

PDU Final Run Report

NREL/Amoco CRADA Task 5

August, 1996

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Run number: P960506CF
Dates of run: 5/14/96 to 6/28/96
Feedstock: Blended Corn fiber

1.0 Executive *Summary*

The Process Development Unit (PDU) **was** operated with a corn fiber blend (corn fiber and corn screenings) for approximately **six** weeks using the Amoco Pretreatment Reactor (APR), the seed fermentation **train**, fermentation support equipment, three of the main 9000-L fermenters, the **fourth** fermenter as a *kill tank*, and distillation and centrifugation systems. The run utilized the recombinant yeast **strain**, LNHST2, to simultaneously co-ferment glucose and xylose to ethanol (SSCF). **The** APR and fermentation equipment operated continuously from May **14** through June 24 with few problems. The major objective for this run, to produce 10 tons of dry solid product for animal feed testing, was completed.

Pretreatment severity was **high** at the **start** of the run and the resulting high inhibitor levels caused cells in the first 9000-L fermenter to wash out. After a subsequent decline in pretreatment severity and reinoculation of the first fermenter, cell growth and fermentation occurred in the first fermenter. There was a decline in pretreatment severity throughout the run.

A more sensitive indicator of pretreatment severity during this run was inhibitor (i.e., acetic acid, HMF, and furfural) concentration. Throughout most of the run, conversion of starch (at 100%) and xylan (at 85%—100%) were high.

A *lactobacillus* contaminant **was** detected in all the fermenters throughout the run. Major outbreaks, characterized by rapidly rising acetic and lactic acid concentrations, were successfully controlled by the addition of an antibiotic (Lactrol). The high acid levels did not affect glucose fermentation, but did inhibit xylose fermentation.

Two complete **mass** balances were performed during this run. The first **was** done at a fermentation solids concentration of 25%, where 67% of the C6 sugars (i.e., monomeric and oligomeric glucose and galactose) and 26% of monomeric xylose were converted to ethanol (34% overall conversion of monomeric xylose to ethanol and by-products). Later in the run when acid levels were lower (due to wash out of the acids earlier produced by the contaminant), monomeric xylose conversion increased to 50%. At the second **mass** balance point (15% solids concentration), 80% of the C6 sugars and 53% of the xylose was converted to ethanol and total xylose conversion increased to 80%. Process yields for the first and second points were low at 47% and 55%, respectively, primarily because unconverted sugars were leaving the process in the form of cellulose, oligomeric glucose, and monomeric and oligomeric xylose.

Areas where additional work is required include characterizing the performance of the **APR** and improving knowledge of pretreatment conditions. **Varying** pretreatment conditions have not allowed a good comparison of **PDU** data with bench **scale** SSCF. Characterization of fermentation performance is necessary to both

improve and optimize performance and improve the predictive capability of the kinetic model. Work should also be done to characterize and limit the amount of unconverted sugars (i.e., potential ethanol) leaving fermentation. All these factors are necessary to improve the operation or economics of the process.

2.0 Introduction

The **PDU** was operated for a period of 6 weeks using the **APR** to pretreat a corn fiber/corn screenings feedstock and the **Purdue** recombinant yeast (LNHST2) for simultaneous saccharification and co-fermentation (**SSCF**) of glucose and xylose to ethanol. The **primary** purpose of this run was to finish collecting 10 dry tons of representative solid product to test as an animal feed. This is the last task in the **PDU** of Phase 3 of the NREL/Amoco CRADA. The task followed closely after the finish of Task 4, in which the first 5 tons of solid product were collected. In addition, the run should show steady state operation at a high solids level (25%) while avoiding severe levels of contamination, demonstrate adequacy of the kinetics model and/or improve the kinetic model to match experimental data, and collect any other data necessary for design of a commercial plant. An additional unit operation (cross-flow filtration) was also tested during this run.

At the end of Task 4, several problems and issues were identified that needed to be resolved and/or investigated during this run. Major problems included the automated feed system operation and removal of foreign objects from the feed, identification of feedstock lots, questionable feedstock composition measurements, contamination of the CSL system, lack of integrated mass balance data, and poor centrifuge performance. All of these problems were resolved except for the centrifuge. The new back drive installed on the centrifuge did not improve performance as expected. Several major issues included improving the fermentation exhaust gas measurements for mass balance calculations, investigating the poor xylose conversion, investigating the high levels of oligomeric glucose at the end of the fermentation, and improving fermentation cell counts. Some progress was made in each of these areas and will be discussed in the report.

3.0 Pilot Plant Operations

Operation of the pilot plant began on May 14 and continued until June 28. The new automated feed system, APR, fermentation, distillation, and centrifugation equipment were used. Operating conditions were specified before the run and are presented in subsequent sections. Additionally, a run history and significant operational notes are presented.

