem during continuous fermentation, since there are high concentrations of glucose and xylose. The corn fiber blend will probably supply enough nutrients, but this problem should be watched for during Task 4.

4.2.2 SSCF

4.2.2.1 Run 3

Figure 6 shows monomeric sugar concentrations in the 1450-L fermenter for run 3. All fermentation data in tabular format is shown in appendix A. This fermentation required 60 h to completely consume all of the glucose, whereas bench scale fermentations only required 20–24 h. It is possible that because this run was inoculated with cell grown with a nutrient limitation as described above, that the cells were not healthy and this produced a significant lag in glucose consumption. Approximately 66% of the monomeric xylose was converted, with consumption beginning after most of the glucose was consumed. Cellobiose concentration increased initially and then decreased after glucose was consumed. There was only a small and constant concentration of xylose oligomers at the beginning and end of the fermentation. The final oligomeric glucose concentration was 9.4 g/L, which is a large amount of unconverted oligomers.

The concentration of products are shown in Figure 7 for run 3. Ethanol concentration increased rapidly during the period of rapid glucose consumption reaching a peak value of 28 g/L. Both glycerol and xylitol increased throughout the fermentation as expected, but there was no increase in either acetic or lactic (not shown) acid concentrations.

4.2.2.2 Run 4

The results for run 4 in the 1450-L fermenter are shown in Figures 8 and 9. Note that there are some spikes and dips in this data that are probably from analytical errors. Glucose was consumed in 24 h followed by consumption of approximately half of the monomeric xylose. The final ethanol concentration in this run was 41 g/L. Note the period of rapid ethanol production during the first 20 h corresponding to glucose consumption, followed by a slower production rate corresponding to xylose consumption. Cell counts increased rapidly to a maximum at 36 hours, then began to decrease during the rest of the run. Even though ethanol concentration was higher and glucose consumption was rapid for this run, the results are disappointing because there is still a large concentration of glucose oligomers and unconverted monomeric xylose.

4.2.2.3 Run 5

Figure 10 shows monomeric sugar concentrations and cell counts for the 9000-L batch fermentation (run 5). This was the best run in the PDU because of the rapid glucose consumption and 70% utilization of the monomeric xylose. Oligomeric glucose level was high at 14.3 g/L at the end of the run. Oligomeric xylose levels are also high, but only because oligomeric xylose was higher at the beginning of the run. No oligomeric xylose was converted to monomer xylose during any of the three batch runs. Cell counts did not follow the pattern of run 4, however, cell counts are not an accurate measurement. Future work will look at direct cell counting techniques.

As with the previous runs, there was a large amount of unconverted oligomeric glucose. A simple experiment was performed with the final broth from run 5 to test for the possible origin of this sugar (cellulose or starch) by adding glucoamylase and cellulase to the fermentation broth treated with antibiotics (tetracycline and nystatin). The results for each enzyme addition as well as a control flask (no enzyme addition) and time zero flask are shown in Figure 11. The top of the bars show total soluble glucose concentration and the shading shows the split between monomeric and oligomeric glucose. Amylase had no effect on glucose level, while cellulase produced some additional monomeric glucose. No cellobiose was detected in any of the flask. If there are starch oligomers present, the glucoamylase did not produce any more conversion. The increase in monomeric glucose in the cellulase flask suggest that at least some of the oligomers originate from cellulose. Since no cellobiose was detected before or after cellulase addition, this suggest that activity of the endo- and/or exo-glucanases may have been limiting at the end of the fermentation.

There are several possible explanations for the increase in total soluble glucose. There may have been a small amount of cellulose in the flask. The broth was taken from a container in which the solids had settled out, however, a small amount of suspended cellulose particles may have been in the liquor. Hydrolysis of this residual cellulose would cause an increase in the total soluble glucose. If there was no cellulose present to produce the extra glucose, there may indicate a problem with the total soluble sugar assay. The assay, which uses dilute sulfuric acid to hydrolyze all oligomeric sugars to monomers for detection by HPLC, may not be detecting high molecular weight oligomers until after they are reduced in size by cellulase. Either way, a better designed experiment in the future could answer these questions as well as explain the loss of cellulase activity.

Figure 12 shows product concentrations for run 5. Ethanol reached a maximum value of 45 g/L after 108 h and probably would have gone slightly higher from additional xylose consumption had the run continued. Both glycerol and xylitol concentrations increased as expected and there was no additional acetic acid production.

During Task 3 fermentations, xylose was not completely utilized by the recombinant yeast. Incomplete xylose utilization could be caused by acetic acid inhibition, ethanol inhibition, a nutrient limitation, contamination, or the presence of other unknown inhibitors produced during pretreatment of the corn fiber. A quick study was completed after Task 3 comparing the effect of acetic acid concentration on xylose utilization by the recombinant yeast. Acetic acid concentrations from 2—10 g/L were evaluated at pH 5 and 6. The recombinant yeast was incubated in 60 g/L glucose, 30 g/L xylose, and 10 g/L corn steep liquor with the varying acetic acid concentrations. Figure 13 shows the effect of acetic acid on xylose utilization at pH 5 and Figure 14 shows the effect at pH 6. At either pH, higher acetic acid concentrations reduce the xylose utilization rate. Figure 15 compares pH 5 to pH 6 at varying acetic acid concentrations. In all cases, pH 6 increased the xylose utilization rate, although, the ethanol yield was lower because of more glycerol and cell mass production at the higher pH. There was not a significant difference in final xylose consumption for pH 5 at an acetic acid concentration below 5 g/L. However, the combined concentrations of acetic and lactic acid were above 5 g/L in the Task 3 runs and this may have produced the incomplete xylose utilization seen in the Task 3 fermentations. Work planned for Purdue will investigate the effect of the two acids on fermentation performance.

Table 6 shows material balance information for run 5. This run achieved 76% conversion of the total available glucose (as monomeric and oligomeric glucose and cellulose) and 61% conversion of the xylose (as monomeric

and oligomeric xylose). Xylose conversion is low because of the residual monomeric xylose and oligomeric xylose that is not converted during fermentation. The large amount of oligomeric glucose (14.3 g/L) also significantly reduces the overall glucose conversion and may be due to a cellulase problem discussed above. No significant conversion of galactose or arabinose occurred and no conversion of lignin occurred.

Also shown are the yields based on grams of product per 100 g of six carbon sugars and xylose consumed. 91.6 g of product out of 100 g have been accounted for in this material balance. Carbon dioxide is based on ethanol stoichiometry. The ethanol metabolic yield (based on sugars consumed) is 83%, however, the ethanol process yield is only 57%. This is due to the large amount of unconverted glucose oligomers and xylose monomers and oligomers at the end of the fermentation. Cell mass was calculated from the highest cell count achieved during the run and then converted to cell mass. Admittedly, this is a low estimate because cell mass is greater than viable cell counts. It is difficult to measure cell mass production in the presence of a solid substrate. If an assumed cell yield of 5% is used, the total grams of products becomes 95.4. A more reliable estimate of cell mass would improve the material balance closure. We will continue to investigate possible improvements in estimating cell mass production. Accounting for ethanol lost in the exhaust gas may also improve the material balance closure. However, the mass spectrometer did not produce reliable measurements of exhaust gas composition during Task 3. The overall carbon recovery for this run was 94.5%.

Table 6. SSF material balance information showing percent conversion and product yields.

Conversions (% consumed/total in):	
Glucose	75.7
Galactose	20.3
Xylose	61.0
Arabinose	13.7
Lignin	1.1
Cellulose	62.3
Yields (g/100 g C6 + xylose consumed)	
Ethanol	42.5
Carbon Dioxide	40.6
Cell Mass	1.2
Glycerol	4.6
Xylitol	2.7
Total	91.6
Overall Carbon Recovery (%)	94.5

4.2.2.4 Comparison to Bench Scale Results

Two bench-scale fermentations were performed during Task 3 (see Appendix B for detailed results). The results of the first bench fermentation are shown in Figure 16. The pretreated material was taken from the material used to fill the 1450-L fermenter for run 1. This material was sterilized in the bench scale fermenter, then sterile CSL and inoculum (also the same used in run 1) were added to the fermenter. Glucose was rapidly consumed (within 20 h) and almost all the monomeric xylose was consumed in about 90 h. The final ethanol concentration was near 40 g/L and the acetic and lactic acid concentrations were approximately 2.5 g/L.

The second bench-scale fermentation used combined pretreated feedstock and CSL taken directly from the 1450-L fermenter (run 4) after pH adjustment and before inoculation. The same inoculum use in run 4 was added later to the bench scale fermenter. The results of this fermentation along with the comparable fermentation in the PDU are shown in Figure 17. Each fermentation has nearly identical rates and product and substrate concentrations. The acetic and lactic acid concentrations were about 5.0 g/L and 2.5 g/L, respectively.

The reproducibility of the two fermentations at the two different scales is encouraging. The results suggest that the incomplete xylose utilization may be affected of other factors, such as total organic acids. Shake flask work showed slower utilization of xylose at acetic acid concentrations greater than 5 g/L (Figure 13). It was also noted that none of the fermentations achieved an ethanol concentration much higher than 45 g/L. This may suggest an ethanol inhibition problem. The study being done by Purdue should identify any inhibition by organic acids and/or ethanol.

4.2.3 Comparison to Modelling Results

The predicted (lines) and measured (points) concentrations of glucose, xylose, and ethanol are shown on Figure 18. The predicted cell mass concentration is also shown on Figure 18 without measured cell mass concentrations, because it is difficult to obtain accurate cell mass measurements. Low monomeric glucose concentrations are detected by the HPLC, whereas, YSI measurements show no or very low levels of glucose present. The HPLC glucose levels are probably elevated due to baseline problems with corn fiber chromatography that typically show up with Biorad columns. Figure 19 shows predicted and measured glucose, cellulose, and cellobiose concentrations. The cellulose concentration is the sum of insoluble cellulose and soluble cellulosic oligomers.

The kinetic model's parameters for both figures were developed from hydrolysis experiments done on corn fiber and with initial batch data from early bench-scale experiments on LNHST2 done with pure sugars. Since the intent of these early experiments were not to develop kinetic parameters, the experiments were done with pure sugars and few data points were available for fitting. Because of this problem, the fermentation parameters have a lot of uncertainty. The predicted fermentation rate of both glucose and xylose is too fast and the predicted cell mass yield may be too high, so the fermentation parameters were adjusted to fit fermentation run 5 experimental data. The cell mass yield was adjusted to 0.05 g cell mass/g glucose from 0.155. The new predictions are shown in Figures 20 and 21. The fit is good with the exceptions of cellobiose concentration and with data at the 18 hour time point.

4.2.4 Contamination

A low level of contamination was detected in the 1450-L fermenters (run 3 and 4) and in the 9000-L fermenter (run 5). The contaminant in run 3 was typed as *Lactobacillus buchneri* and the contaminant in run 4 was typed as *Lactobacillus brevis*. Both organisms have been seen in previous fermentations. The source is unknown, but could have come from the transfer line from the APR to the fermenters or was contained in the pretreated material. The level was very low and was cultured in liquid medium only. No by-products, namely lactic acid or acetic acid, were produced during the runs. Because the contaminant cell levels were low and there were no detectable levels of by-products, the contamination was at an acceptable level.

Six APR samples from Task 3 were tested for contaminants. After slurring the material in growth medium, adjusting the pH and incubating in a shaker incubator for 7 days, no contaminants were found. The corn fiber material looked to be more consistent, with fewer pockets of unpretreated material. This improvement in pretreatment improves the sterilization of the material. The material will continue to be monitored throughout Task 4.

4.3 Centrifugation

The centrifuge was operated on two consecutive days (Mar. 15 and 16) to produce the separated solids. The material from Mar. 15 run was darker than the Mar. 16 run because of overneutralization of the fermentation broth. The solids and protein content of the centrate and cake are shown in Table 7. Soluble protein was measured on clarified liquid (separated using a high speed laboratory centrifuge) separated from the centrate and cake. The remaining solids were washed twice and dried, then measured for protein content. The centrifuge was only able to produced a cake at 24% solids concentration. The fraction of the protein in the solids before centrifugation is 62% and 64% for the Mar. 15 and 16 runs, respectively. However, the protein content in the liquid could be biased high due to absorption of atmospheric nitrogen during the assay. The data also shows 60% more protein in the Mar. 15 sample than the Mar. 16 sample. This doesn't lead to much confidence in the results.

Table 7. Solids concentrations and protein content (by weight) before and after centrifugation in clarified liquid and washed solids.

Sample	Total Solids (%)	Insoluble Solids (%)	Soluble Protein in Clarified Liquid (%)	Protein in Washed Solids (%)
Mar. 15 run				
Centrifuge Feed	11.7	2.9	0.81	40.6
Centrate	10.4	2.1	0.62	
Cake	24.1	17.8		41.6
Mar. 16 run				
Centrifuge Feed	8.9	1.9	0.44	37.8
Centrate	7.1	0.6	0.44	
Cake	24.2	16.6		41.6

5.0 Review of Run Specifications

The following is the list of criteria for success defined in the Task 3 run specification, and a short discussion of how each of these criteria were met.