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 (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 Casco corn wet-milling facility (Cardinal plant, Ontario, Canada) and blended, frozen and shipped to the PDU in 55-gal drums in a refrigerated trailer.

3.1.2 Fermentation Operating Conditions

Operating conditions for the seed **train** are shown in Table 1. 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 flasks. pH was controlled at 5 with 3.0 M NaOH in the 20-L and 160-L fermenters and with 50% NaOH in the 1450-L fermenter. A 20% (w/w) inoculum was transferred from the 1450-L fermenter directly into the first 9000-L fermenter.

Fermentation conditions in the 9000-L fermenters are also presented in Table 1. Corn steep liquor (CSL) and enzyme additions were made to only the first 9000-L fermenter. pH was controlled using 50% NaOH. Level was controlled in the 9000-L fermenters (at 3500 L for 120 lb/h feed rate and at 3850 L for 130 lb/h feed rate for a 25% solids concentration) to maintain a residence time of 36 hours in each vessel. The fermenter level was controlled at 6500 L when the fermentation solids concentration was 15%. Glucoamylase was batched into the first 9000-L fermenter daily at an approximate loading of 2 IU/g starch in the raw feedstock.

pH	5.0	5.0	5.0	5.0	5.0	5.0
Gauge Pressure (barj)			0.33	0.33	0.33	0.33
Airflow (vvm)	-	-	0.5	0.5	0.25	0.03 ^b

a

b

d

3.2 Run History and Operational Notes

A time line for this run showing major events is presented in Figure 1. Feedstock lots 2—8 were used during this run at the approximate times shown on the time line.

The first 9000-L fermenter was inoculated on May 17 at 10:00 (run time 0 hours). Transfers to the second and third 9000-L fermenters began shortly thereafter. After 36 hours, there was no fermentation occurring in the first fermenter. Therefore, the solids concentration was lowered to 15% from the targeted 25% to lower inhibitor concentrations. The results of bench scale fermentations with pretreated material produced during this time period confirmed that pretreatment inhibitors were responsible for the poor fermentation performance (see Section 4.3.2.1).

Since there was no cell growth in the first fermenter because of the high inhibitor levels, it was reinoculated on May 21 at 10:00 (run time 96 hours). This was the last inoculum used during this run. Solids concentration in the fermenters was increased back to 25% on May 27 at 12:00 (run time 242 hours) so that performance could be tested at the higher solids concentration. Inhibitor concentrations were lower because of decreasing pretreatment severity. Solids concentration was again decreased to 15% on June 12 at 16:00 (run time 630 hours) to test fermentation performance at the lower level.

On May 27 at 12:00 (run time 242 hours), the third 9000-L fermenter was drained to replace a bad pH probe. On May 30 at 4:00 (run time 306 hours) sterile water addition to the first fermenter was lost for 12 hours. This was caused by a control system problem that was not caught by the operators. The time line also shows Lactrol addition made during the run to control contamination. Each arrow indicates an addition to all three fermenters.

Analysis of the data after the fact revealed that reinoculation may not have been necessary. The laboratory data discussed in section 4.3.2.3 (and Appendix D) show that concentrations of inhibitors fall during the lag phase, and that yeast cells remaining in the first fermenter would be expected to grow once concentrations of inhibitors fell to low enough levels. There was evidence of fermentation in the second and third fermenters prior to reinoculation of the first vessel. However, it is likely that washout of cells during the lag phase would have resulted in a very long delay in the establishment of steady state, and reinoculation eliminated this delay.

The most significant operational problem with the fermentation system was plugging of the transfer pump between the first and second fermenter. At the time, it was not clear if large chunks of material were plugging the pump or if the pump was failing. After the run, it was found that metal and Teflon parts attached to the capacitance probe in the first fermenter had broken off and lodged in the suction pipe to the pump, blocking a large fraction of the pipe area.

Distillation operated well most of the run with only a few plugging problems. Centrifugation also operated well except for an occasional spill over of centrate into the cake tank. This was caused low density material that would plug the centrate line.

4.0 Key Results

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

4.1 Feedstock

The composition of corn fiber blends used in this run as well as in Task 4 are shown in Table 2, along with the average values that were used in mass balance and yield calculations. These averages include Task 4 and 5 data. Some of the glucose values produced by the outside laboratory were high. Repeat analysis of the feedstock by NREL personnel gave more reasonable values, which are also closer to values generated by Amoco's analytical laboratory. Both CAT and Amoco values and some of the more reasonable values generated by the outside laboratory have been used to calculate averages. Starch measurements were the most variable ranging from 17.5%—39.0% with an average of 27.2% and a standard deviation of 6.8%. Starch measurements have been a problem with the blended feedstock. This could be due to problems obtaining a representative sample of feedstock or obtaining a representative sample from the sample bag for analysis. CAT processing procedures included milling the entire sample and thoroughly blending before withdrawing a sample for analysis. Although not shown, acetate is assumed to be 2.5% of the feedstock, based on previous acetate values reported by the Amoco Analytical Laboratory.

4.2 Pretreatment

A new phenomenon that occurred during this run was deposition of a solid product.

This residue coated the inside of the pipes and varied from near the same color to much darker in color than the normal pretreated material. The darker residue coated the inside wall of the pipe and the lighter residue was deposited on top of the darker residue.

The results of an analysis of some of the residue using methods for feedstock analysis are shown in Table 3.

There are significant differences between each of the above samples as well as a significant difference from pretreated material. The deposited residue is low in carbohydrates and high in lignin and protein when compared to pretreated material. The residue may be deposited after cooling during the flash step. However, the presence of solids in the high temperature environment and deposition of similar solids in reactors conducting high-temperature, dilute acid hydrolysis of cellulose suggest that cooling may not be important. The more severe pretreatment conditions during this run are likely responsible for this lignin-rich residue that

Table 2. Corn Fiber Composition

Lot #	Used In	Source	Moisture (%)	Glucose (%)	Xylose (%)	Gal. (%)	Ara. (%)	Lignin (%)	ASL (1) (%)	Ash (%)	Ext. (2) (%)	Starch (3) (%)	Protein (4) (%)	Analysis Source
15-Mar	P960314CF	Casco	52.6	62.2	11.8	2.3	9.5	3.3	3.8	0.9	10.3	19.9	9.2	CAT/Out(5)
16-Mar	P960314CF	Casco	53.7	62.0	12.7	2.5	10.1	3.3	4.0	0.9	10.1	13.9	8.4	CAT/Out
17-Mar	P960314CF	Casco	54.3	62.1	12.9	2.6	9.9	3.1	4.2	0.8	10.6	18.9	9.1	CAT/Out
28-Mar	P960314CF	Casco	53.6	42.0	19.3	3.8	12.8	3.5	4.7	0.8	8.9	20.0	7.3	CAT/Out
30-Mar	P960314CF	Casco	53.3	45.9	14.8	3.2	11.8	3.5	4.2	0.9	10.1	18.7	7.8	CAT/Out
3-Apr	P960314CF	Casco	52.7	43.1	15.3	3.3	12.0	3.4	4.2	0.8	11.4	19.4	7.5	CAT/Out
26-Mar	P960314CF	Casco	53.8	47.0	18.9	3.6	11.8	2.9	3.6	0.7	12.6	27.8	5.7	CAT/Out
6-Apr	P960314CF	Casco	54.7	42.2	18.2	3.5	12.5	3.7	4.0	0.7	10.6	19.8	7.2	CAT/Out
10-Apr	P960314CF	Casco	55.2	42.8	18.4	3.6	11.9	3.9	3.9	0.8	12.3	20.8	7.1	CAT/Out
13-Apr	P960314CF	Casco	54.7	44.4	17.6	3.3	10.4	3.5	3.6	0.7	11.1	27.3	6.3	CAT/Out
16-Apr	P960314CF	Casco	54.9	45.8	17.6	3.2	10.9	3.7	3.8	0.6	11.9	28.9	6.8	CAT/Out
	P960314CF	Casco		45.3	17.2	2.9	11.0	4.0	3.7	0.7	11.1	24.9	6.7	CAT/Out
16-Mar	P960314CF	Casco	52.3	66.8	11.5	2.3	8.5	2.2	3.1	0.7	9.6		7.6	CAT/Out
22-Apr	P960314CF	Casco	51.9	67.2	8.0	2.1	7.7	2.7	3.1	0.8	8.9		6.7	CAT/Out
3	P960314CF	Casco	54.2	66.0	10.9	2.2	8.3	3.2	3.3	0.7	8.6		7.3	CAT/Out
3	P960314CF	Casco		46.1(6)	20.2		12.6					37.2		Amoco
3	P960314CF	Casco		45.7	21.0	4.2	13.5							CAT
2	P960506CF	Casco	55.3	55.1	13.3	0.6	8.9	2.7	3.1	0.8	13.6		7.3	CAT/Out
2(7)	P960506CF	Casco		45.0	18.3	4.4	11.2					20.1		CAT
4	P960506CF	Casco	54.0	54.1	15.2	0.7	10.0	3.6	3.3	0.7	11.9		7.9	CAT/Out
4(7)	P960506CF	Casco		40.3	21.0	4.7	12.7					17.5		CAT
5	P960506CF	Casco	54.3	59.0	12.0	2.6	10.0	3.8	3.4	0.9	10.1	43.1	5.9	CAT/Out
5	P960506CF	Casco		39.2	16.7	3.8	10.9					32.3		CAT
5	P960506CF	Casco	56.0	43.2(6)	19.3		12.2					33.1		Amoco
6	P960506CF	Casco	55.3	52.0	14.9	3.1	11.9	3.9	3.8	0.7	9.6	38.9	6.0	CAT/Out
6	P960506CF	Casco		39.5	18.5	4.0	11.8					39.0		CAT
6	P960506CF	Casco	54.5	43.2(6)	18.5		12.0					32.2		Amoco
7	P960506CF	Casco	54.1	42.0	19.1	3.4	12.5	3.0	4.5	0.7	7.1	28.1	9.8	CAT/OUT
7(7)	P960506CF	Casco		38.3	21.9	5.0	13.8					35.0		CAT
8	P960506CF	Casco	54.0	48.0	17.0	3.1	11.2	2.5	3.9	0.7	8.5	34.0	10.0	CAT/OUT
8(7)	P960506CF	Casco		43.8	19.1	4.4	11.7					27.3		CAT
Average			54.3	43.7	18.7	3.8	12.1	3.4	4.0	0.7	10.5	27.2	7.5	
Standard Deviation			0.9	2.7	1.9	0.6	1.0	0.5	0.4	0.1	1.7	6.8	1.3	