1) Complete batch fermentation runs in the 1450-L and 9000-L fermenters with no interruption in operation of longer than 6 hours and run contamination free.

Batch fermentation runs were completed with no interruptions and were contamination free (according to the definition of 95% of all products produced are yeast by-products and 90% of the living cells are yeast).

2) Neutralize the solids at the end of the fermentation to pH 7 and separate out the solids for future testing as animal feed.

Solids left after fermentation were separated from the fermentation broth using the Sharples centrifuge and supplied to Swan. The moisture and protein content of the separated components are shown in Table 7. Sulfates were measured at approximately 0.5%.

3) The pretreatment for this run should solubilize at least 85% of the xylose in the feed and acetic acid level should be no more than 25 mg/g dry solids in the feed. The furfural plus HMF concentration should be below 3 mg/g dry solids in the pretreated feed.

At least 85% of the xylan in the feed (measured yields were as high as 95%) was solubilized, the highest acetic acid concentration was 13.5 mg/g and the highest furfural plus HMF concentration was 6.3 mg/g in APR-179. Typical concentrations of furfural plus HMF were approximately 3.0—3.5 mg/g.

4) Close the overall carbon balance around pretreatment and SSF to within $\pm 15\%$.

Overall carbon balance around pretreatment (89.6%) and SSF (94.5%) were closed to within $\pm 15\%$.

6.0 Problem Resolution and Post Run Issues

The following were significant problems that occurred during this run and steps that will be taken to solve the problem.

● Equipment Maintenance

A clogged steam filter and faulty hand valve on a steam line was responsible for problems during fermenter sterilizations that led to over cooking the material. In the future, routine preventative maintenance will be done on steam lines to prevent this problem.

● Fermenter Temperature Control

A problem with controlling temperature in one of the 1450-L fermenters was identified as an incorrect assignment of an I/O point in the data acquisition and control system. This was corrected during the run.

● Inoculum Addition to the 1450-L Fermenters

The transfer line from the 160-L fermenter to the 1450-L fermenter was modified for easier transfers. The diaphragm valve controlling entry into the 1450-L fermenters would get plugged with solids. The transfer line was repiped through a hand valve with a wider opening.

● Carbon Balance Closure

Pretreatment carbon balances may be improved by collecting flow rate data from the APR. This will also provide data for calculating flow rate errors. This information will be used to calculate confidence intervals for both pretreatment and SSF carbon balances. APR measurements are being automated and will be available for Task 4.

● Cell Mass

A measurement of cell mass would improve the material balance, however, there is currently no reliable technique for measuring cell mass in the presence of solids. During Task 4, microscopic cell counts will be done to see if this technique can give a more reasonable cell mass number. Other techniques (e.g., epi-fluorescence) are not developed enough for use.

● Mass Spectrometer

The mass spectrometer system was used during Task 3 but the results were not believable. Between Tasks 3 and 4, the mass spectrometer and flow rate measurements of exhaust gases will be validated.

The goal is to have a reliable exhaust gas component measuring system for use in calculating mass balance information.

- Oligomeric Glucose

The large concentration of oligomeric glucose remaining after the fermentation is troublesome and produces a significant loss of potential ethanol. Some of the oligomers could be from starch that are not convertible, however, some or all also originate from cellulose. Cellulase added to the fermentation broth produced additional monomeric glucose. More experiments are necessary to determine why the oligomers were not converted to monomers (e.g., by adsorption of cellulase onto protein).

7.0 Summary

Four 1450-L batch fermentations and one 9000-L batch fermentation runs were attempted in Task 4. The first two 1450-L runs were not successful probably because fermentation inhibitors were produced from sterilizing pretreated material and CSL together. When this practice was eliminated, conversion of the sugars were achieved in the rest of the fermentation runs.

The highest ethanol concentration of 45 g/L was achieved in the final run in the 9000-L fermenter. This run achieved 76% conversion of the available glucose and 61% conversion of the xylose. The ethanol yield was 83% based on sugars consumed but only 57% based on total sugars available. This is due in large part to a significant amount of unconverted sugars left at the end of the fermentation (14.3 g/L glucose primarily as oligomers and 16.3 g/L xylose half as oligomers). However, this run proved that high solids fermentations can be conducted in the PDU and produce results comparable to bench scale. Potential organic acid and/or ethanol inhibition should be identified by future testing. Kinetic parameters in the model have been changed to match results from run 5.

The distillation system ran well with no plugging, however, additional work is needed to validate distillation as a kill system. Separation of the solids from the fermentation broth after sterilization was completed with the Sharples centrifuge and the solids and liquid were saved for testing by drying/dewatering vendors. A preliminary measurement of protein in the fermentation broth showed that approximately 60%—65% of the protein is in and remains with the solids.

8.0 Acknowledgments

The following staff members contributed either full or part-time help to the operation of the plant during this run: Brian Boynton, Nancy Combs, Kelly Ibsen, Ed Jennings, James Johnson, Tim Johnston, John Lesko (Amoco), Bob Lyons, Sam McWilliams (Stone and Webster), Robert O'Conner, Tim Plummer, Dana Rice, Mark Ruth, Dan Schell, Larry Schwartz (Stone and Webster), Ian Thompson. Analytical support was provided by Larry Brown, Tina Ehrman, Jim Hora, Netta Ingle, Ray Ruiz, and David Templeton. Christos Hatzis supplied the original material balance spreadsheets that were subsequently modified for use with PDU data. This report was put together with written contributions from Nancy Combs, Kelly Ibsen, Mark Ruth, Dan Schell and Susan Toon.