(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 on extracted feedstock

(5) CAT/Out - Carbohydrates, ash, and lignin determined by outside laboratory

(6) Corrected using CAT galactose number

(7) Unextracted feedstock

has not been seen during previous runs. Factors such as lignin melting and/or reactions with lignin that occur at more severe conditions may be responsible for the residue.

APR sample analysis data are shown in Appendix A.

Table 3. Composition of Solids Deposited at Outlet of the APR

	TS	Glucose	Xylose	Galactose	Arabinose	AIL	ASL	Ash	Protein
Normal APR Sample		50.0	3.0			20.0	6.0		20.0
Colored Residue									
Colored Residue									

4.2.1 Component Concentrations and Yields

Figure 3 shows component concentrations in APR samples during the run. The most obvious result was higher pretreatment severity at the beginning of the run as particularly shown by the high inhibitor levels. Also, both glucose and xylose concentrations were relatively high compared to the rest of the run. A comparison with Task 4 data shows that sugar concentrations were on average approximately 50 mg/g TS higher during this run.

There is an obvious decrease in pretreatment severity throughout the run as shown by the declining xylose and inhibitor concentrations. This could be attributed in part to the increase in feed rate that occurred at 80 hours, but inhibitor concentrations were already decreasing before this change occurred.

The APR data was more stable and the decline in component concentration less severe than during Task 4.

It is interesting to note the relatively large drop in inhibitor concentrations (approximately 40%—50%) during the run when compared to xylose concentrations, which shows a much smaller drop (20%—30%).

The implications on fermentation performance will be discussed in Section 4.3.

Figure 4 shows glucose concentrations and feedstock usage during the run. A comparison of feedstock glucose and starch composition for each lot does not show any correlation with the trend on the chart. Although there is no noticeable difference in glucose concentration, there was a noticeable increase in inhibitor concentrations (see Figure 3), particularly at 270 hours.

Figure 5 shows the ratio of monomeric to total soluble sugar for both glucose and xylose. The ratios were high during the first few weeks of the run averaging about 80% for both sugars. As pretreatment severity decreased toward the end of the run as noted above, the ratio also slightly decreased to an average of about 70%.

Yields of total soluble glucose and xylose and acetic acid are shown in Figure 6. Xylose yields during the first 300 hours of the run were high averaging about 110%, and decreased to an average of about 90% by the end of the run. Acetic acid yields were also high during the start of the run (70%) and decreased to about 40% at the end of the run. The greater proportional decrease in acetic acid when compared to xylose suggest that there is not a one-to-one release of acetic acid and xylose during hemicellulose hydrolysis.

Glucose yields do not show any trend and were somewhat constant at about 80% throughout the run. Based on the average feedstock composition, starch is 70% of the total available glucose. Thus, these results suggest nearly complete starch hydrolysis and 30%—50% hydrolysis of the cellulose.

4.3 Fermentation

4.3.1 SSCF Performance

The SSCF train began operation on May 17 at 10:00 with inoculation of the first 9000-L fermenter (run time 0 hours). The third fermenter was filled by May 19 at 20:00 (run time 58 hours). The train operated in a continuous mode except when feed was lost from the APR, at which time operation reverted to batch. Analytical data for all of the fermenters are shown in Appendix B.

4.3.1.1 Sugar and Product Concentrations

Monomeric sugar concentrations in all three fermenters are shown in Figure 7. Note that all of the glucose is consumed after an initial lag. The bump in the glucose curve (at 50 hours) for the first fermenter was caused by changing the solids concentration from 25% to 15% in an attempt to get the fermentation going. Although, there was still no utilization of glucose in the first fermenter until after it was reinoculated at 96 hours. This is also shown in Figure 8, which plots only ethanol and xylose in each of the three fermenters. Ethanol production began in the second and third fermenter before reinoculation. This lag in ethanol production was

caused by the high inhibitor levels in the pretreated material as noted above. Shake flask fermentations with this same pretreated material showed little or no growth or ethanol production after 96 hours (see section 4.3.2.1). Ethanol production or glucose consumption in the shake flask began only after consumption or disappearance of furfural. In the PDU, cells were washed out of the first fermenter during this lag period, but were able to begin growing in the second and third fermenters. The decrease in solids concentration (dilution of the inhibitors) and reinoculation of the first fermenter with new cells allowed fermentation to begin in the first fermenter.

The period from 100—150 hours shows increasing xylose consumption due to both decreasing inhibitor concentrations in the pretreated material (see Figure 3) and lower solids concentrations in the fermenters. At 150 hours, the xylose concentration began rising in the first fermenter. This was probably the system seeking a new steady state after the changes in pretreated material composition and dilution water addition rate. When the dilution water rate was first changed at 36 hours, the water addition rate was much higher than it should have been (by 50%—100%) for approximately 12 hours. So solids concentration was lower than 15% in the first fermenter for a while, which significantly increased xylose utilization. Once the correct water addition rate was established, xylose was not utilized as rapidly and xylose concentrations in the fermenters began rising. However, before a steady state was established, the solids concentration was increased back to 25% (at 242 hours) to determine the performance at this level.

After the change back to 25% solids, the xylose concentration continued to increase as expected and peaked in the first fermenter at 310 hours. The decline in xylose concentration following this is from both decreasing xylose concentration in the pretreated material (see Figure 3) and increasing xylose consumption by the yeast, which is clearly shown by the rising ethanol levels. During the period from 300—600 hours, the average calculated xylose concentration in the pretreated material (after accounting for dilution in the fermenters) dropped approximately 5 g/L. But during this same period, xylose concentration dropped by 10 g/L in each of the fermenters indicating that xylose consumption increased during this period. Xylose consumption was at first inhibited near 300 hours because of high levels of acetic and lactic acid produced by a *lactobacillus* contaminant. Figure 9 shows the acid concentrations in all three fermenters. The calculated xylose concentration at 600 hours in the first fermenter in absence of any conversion would be 35 g/L. The measured level was approximately 25 g/L in the first fermenter and 18 g/L in the third fermenter. Approximately half of the xylose was consumed and a major fraction (60%) was consumed in the first fermenter. A mass balance point was taken near the middle of this period (480 hours).

At 630 hours, solids concentration in the fermenters was lowered to 15% to test performance at this level. Component concentrations began to decrease because of the extra dilution water. At near the same time, acetic and lactic acid levels also began to increase because of contamination (see Figure 9). Lactrol was quickly added to the fermenters and acid levels approached normal levels by 820 hours. At the same time, the system appeared to reach a steady state as shown by leveling off of ethanol and xylose concentrations (see Figure 8). The calculated xylose concentration at this time in the first fermenter in absence of any conversion would be 22 g/L. The actual level in the first fermenter is 11 g/L (50% conversion) and 6 g/L in the third fermenter (73% total conversion). A mass balance point was taken at 845 hours. It is interesting to note that a majority of the xylose is consumed in the first vessel (approximately 60%—70%) and very little is used in last vessel. This would suggest that it may be more economical to operate at shorter residence times.

4.3.1.2 Identification of Unconverted Oligomeric Glucose

Figure 10 shows chromatograms of fermenter and centrifuge feed samples analyzed specifically to determine the presence of disaccharides that could occur because of reversion reactions. Several disaccharides were

identified and quantified as shown in Table 4. There are also peaks of other possible disaccharides, but standards do not exist or are not readily available. The results show that significant levels of disaccharides were present, and some consumption of these disaccharides occurred along the fermentation train. Heating during the distillation step has also caused a significant loss of α,α -trehalose and nigerose. However, the sum of all of these sugars is not enough to explain the significant levels (15—20 g/L) of oligomeric sugars left at the end of fermentation.

Table 4. Disaccharide Concentrations (g/L) in Fermenters and Centrifuge Feed

Disaccharide	First Fermenter	Second Fermenter	Third Fermenter	Centrifuge Feed
α,α -Trehalose	0.84	0.63	0.36	0.08
Isomaltose	1.46	1.34	1.33	1.05
Gentiobiose	0.85	0.72	0.64	0.41
Nigerose	0.42	0.37	0.35	trace
α -Maltose	0.76	0.9	0.9	0.72

The same sample from the third fermenter was also analyzed on a TSK 1000 column for detection of higher molecular weight polymers. The results showed the presence of disaccharides as well as a significant level of an octamer. A semi-quantitative estimate of the octamer concentration was 31 g/L. This is much larger than expected, but the measurement was well outside the range of the standards (highest standard used was a pentamer). This doesn't positively identify the compound as a carbohydrate and the measured value is suspect, but it does suggest a significant level of a high molecular weight sugar was left unconverted. Further work is necessary to identify the origin of the polymer (cellulose or starch) and determine if it can be broken down to monomeric form.