Figure 1. Monomeric Sugars in APR Samples

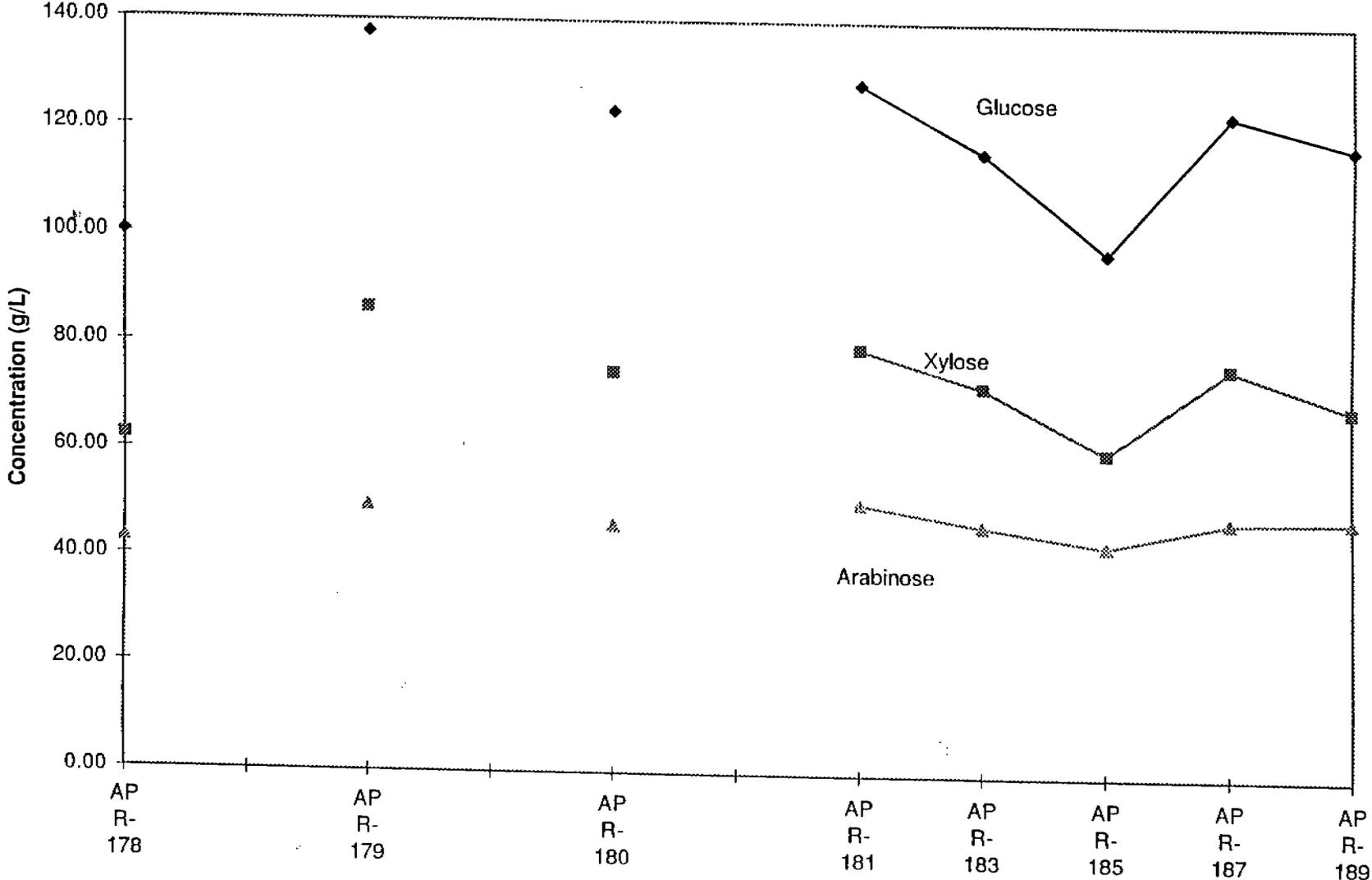


Figure 2. Inhibitors in APR Samples

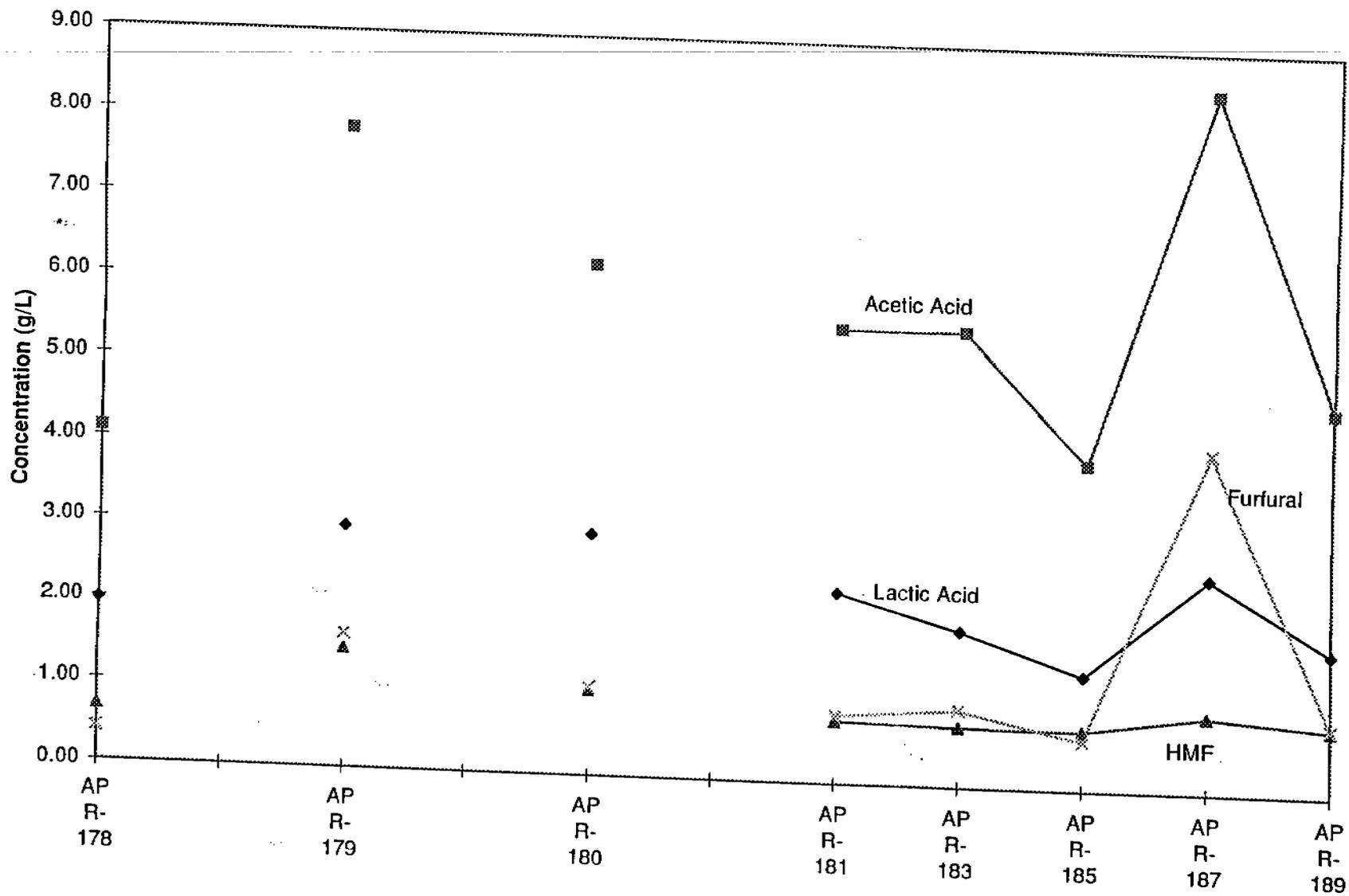


Figure 3. Sugar Yields in APR Samples

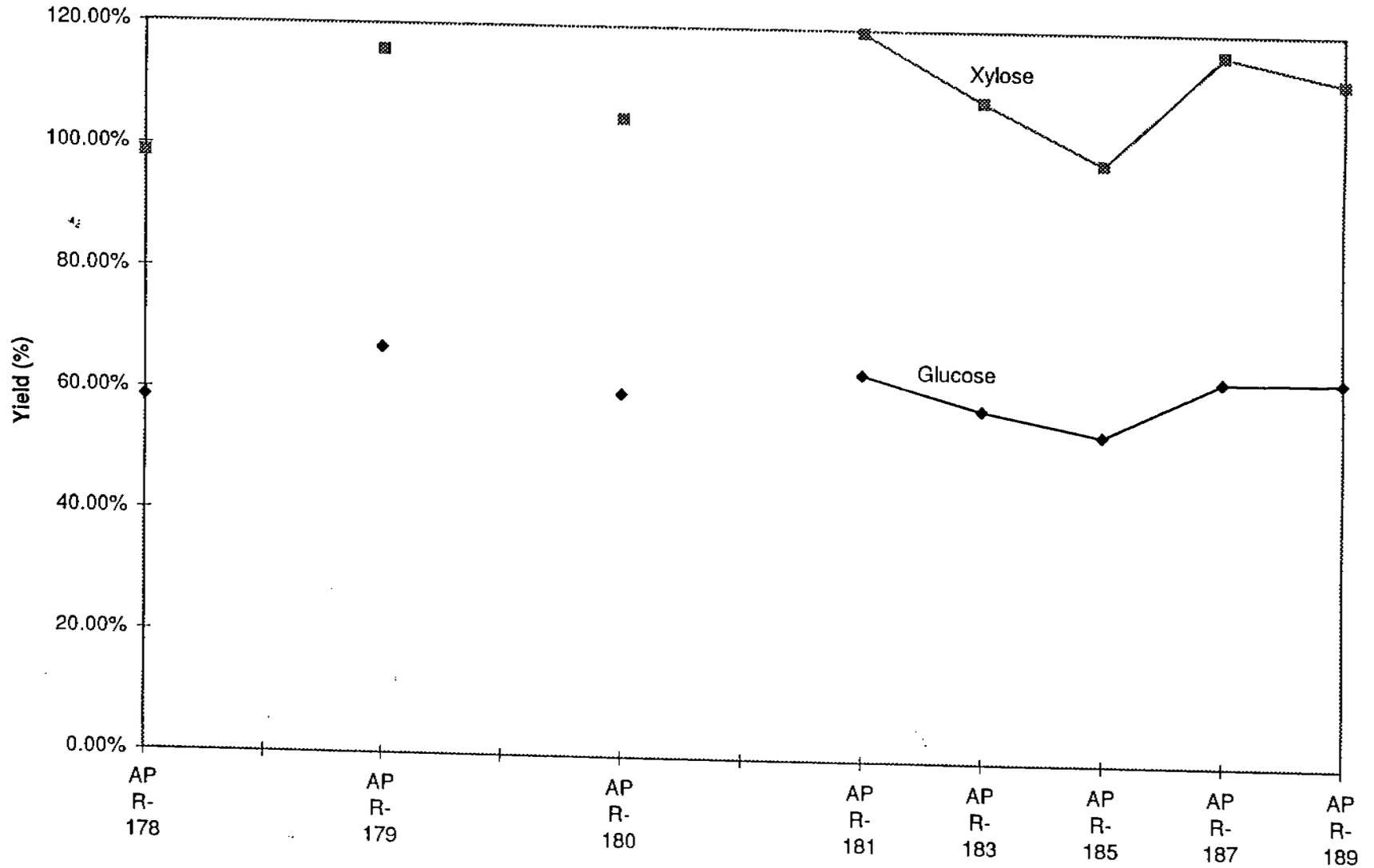


Figure 5. CSL Addition Test with Culture From 160-L Fermenter

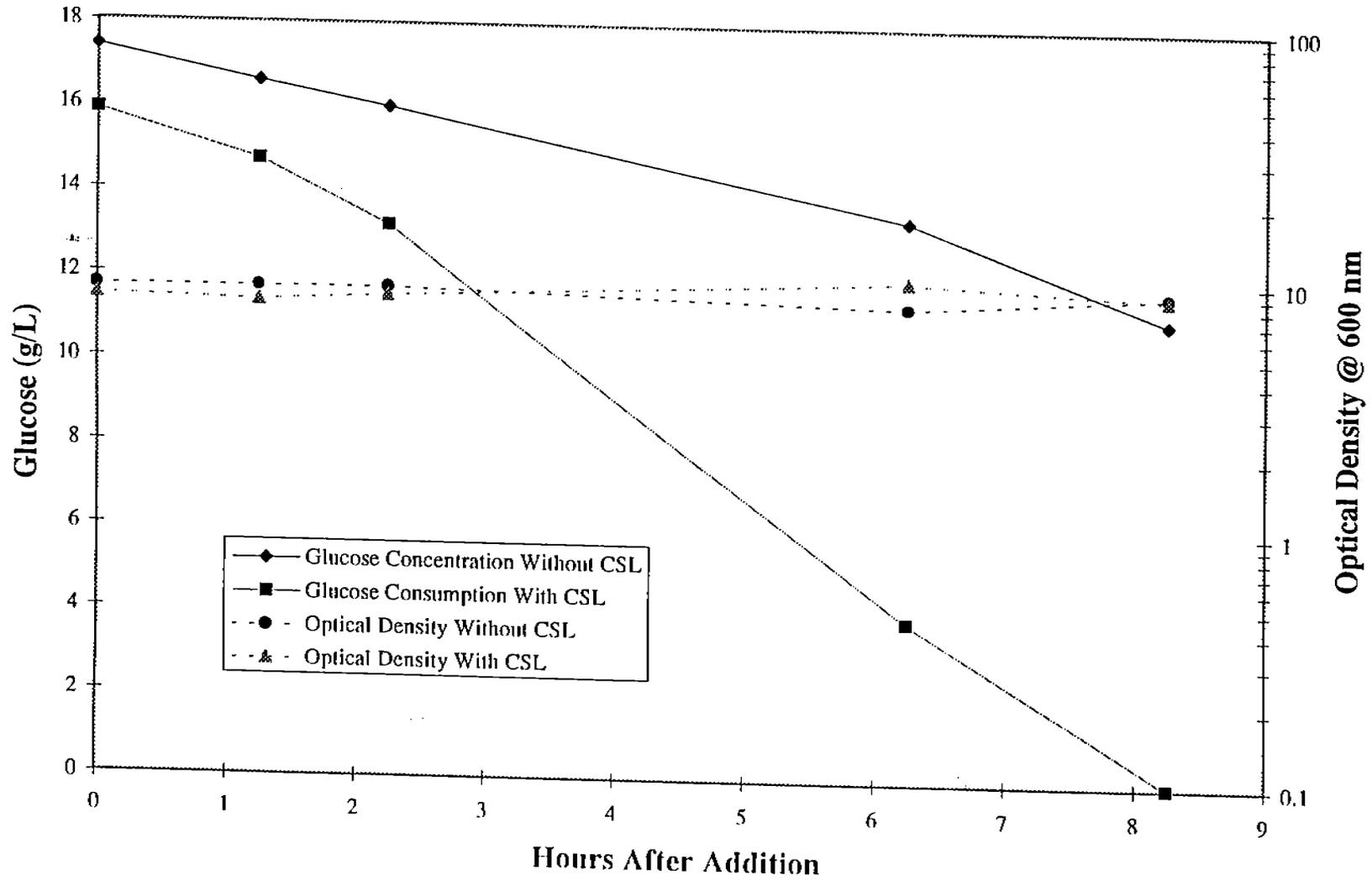


Figure 6. Monomeric Sugars in the 1450-L Batch Fermentation (Run 3)

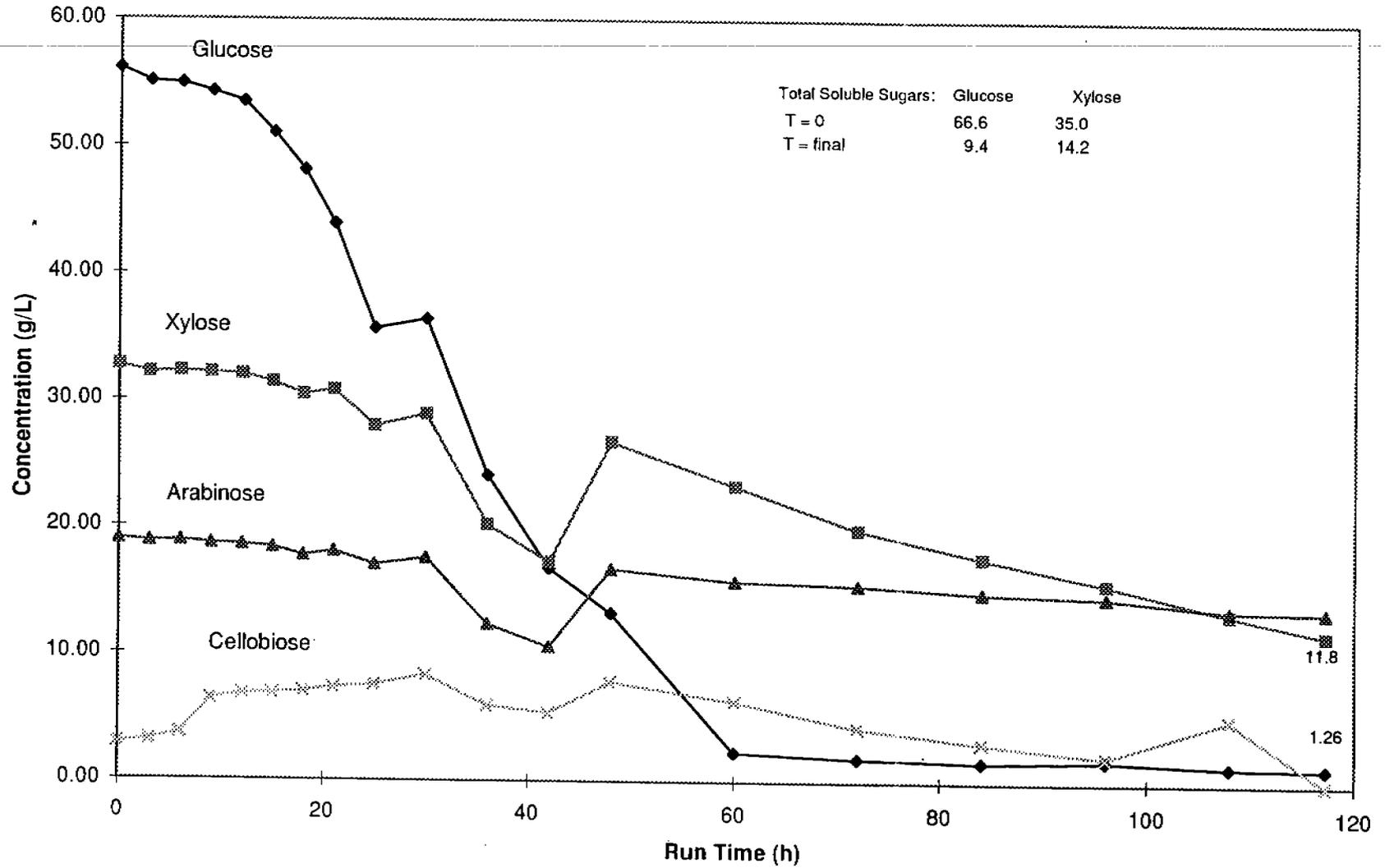


Figure 7. Product Concentrations in the 1450-L Batch Fermentation (Run 3)

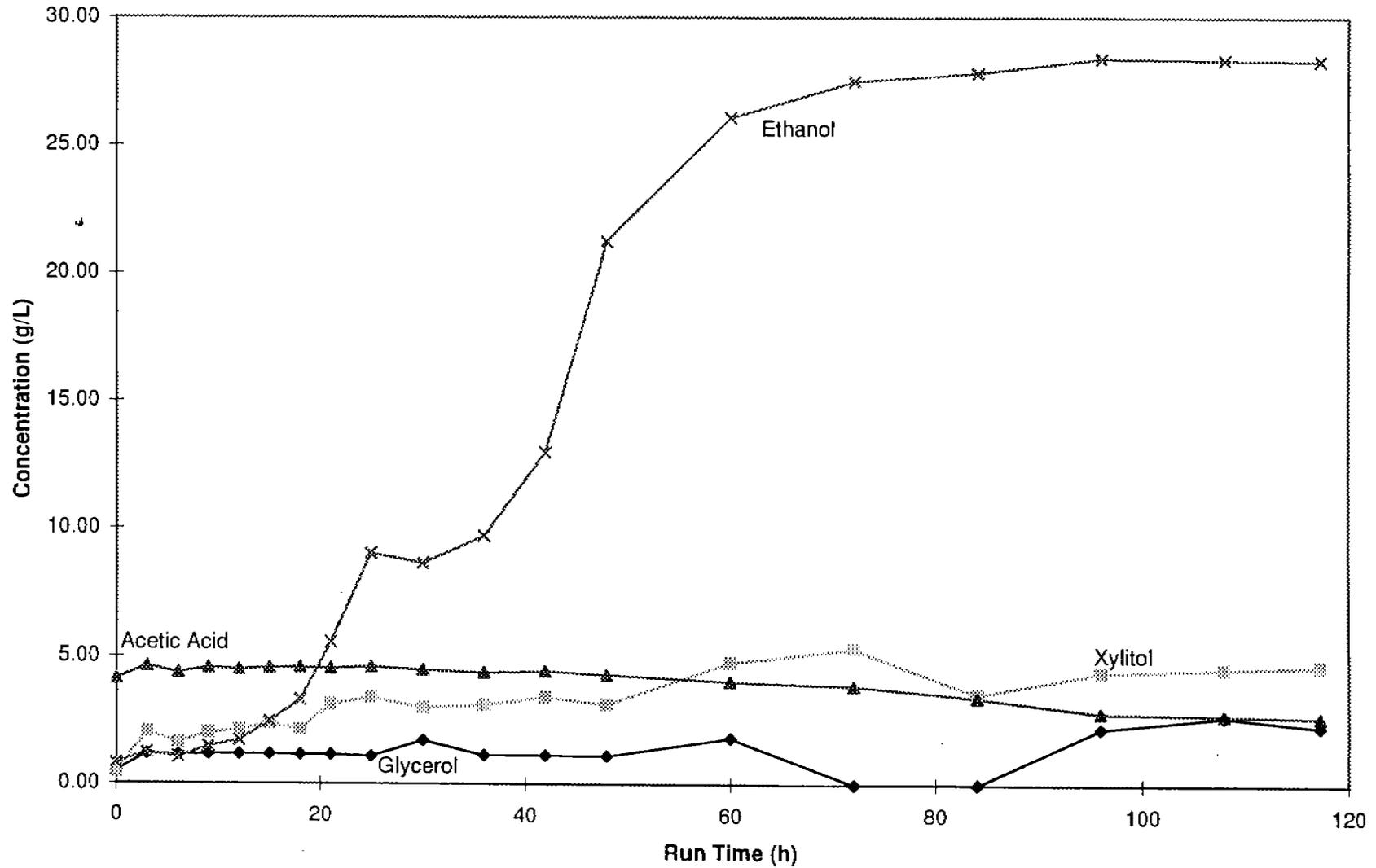


Figure 8. Monomeric Sugars in the 1450-L Batch Fermentation (Run 4)

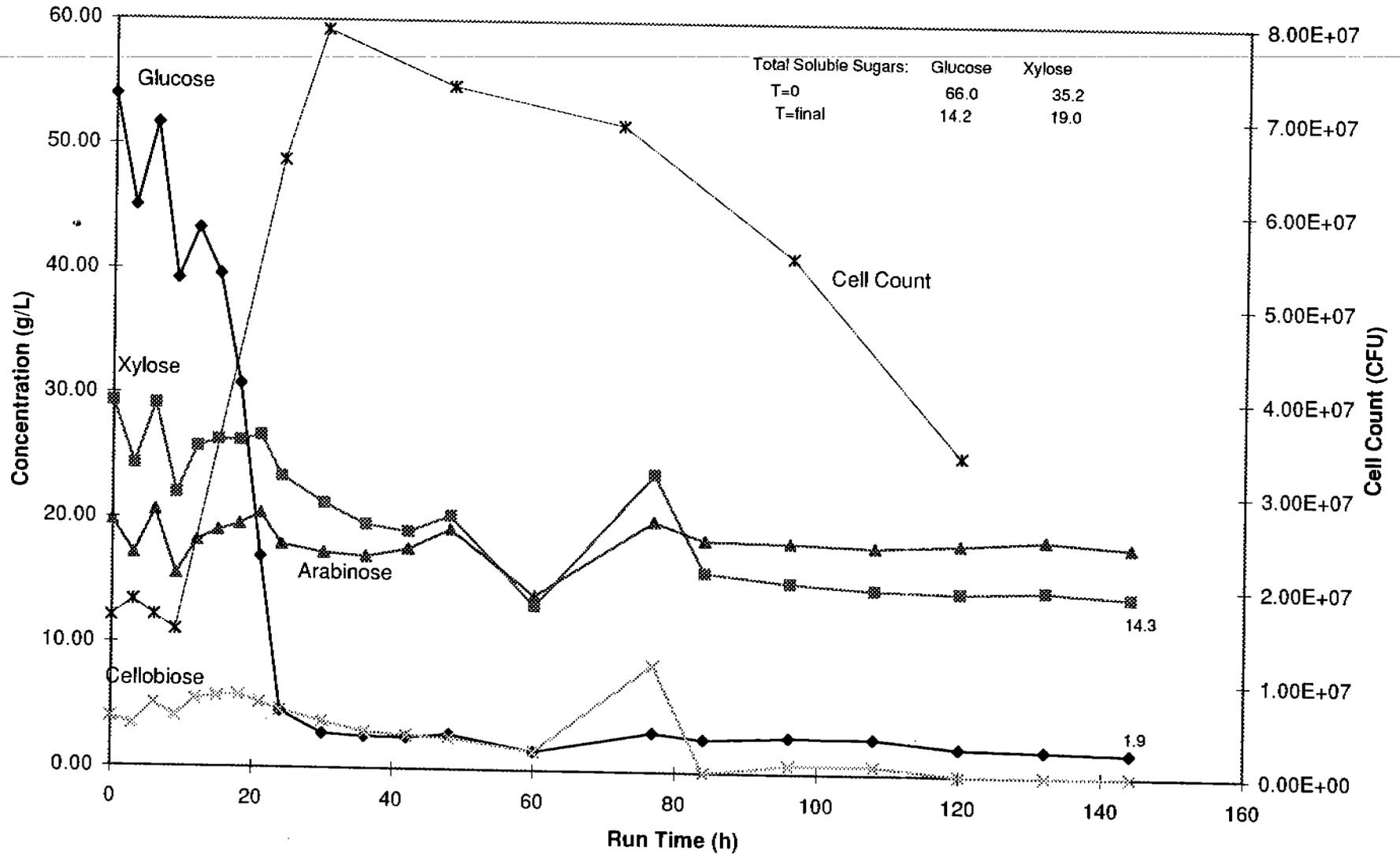


Figure 9. Product Concentrations in the 1450-L Batch Fermentation (Run 4)

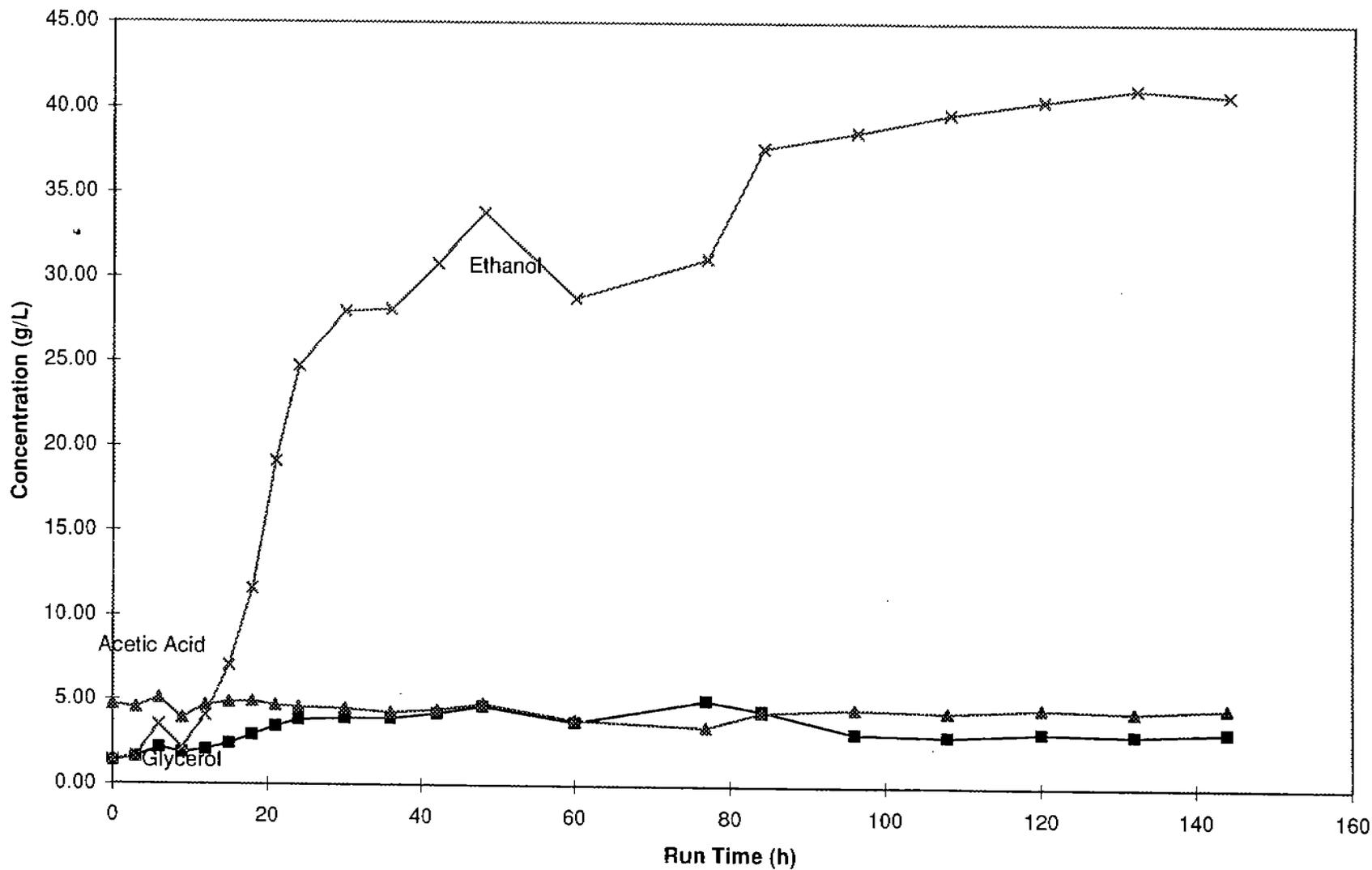


Figure 10. Monomeric Sugars in the 9000-L Batch Fermentation (Run 5)