4.3.1.3 Cell Mass Determination

Quantifying cell mass production during these fermentations has been difficult as discussed in previous task reports. The cells and corn fiber solids cannot be separated well enough to give an accurate cell mass measurement. Previous estimates of cell mass based on data from liquid cultures was suspiciously high and viable cell counts are known to be low. For this run, the cell mass was determined by counting the number of cells using a hemacytometer and microscope. The fermentation broth was diluted to a point where the solids were dilute enough and yet the number of cells was high enough to count. Cell counts can then be converted to cell mass assuming the method accurately counts all of the cells.

To determine the correlation between cell mass and cell counts, liquid YPD was inoculated with LNHST2 frozen culture. The flask was sampled over a 24 hour period. At each sample point, the number of yeast were counted on the hemacytometer and the dry cell weight was measured. Figure 11 shows the strong correlation between dry cell weight and cell counts that can be used to calculate cell mass.

To determine the accuracy of the counting method in the presence of corn fiber solids, pretreated corn fiber was diluted to 15% solids and spiked with a known number of yeast cells. The percentage of yeast counted to total number expected was calculated. The average determined from six separate samples was 89.5% (standard deviation of about 6.0 percentage points). Thus, cell counts were corrected by dividing by 0.895 and then multiplying by 1.6×10^{-8} to calculate cell mass.

4.3.1.4 Contamination

The 9000-L fermenters were contaminated throughout the run. A contaminant found at the beginning of the run was a bacillus, but this organism did not get established in the fermenters because of the high toxicity of pretreated material. Once the APR conditions were changed and the environment improved in the fermenters, a second contaminant was isolated at 96 hours into the fermentation. This organism was typed as a *Lactobacillus plantarum*. The contaminant, which became a problem around 200 hours was brought under control, though not totally eliminated, by several days of Lactrol treatment at 50 ppm every 12 hours into all three fermenters. The concentration of the contaminants remained at very low levels until the solids concentration in the first 9000-L fermenter was lowered to 15% (at 630 hours). The increase in bacterial contaminants were quickly detected by increases in lactic acid concentrations measured by the YSI. Lactrol was added at the same levels as before, until lactic acid concentrations decreased. Finally, contamination and the resulting by-products began increasing around 875 hours in all three 9000-L fermenters. The run ended before the contamination could be treated with antibiotics.

A fungal contaminant was also isolated from the second and third 9000-L fermenters around 500 hours, and may have been present in first fermenter at very low levels. The number of contaminant yeast cells never reached levels greater than 2×10^7 /mL and decreased below detection by the end of the run. The organism only consumed glucose, fructose and mannose.

Several areas were sampled during the run to troubleshoot the contamination issue, and some of those areas did prove to be sources of bacteria and fungi. Since the first 9000-L fermenter was contaminated early in the run, the inoculum was considered a likely source. In the first inoculum batch, bacteria were isolated from the tank. Using a bacterial typing kit, the bacterium was determined to be *Bacillus cereus*. A bacillus organism was found in the first 9000-L at the start of the run, but never established itself. The first 9000-L fermenter was reinoculated with a second inoculum batch that was also contaminated with a *Lactobacillus brevis*. This did not match the contaminant (*Lactobacillus plantarum*) present in the 9000-L fermenters at 96 hours. The cause of the contamination in seed fermenter was probably a plugged steam trap located on a line attached to the headplate. Since the CSL tanks and APR feed were clean during this time, it was possible that the seed vessel may have also been contaminated with *Lactobacillus plantarum*, but this was not confirmed.

Even though the CSL tanks were clean during the early part of the run, 400 hours into operation both feed tanks were continually contaminated with bacteria and fungi. The bacteria typed consistently as *Lactobacillus brevis*. The fungi, found in the multicellular (hyphae) and unicellular (yeast) form, exhibited the same characteristics as the one found in the 9000-L fermenters. Contamination was also found in the CSL feed line. Several issues regarding sterilization procedures are being looked at closely, although, the exact cause of the problem is unknown. The source of the contamination may not have been the CSL tanks, because *lactobacillus plantarum* was the only contaminant found in the 9000-L fermenters during this period. Except at the end of the run, *Lactobacillus brevis* was isolated from the 9000-L fermenters. This suggest that this final episode of contamination may have come from the CSL system.