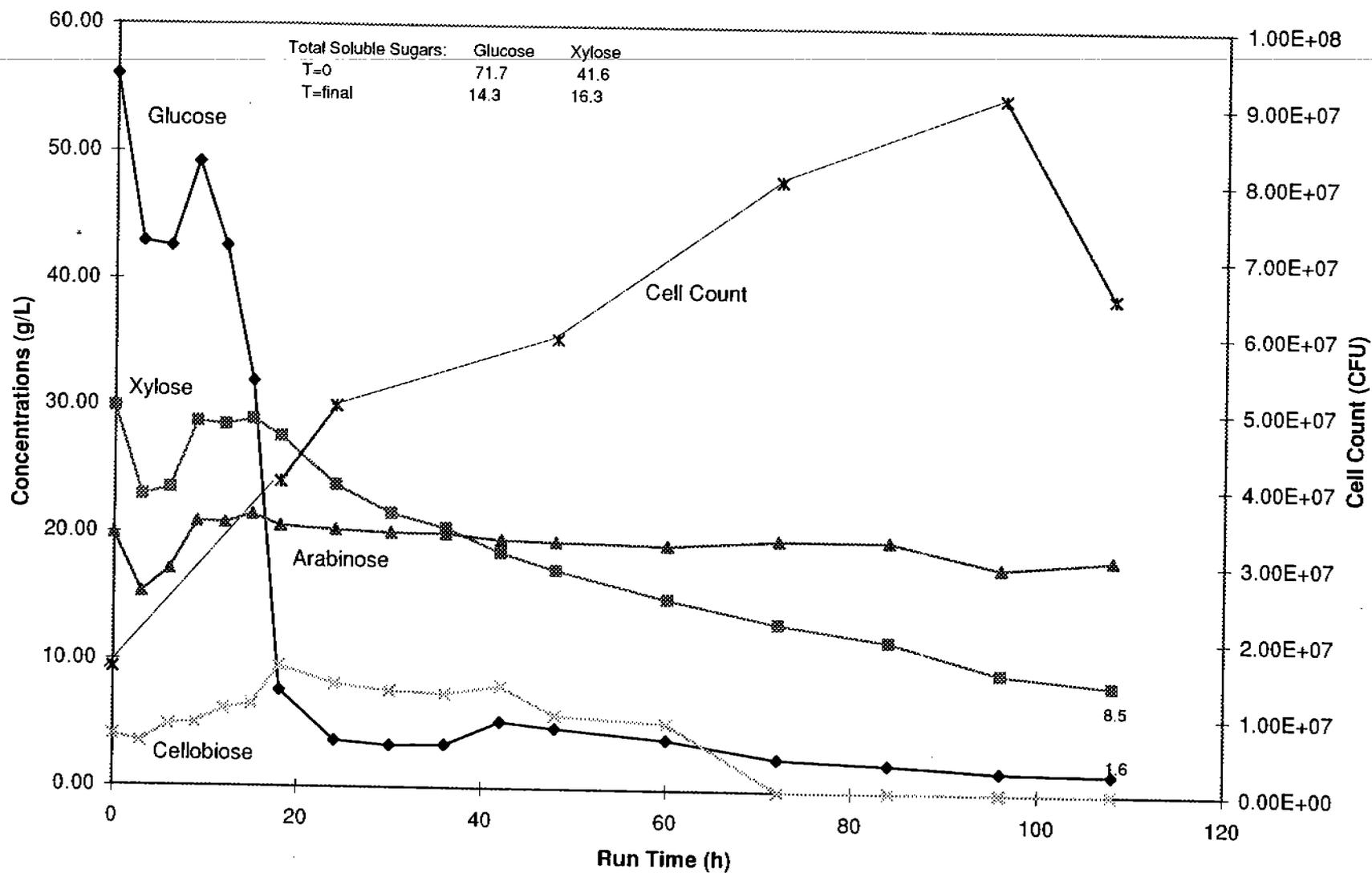


Figure 11. Effect of Added Enzyme on Glucose Levels in Spent Fermentation Broth

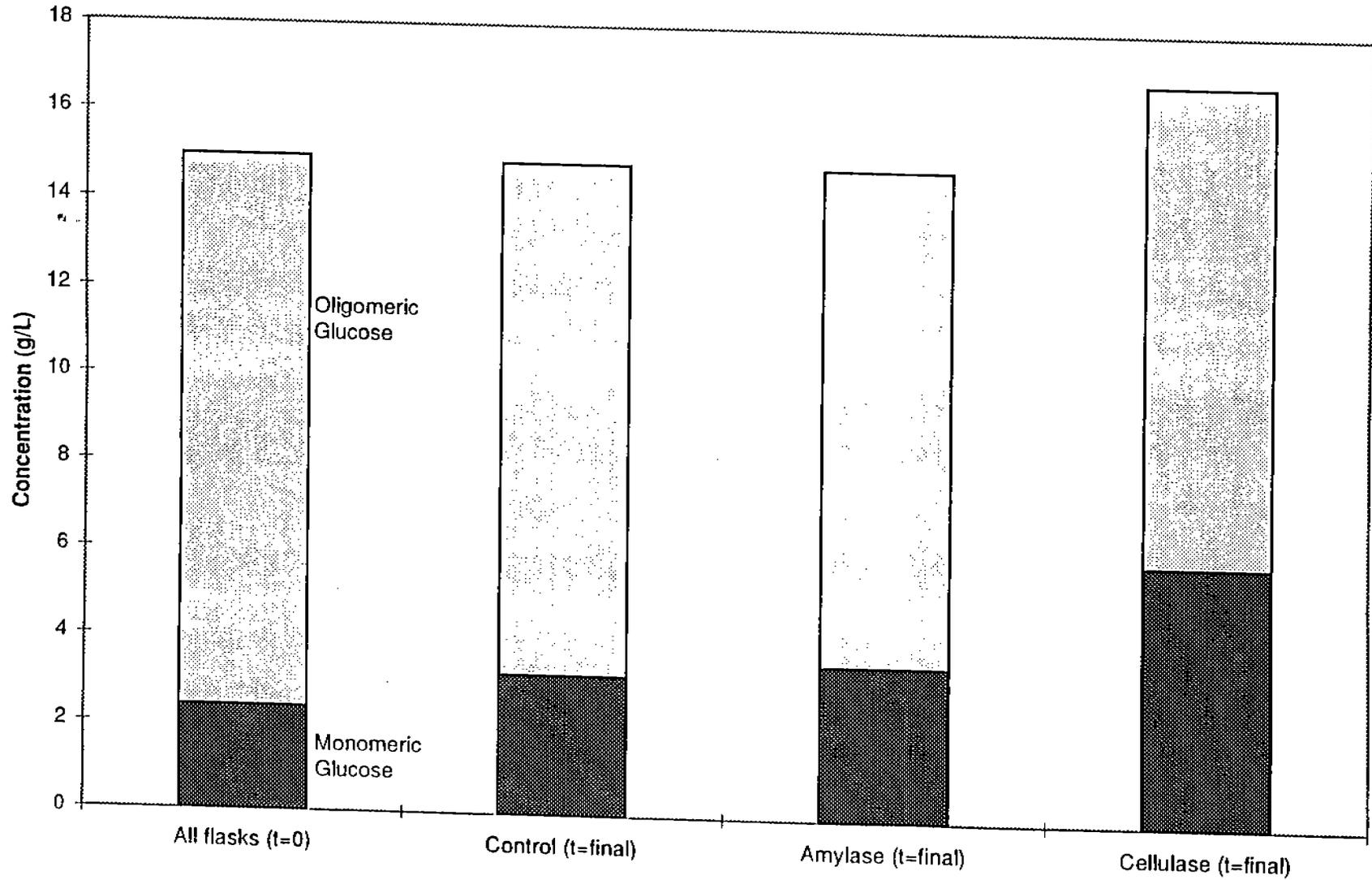


Figure 12. Product Concentrations in the 9000-L Batch Fermentation (Run 5)

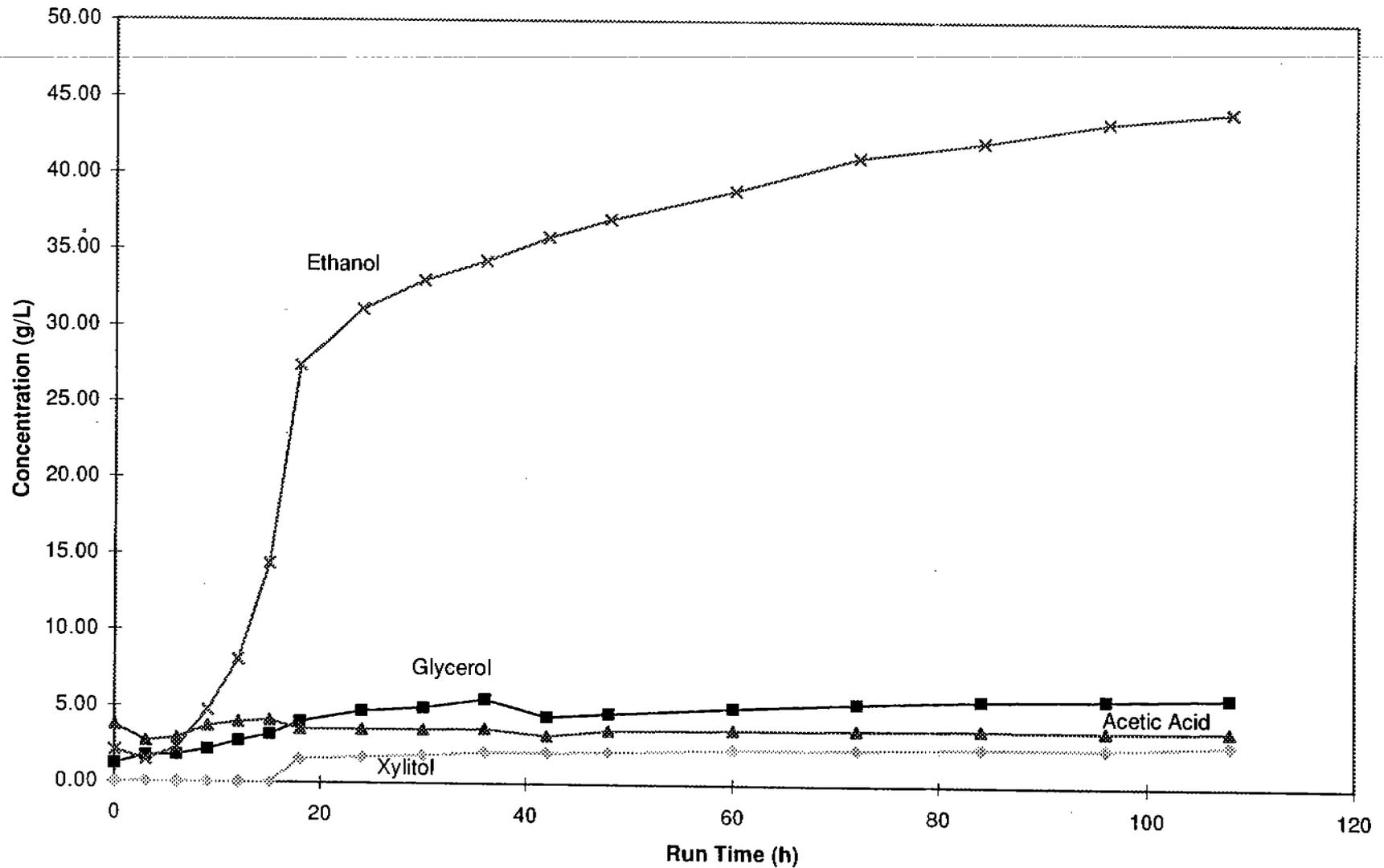


Figure 13. Xylose Utilization with Acetic Acid Present (pH 5.0)

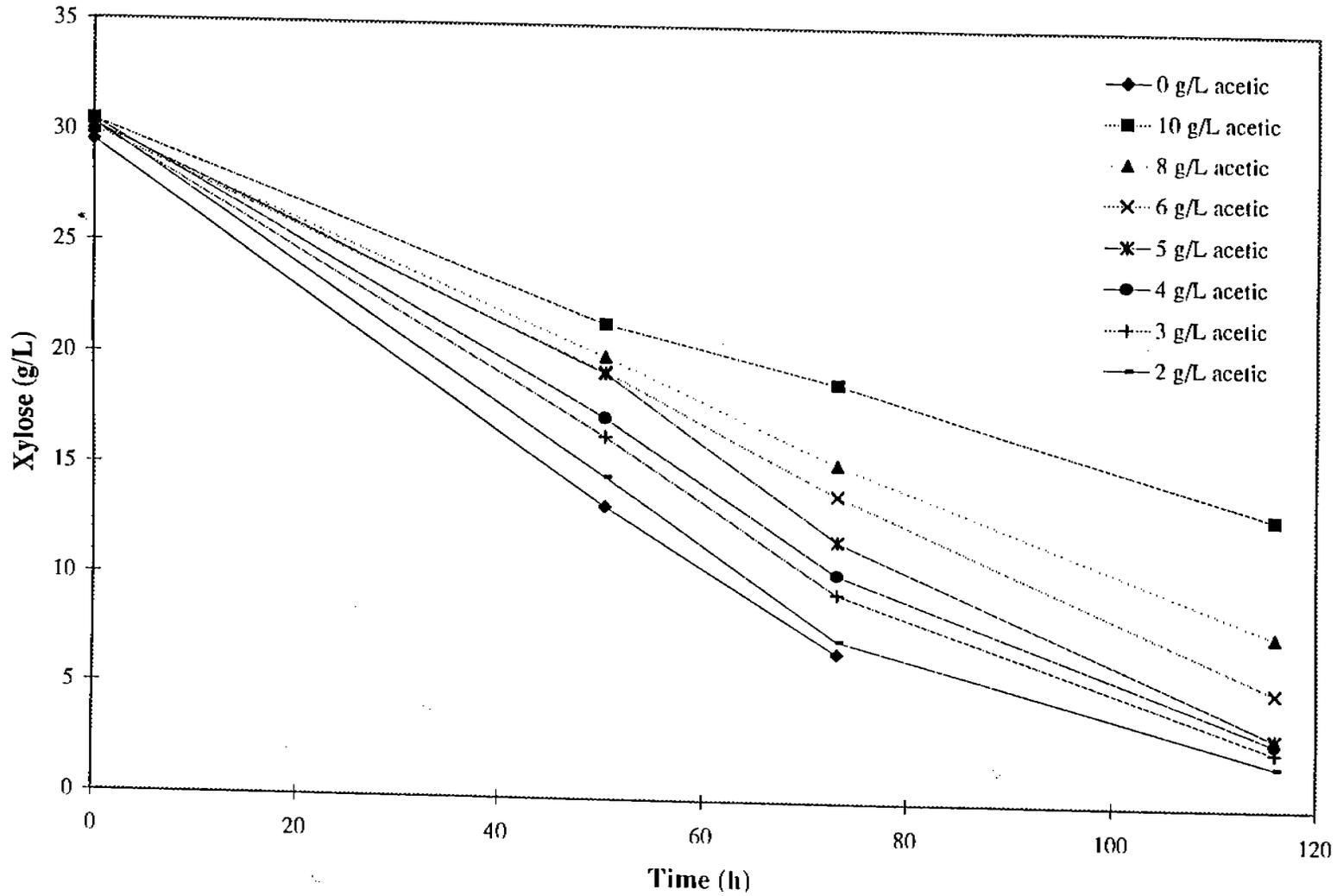


Figure 14. Xylose Utilization with Acetic Acid Present (pH 6.0)

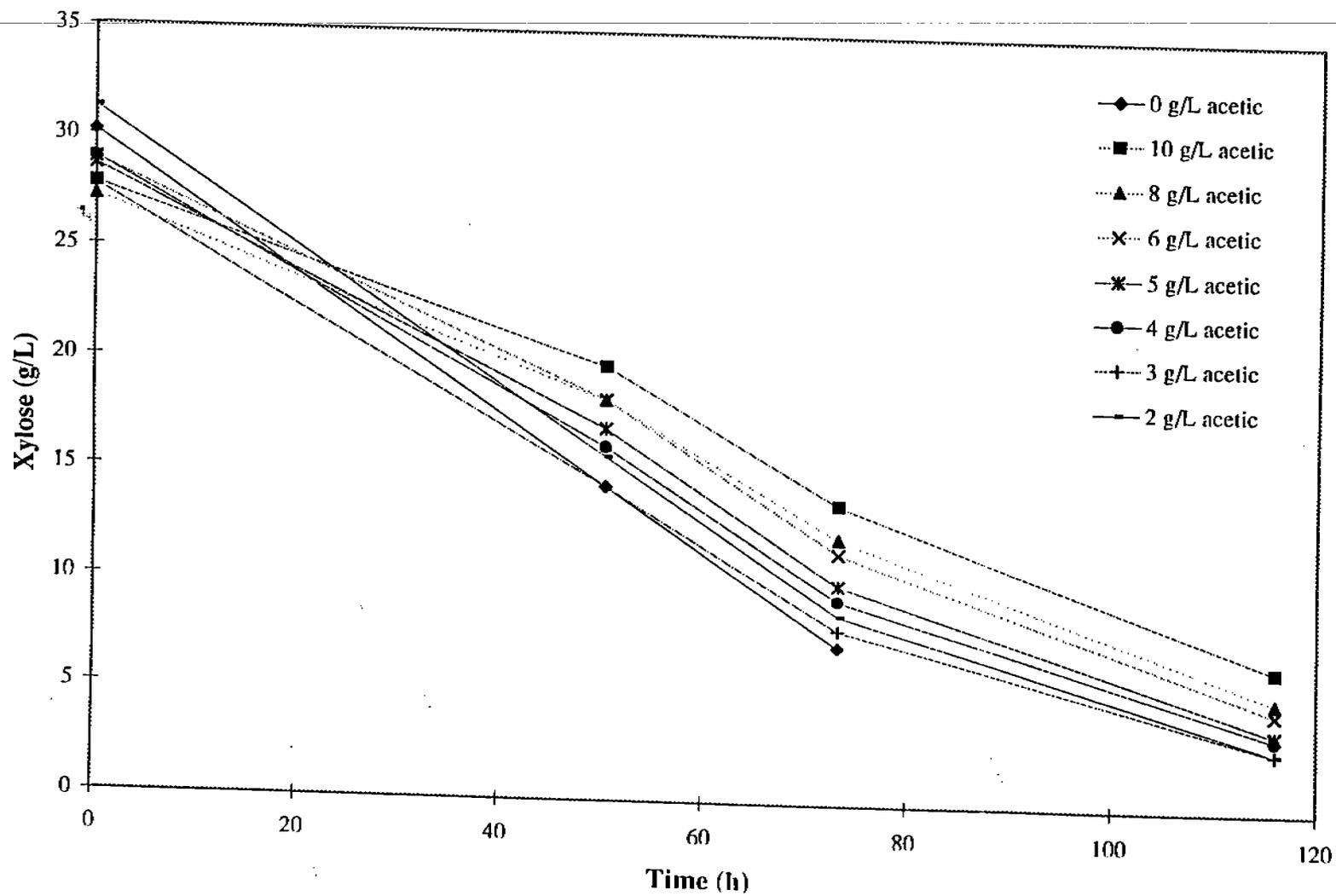


Figure 15. Xylose Utilization with Acetic Acid Present (pH 5 and 6)