The APR feed is another possible source of contamination, but up until Task 5 no contaminant was isolated **from** pretreated material. After several modifications to the laboratory test procedure, including increasing the incubation time to 7 days, several samples from the APR contained both bacteria and fungi. Bacteria and fungi were isolated in samples taken at 220 hours, 400 hours, 450 hours and all samples taken between 760 and 840 hours. The bacterial isolates typed from the 450 and 840 hour samples were *Lactobacillus plantarum*, matching the organism found in the fermenter during that same time frame. The yeast isolated from the sample taken at 220 hours exhibited the same characteristics **as** the yeast in fermenter and **from** the CSL tanks. No obvious loss of APR operating conditions were noted at these times, although many small problems may have contributed to contaminating the samples. Finding the same type of yeast in the APR material, fermenter and CSL samples may point to **an** airborne contaminant. Contaminated APR feed would explain the continued presence of contamination in the fermenters.

Other areas checked for contamination included sterile water added to the first 9000-L fermenter, the cellulase feed bottle and pump seal water. Pump seal water samples were positive for a **gram** negative bacteria, which **was** not found in the fermenters but is very common in process and chilled water. Sterile water and the cellulase enzyme systems were both clean. Nisin addition to the enzyme worked well at eliminating bacteria **from** the enzyme. Other areas to consider sampling in the future would be the fermenter agitation seals and plant air.

Two areas need to be addressed to improve contamination control are changes in fermentation conditions and PDU procedures and equipment. Higher ethanol concentrations and lower operating pH would significantly reduce bacterial contaminants. PDU personnel need to be more diligent in insuring equipment and associated feed lines are thoroughly cleaned. Improvements have been made to the CSL system, but there are still contamination problems. Determining microbial load in the CSL may be necessary to effectively determine sterilization times and temperature. Finally, another sterility validation test should be performed on all of the fermenters.

4.3.2 Supporting Bench Scale Work

The following sections report on bench scale work carried out during **Task 5** in support of PDU operations.

4.3.2.1 Fermentation of APR Samples From Start of Run

At the **start** of the run no glucose conversion by LNHST2 was observed in the 9000-L fermenters. It was suspected that high levels of inhibitors in the pretreated material produced **during** the first week of Task 5 (see Figure 3) was **inhibiting** the fermentation. To confirm this, a set of shake **flask** fermentations were done using APR material from this period. Table 5 shows HMF, furfural, and acetic acid in the pretreated samples. Each **flask** contained pretreated corn fiber at 25% (w/w) solids (**33%** solids were **assumed** for all pretreated corn fiber samples), 1% CSL, and 10% inoculum. The pH in each **flask** was adjusted to 5.0 using NaOH. A control flask containing 8% glucose, **4%** xylose, 1% CSL, and 10% inoculum was also included. Samples taken over a week long period were analyzed for sugars, ethanol, organic acids, HMF, and furfural.

Figure 12 summarizes sugar consumption by LNHST2 for each of the APR samples. The results show a strong correlation between inhibition of glucose and xylose consumption and increased furfural and HMF levels. The flask with the highest furfural and HMF at the **start** of the fermentation still had small quantities of the two chemicals detected **at** 150 hours and had yet to utilize any sugar. The disappearance of HMF and furfural during fermentation **was** later studied in more detail (see section 4.3.2.3). The presence of 3.7 g/L acetic acid,

with little HMF and furfural detected, did not inhibit glucose consumption but did inhibit xylose uptake (flask 7). These results explained the lack of fermentation performance at the start of the run.

Table 5. Inhibitor Concentrations in Tested Pretreated Samples

APR Sample Run Time	Flask Number	HMF (g/L)	Furfural (g/L)	Acetic Acid (g/L)
-53	1	<0.1	0.39	5.60
-29	2	0.55	0.49	5.37
-5	3	0.72	1.10	5.23
19	4	0.55	0.39	4.83
43	5	0.47	0.81	4.88
67	6	0.3	<0.1	5.01
91	7	<0.1	<0.1	3.74
Control	8	0.0	0.0	0.0

4.3.2.2 Study of Fermentation Performance at Different Solids Concentrations

A shake flask study was performed to compare the fermentation performance of two hydrolyzates (APR-330 produced on 4/29/96 at 21:00, run time 1040.5 hours [Task 4] and APR-392 produced on 6/5/96 at 4:00, run time 450 hours) at different solids concentrations (25%, 18% and 12%). This was done to determine how solids level was affecting xylose fermentation. The experiment also compared the performance of liquor versus whole slurry at the same equivalent solids level.

At a solids loading of 25% with APR-330, a lag phase of 96 hours was observed before glucose utilization began. Once glucose consumption began, over 90% was utilized within 24 hours. Minimal xylose utilization was observed within 120 hours. At 18% solids, a lag phase of less than five hours was observed, and 100% glucose conversion was observed within 24 hours. After 120 hours, 84.6% of the xylose was utilized. No appreciable lag phase was observed at 12% solids, and 97% of the xylose was utilized in 120 hours. The rate of glucose utilization appears to be similar in each flask after the lag phase. The xylose utilization rate was similar at the two lower solids levels.