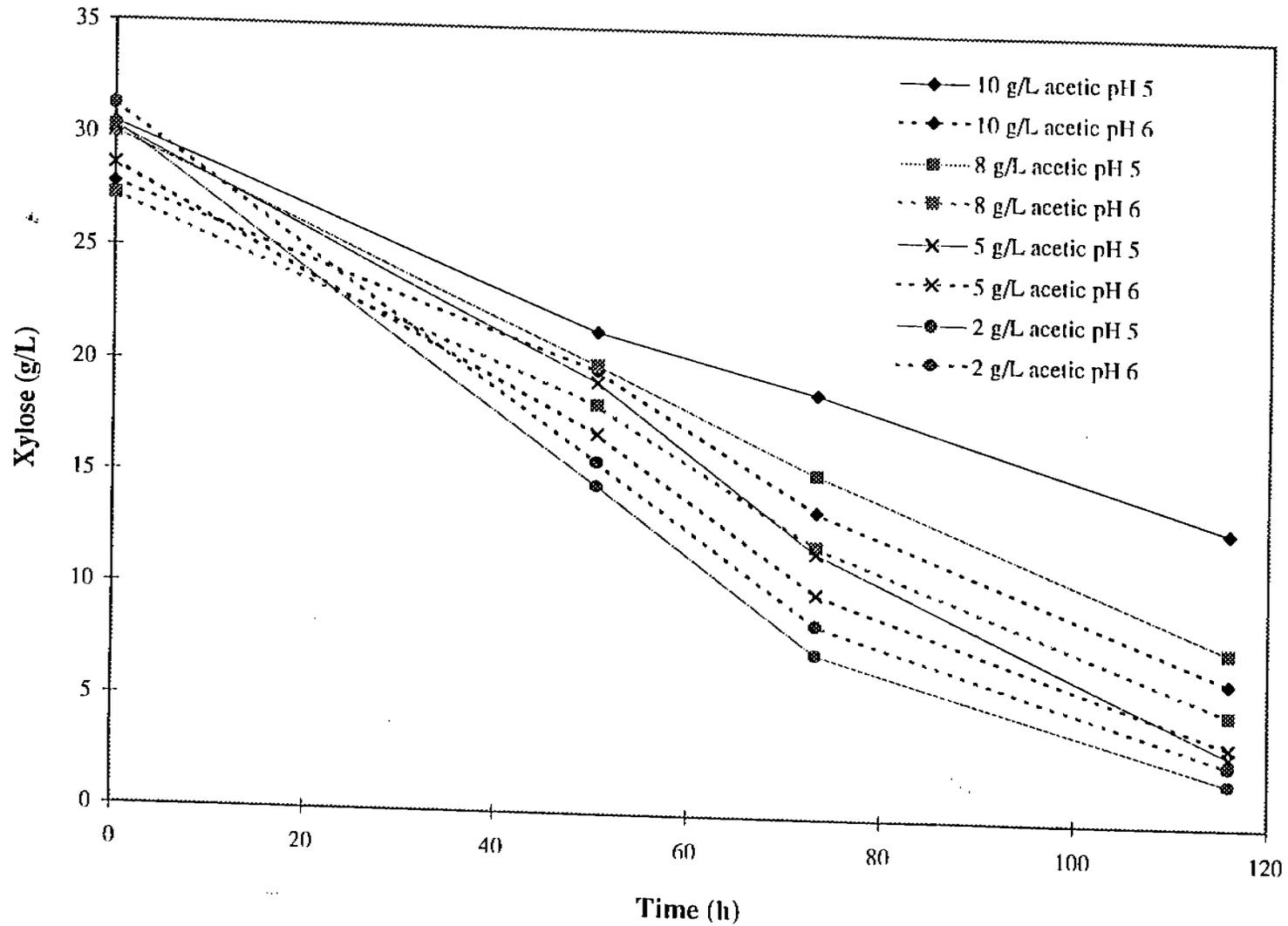


Figure 16. Bench-Scale SSCF Results

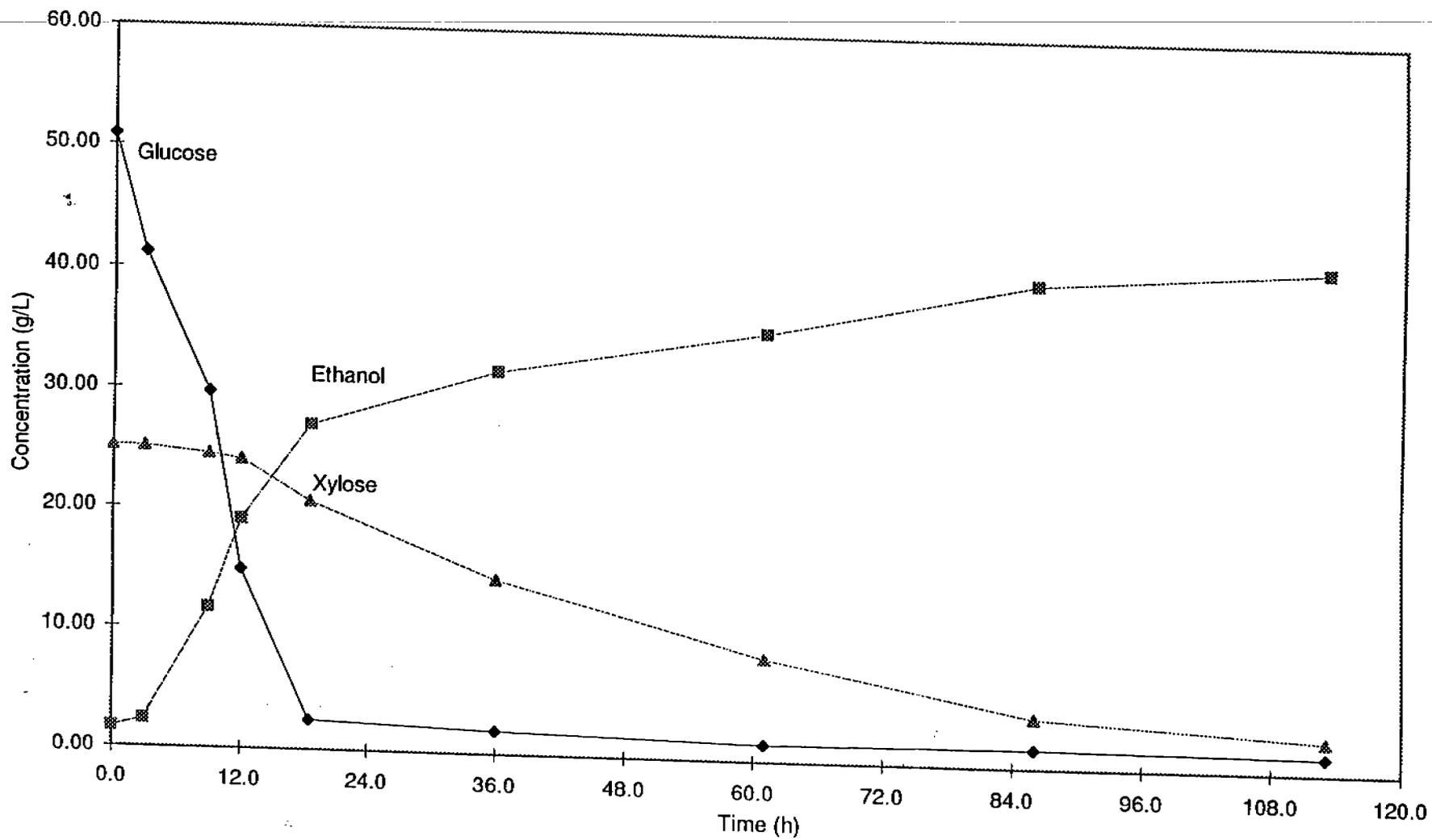


Figure 17. Comparison of Bench-Scale SSCF Results With Corresponding PDU Run 4

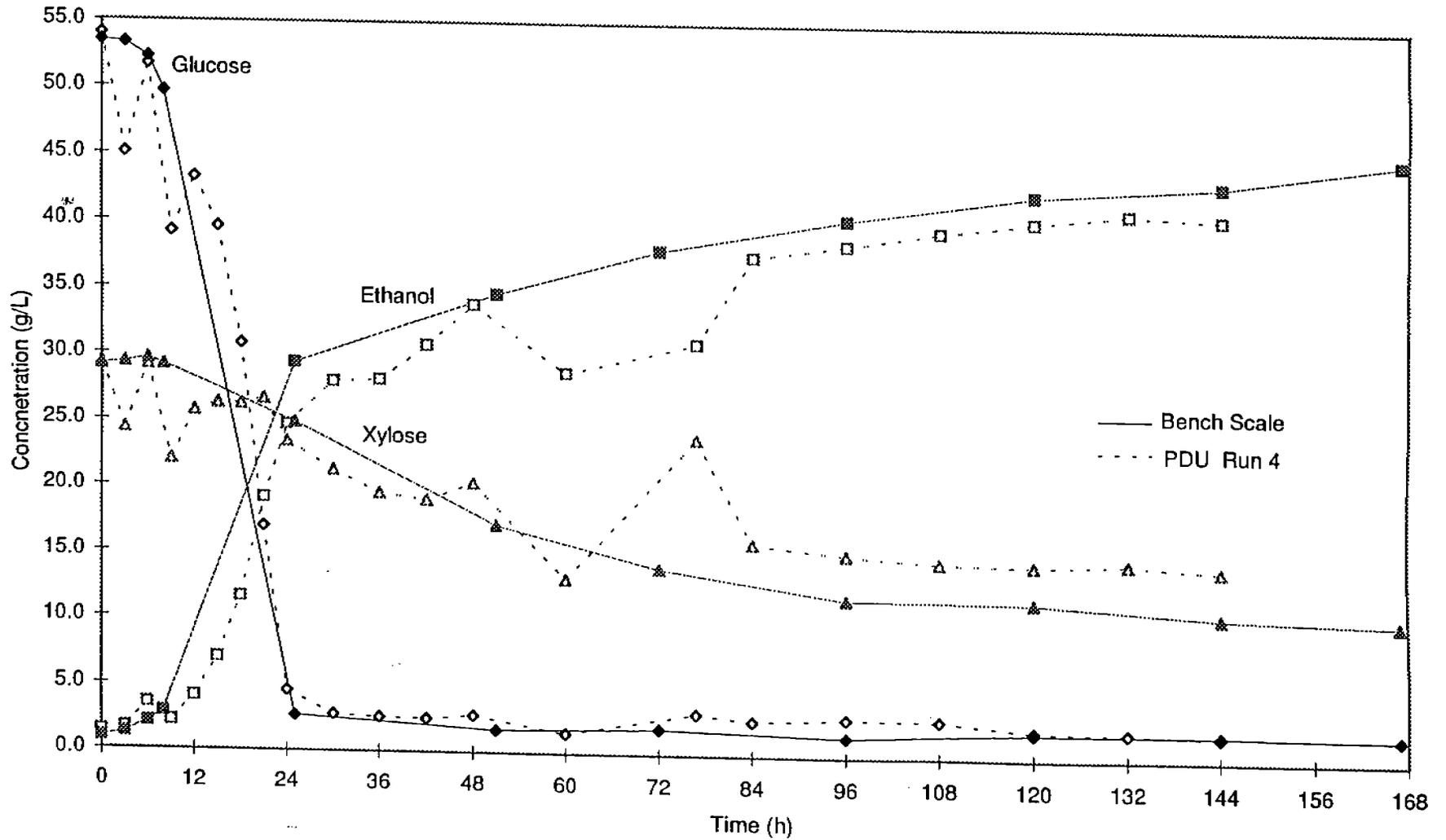


Figure 18. Fermentation Run 5 Data and Predicted Data From the Model

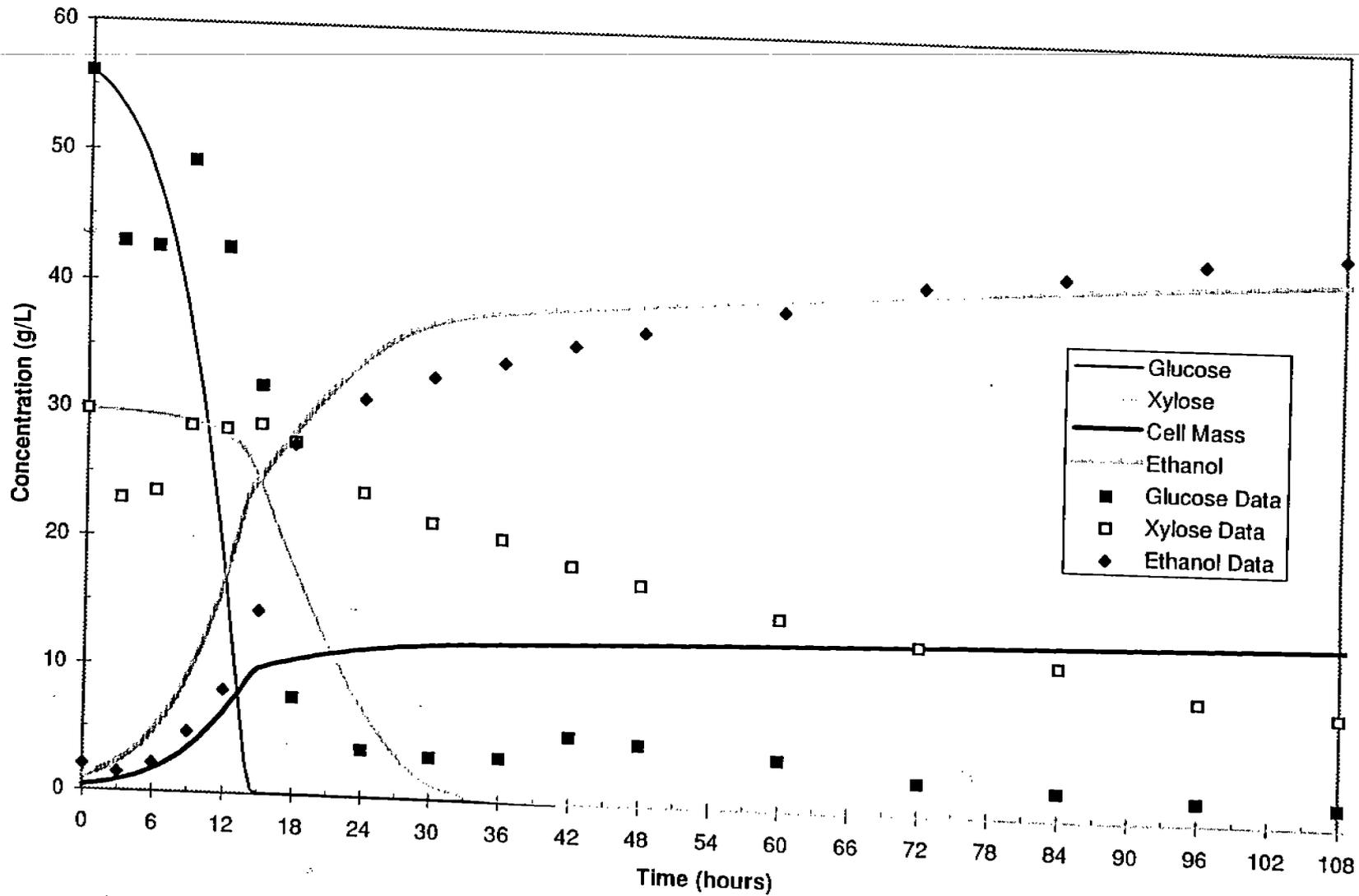


Figure 19. Fermentation Run 5 Data and Predicted Data From the Model

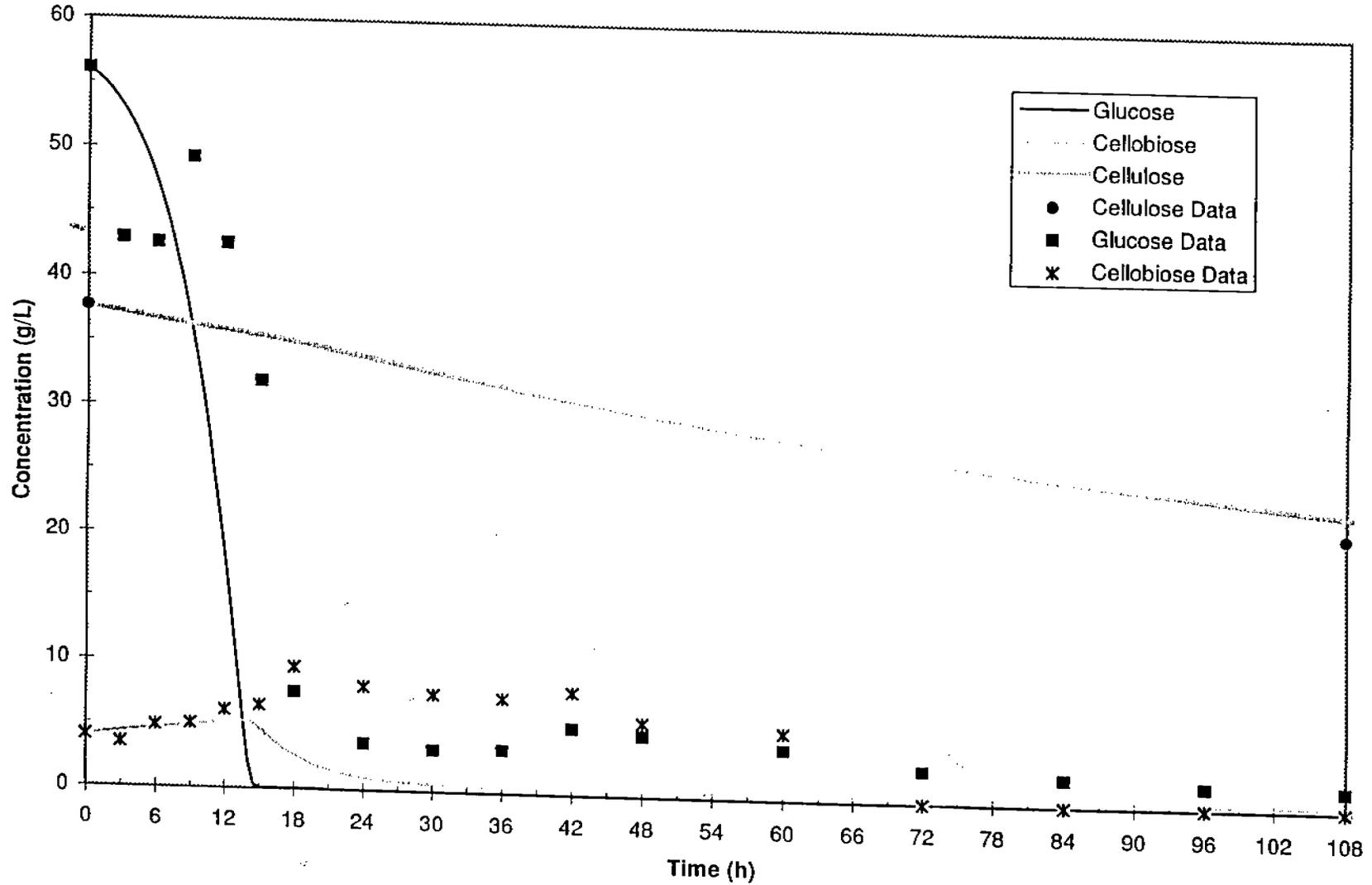


Figure 20. Fermentation Run 5 Data Compared to Model After Adjustment of Kinetic Parameters

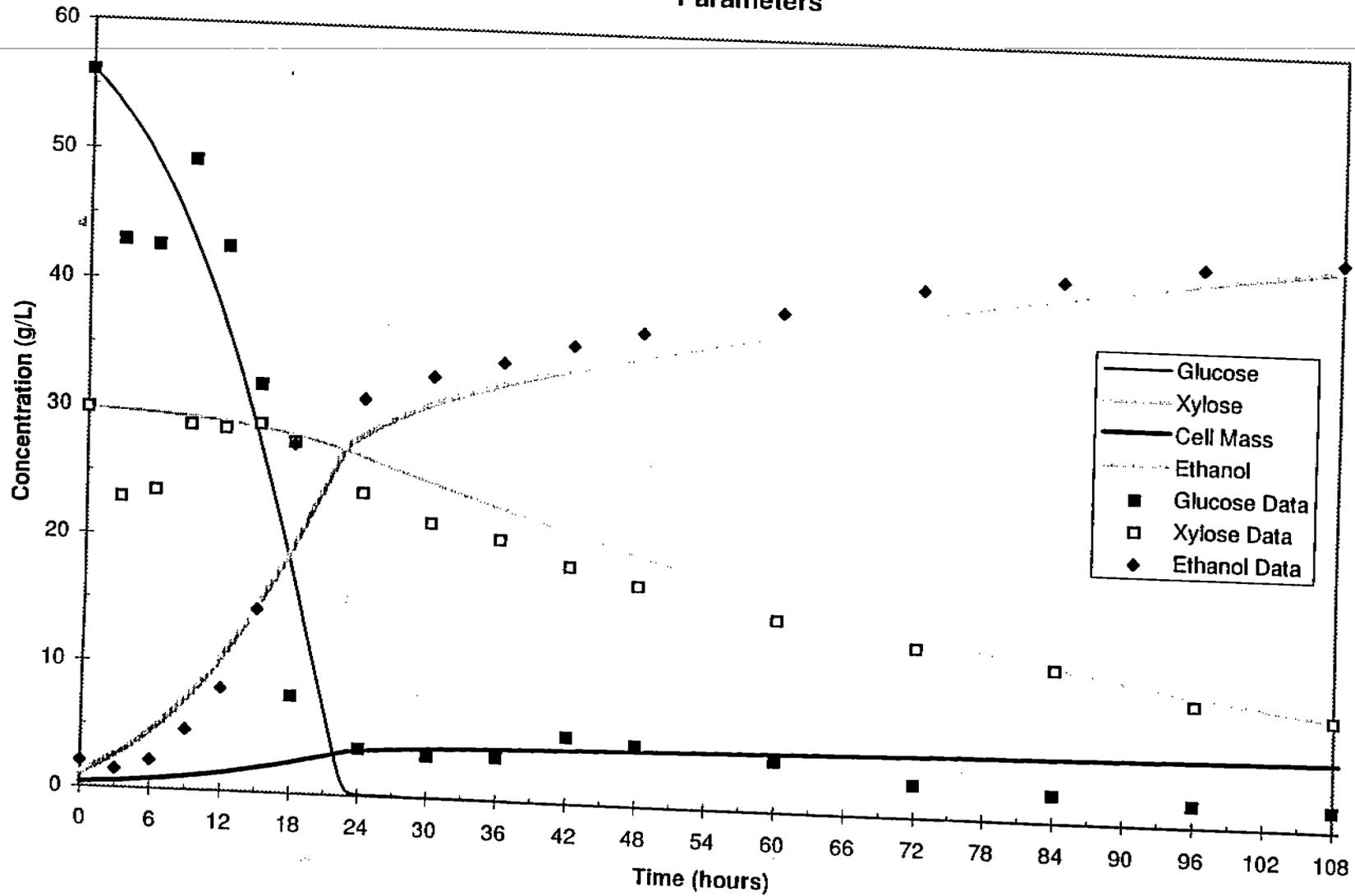
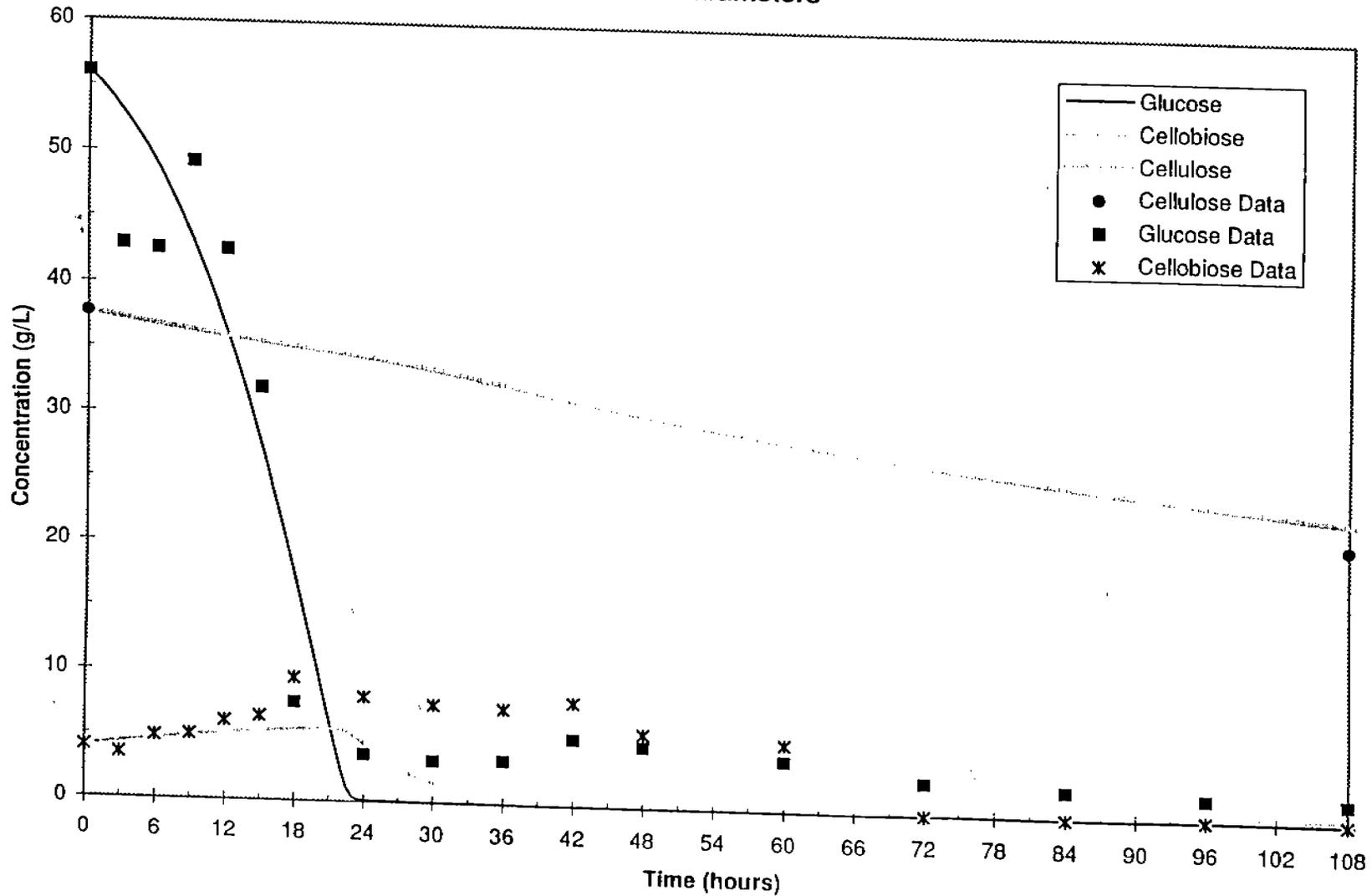


Figure 21. Fermentation Run 5 Data Compared to Model After Adjustment of Kinetic Parameters



Run start data

2-Feb-96

Run Name:

CRADA Task 3

Run ID#:

P960122CF

APR Data

Date	Time	APR Sample #	Tot. Solids Oven (%)	TDS Liquid (%)	Ins. Solids (%)	Sample Wt. (g)	HPLC (g/L)					
							Glucose	Xylose	Gal.	Arab.	Man.	Cello.
23-Jan-96	11:00	APR-178	35.51%	0	9.21%	25.07	100.56	62.43	14.33	43.03	0.00	0.00
23-Jan-96	11:05	APR-178	35.51%	0	9.51%	25.05	99.76	61.94	14.10	42.94	0.00	0.00
23-Jan-96	11:10	APR-178	35.51%	0	9.33%	25.02	100.28	62.38	14.21	43.10	0.00	0.00
26-Jan-96	22:30	APR-179	32.46%	0	10.18%	19.55	137.76	86.62	16.78	49.58	0.00	0.00
30-Jan-96	19:00	APR-180	31.51%	0	11.97%	21.71	123.33	74.89	15.61	46.22	0.00	0.00
2-Feb-96	11:00	APR-181	33.27%	0	7.06%	21.63	128.56	79.52	16.81	50.61	0.00	0.00
2-Feb-96	19:00	APR-183	34.28%	0	9.18%	22.90	116.02	72.54	15.50	46.73	0.00	0.00
3-Feb-96	3:00	APR-185	35.39%	0	10.41%	26.85	97.68	60.57	14.06	43.17	0.00	0.00
3-Feb-96	11:00	APR-187	34.70%	0	9.70%	20.89	123.26	76.54	14.03	47.84	0.00	0.00
3-Feb-96	19:00	APR-189	34.93%	0	11.62%	21.72	117.39	68.90	15.80	48.20	0.00	0.00

Run start data

2-Feb-96

Run Name:

CRADA Task

Run ID#:

P960122CF

APR Data

Date	Time	APR Sample #	HPLC (g/L)							
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
23-Jan-96	11:00	APR-178	2.85	0.00	2.09	4.47	4.21	0.00	0.68	0.41
23-Jan-96	11:05	APR-178	2.60	0.00	1.88	5.32	4.32	0.00	0.67	0.42
23-Jan-96	11:10	APR-178	2.75	0.00	1.98	5.03	4.10	0.00	0.67	0.40
26-Jan-96	22:30	APR-179	3.96	0.00	2.95	1.33	7.81	0.00	1.44	1.61
30-Jan-96	19:00	APR-180	4.91	0.00	2.95	2.00	6.23	0.00	1.02	1.07
2-Feb-96	11:00	APR-181	0.00	0.00	2.31	2.20	5.53	0.00	0.75	0.82
2-Feb-96	19:00	APR-183	0.00	0.00	1.89	8.02	5.54	0.00	0.72	0.92
3-Feb-96	3:00	APR-185	0.00	0.00	1.38	7.17	3.98	0.00	0.72	0.60
3-Feb-96	11:00	APR-187	0.00	0.00	2.60	1.51	8.50	0.00	0.92	4.14
3-Feb-96	19:00	APR-189	0.00	0.00	1.72	10.07	4.67	0.00	0.80	0.82

Run start data 2-Feb-96
 Run Name: CRADA Task
 Run ID#: P960122CF

APR Data

Date	Time	APR Sample #	Liquor Analysis (Total Sugars g/L)				
			Glucose	Xylose	Galactose	Arabinose	Mannose
23-Jan-96	11:00	APR-178	155.24	81.71	17.20	54.76	0.00
23-Jan-96	11:05	APR-178	156.39	82.36	17.55	55.18	0.00
23-Jan-96	11:10	APR-178	156.72	82.50	17.47	55.14	0.00
26-Jan-96	22:30	APR-179	180.78	97.53	22.43	63.79	0.00
30-Jan-96	19:00	APR-180	164.69	90.28	18.91	58.20	0.00
2-Feb-96	11:00	APR-181	173.94	102.06	21.31	65.15	0.00
2-Feb-96	19:00	APR-183	158.32	92.45	19.46	59.72	0.00
3-Feb-96	3:00	APR-185	148.67	84.64	17.68	55.54	0.00
3-Feb-96	11:00	APR-187	173.54	100.08	22.83	67.62	17.84
3-Feb-96	19:00	APR-189	173.44	96.01	21.83	69.64	0.00

Run start data
Run Name:
Run ID#:

2-Feb-96
CRADA Task
P960122CF

APR Data

Date	Time	APR Sample #	Feed Solids (%)	Feed Flow (kg/h)	Steam Flow (kg/h)	Acid Flow (kg/h)	Valve Water Flow (kg/h)	Extruder Temp. (C)	pH
23-Jan-96	11:00	APR-178	0.45	54.50	10.30	16.70			
23-Jan-96	11:05	APR-178	0.45	54.50	10.30	16.70	6.15	240.00	0.92
23-Jan-96	11:10	APR-178	0.45	54.50	10.30	16.70	6.15	240.00	0.92
26-Jan-96	22:30	APR-179	0.45	54.50	10.50	17.00	6.10	245.00	0.78
30-Jan-96	19:00	APR-180	0.45	54.50	10.50	15.00	6.20	241.00	1.03
2-Feb-96	11:00	APR-181	0.45	54.50	10.50	16.20	6.15	245.00	0.92
2-Feb-96	19:00	APR-183	0.45	54.50	11.00	16.00	6.15	246.00	0.98
3-Feb-96	3:00	APR-185	0.45	54.50	10.70	15.90	6.20	247.00	0.98
3-Feb-96	11:00	APR-187	0.45	54.50	10.30	16.20	6.15	246.00	0.92
3-Feb-96	19:00	APR-189	0.45	54.50	10.90	16.00	6.15	247.00	0.92

Run start data 2-Feb-96
 Run Name: CRADA Ta
 Run ID#: P960122CI

Fermentation Results - Run 3

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt. (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
28-Jan-96	10:40	0		18.62%			52.03	16.84%		63.65	56.13	32.72	6.50	18.99	0.00	2.90
28-Jan-96	13:50	3									55.09	32.13	6.35	18.81	0.00	3.14
28-Jan-96	16:40	6									54.96	32.21	6.36	18.86	0.00	3.66
28-Jan-96	19:40	9									54.31	32.13	6.37	18.64	0.00	6.39
28-Jan-96	22:40	12									53.49	31.99	6.31	18.56	0.00	6.81
29-Jan-96	1:40	15									51.06	31.39	6.18	18.36	0.00	6.89
29-Jan-96	4:40	18									48.11	30.43	5.97	17.76	0.00	7.04
29-Jan-96	7:40	21									43.91	30.79	6.00	18.08	0.00	7.37
29-Jan-96	11:40	25									35.68	27.94	5.63	17.03	0.00	7.55
29-Jan-96	14:40	28														
29-Jan-96	16:40	30														
29-Jan-96	22:40	36														
30-Jan-96	4:40	42									36.44	28.89	5.87	17.58	0.00	8.34
30-Jan-96	10:40	48									24.05	20.20	4.20	12.35	0.00	5.96
30-Jan-96	15:00	52									16.78	17.27	3.54	10.60	0.00	5.36
30-Jan-96	22:40	60									13.28	26.75	5.62	16.77	0.00	7.84
31-Jan-96	10:40	72														
31-Jan-96	22:40	84									2.31	23.26	5.45	15.79	0.00	6.34
1-Feb-96	10:40	96									1.87	19.90	4.87	15.49	0.00	4.28
1-Feb-96	22:40	108									1.60	17.70	4.43	14.91	0.00	3.09
2-Feb-96	8:00	117.3		10.37%			29.36	22.29%		36.50	1.78	15.68	3.35	14.62	0.00	2.06
											1.36	13.40	2.31	13.70	0.00	5.08
											1.26	11.77	2.19	13.61	0.00	0.00