At a solids loading of 25% with APR-392, a lag phase of only 12 hours was observed before glucose utilization began. At all three solid loadings, the rate of glucose utilization is the same after the lag phase. Similarly, the rate of xylose utilization is not affected by solids level after the lag phase. After 120 hours, 66.1%, 92.6%, and 95.3% of the available xylose was consumed in the 25%, 18%, and 12% solids level flasks, respectively. Even though, the xylose utilization rates were the same, all the xylose in the 25% flask was not consumed after 120 hours because of the extra xylose present.

The longest lag phase (96 hours) was observed in the flask containing the highest amount of inhibitors (i.e., 5.9 g/L acetic acid, 0.5 g/L furfural, and 0.5 g/L HMF). As the concentration of these compounds decreased with dilution in the different flask, the duration of the lag phase decreased. In both sets of flasks, furfural present at the beginning of the experiment decreased to zero and HMF started to decrease before glucose was consumed. HMF was below detection limits by the time all of the glucose was consumed.

The results show that a reduction in inhibitor concentrations either by dilution or from reduced pretreatment severity (e.g., APR-392) increases xylose utilization. If the organism cannot adapt to high inhibitor concentrations, then the best operating condition may be a trade off between operating at reduced pretreatment severity and higher solids concentrations or increasing the severity and operating at lower solids concentration.

A set of flasks containing the whole slurry was compared to liquor at the same solids concentration (i.e., approximately the same sugar concentrations). The glucose and xylose utilization rates were similar and shows that the organism performance is the same in liquor or whole slurry.

Detailed experimental results are shown in Appendix C.

4.3.2.3 Study on the Effect of HMF and Furfural on Fermentation Performance

Because of the effect of HMF and furfural on fermentation performance as noted above, a shake flask study at effective solids concentrations of 25%, 18%, and 12% was performed to examine the effect of furfural and HMF on the fermentation performance of LNHST2 and to provide data for kinetic modeling. The substrate used in this study was liquor separated from hydrolyzate generated on 6/14/96 (between APR samples 417 and 418) during Task 5 and was also used for a bench-scale continuous fermentation experiment.

At the 25% effective solids level, an initial lag phase of 6 hours was observed. The duration of the lag phase decreased at the lower solids levels. Onset of exponential growth and glucose utilization began after depletion of furfural. At all three solids levels, depletion of furfural was followed by depletion of HMF. However, exponential growth and glucose utilization occurred while HMF was being used. This may explain why the cells washed out of the first fermenter during the start of Task 5, as furfural levels were at their highest levels seen during this run.

Detailed experimental results are shown in Appendix D.

4.3.2.4 Bench-Scale Continuous Fermentation Work

Four bench-scale continuous fermentations were initiated to verify PDU performance on the bench scale and thus provide a cheaper and quicker method for investigating continuous fermentation performance with pretreated material. A two-stage fermentation using LNHST2 with clarified hydrolyzate liquor, obtained after sample APR-330 (for continuous runs 1—3), was performed to first duplicate PDU performance at the end of Task 4 and then, by modifying parameters, establish operating conditions in which both glucose and xylose are fermented to ethanol. Run 4 used pretreated material taken between APR samples 417 and 418 during Task 5. The fermentations were carried out in 1.3-L chemostats at 30°C, with an agitation rate of 150 rpm, and a targeted residence time of 36 hours per stage. Caustic (50 % sodium hydroxide) was used to control pH to 5. After analysis of the hydrolyzate liquor (via HPLC) it was noticed that the concentrations of all sugars, acid salts, and reversion products were approximately 40% higher than those recorded for APR samples 417 and 418 (i.e., glucose concentration in clarified hydrolyzate was 130 g/L whereas the PDU samples were recorded at 95 g/L). Therefore, solids concentrations reported in this sections have been adjusted to compensate for this concentration effect. It is suspected that concentration occurred during operation of the batch centrifuge used to obtain the liquid fraction.

Liquor for Run 1 and 2 were diluted to an equivalent solids loading of 23%. Run 2 employed the same temperature, agitation rate, and residence time, but was run at pH 6.5 to determine the effect of elevated pH on the fermentation. Run 3 was run at a pH of 5.0 and the hydrolyzate was diluted to a solids concentration

of 24%. Run 4 was also at a pH of 5, but at a residence time of 24 hours and a solids concentration of 21%.

During Run 1, after the batch phase, there was a continuous decrease in ethanol concentration and increase in glucose concentration during the entire run. Cell counts were low during this run as well. Ethanol concentration peaked at 42.5 g/L in the second vessel before dilution occurred. In Run 2, glycerol concentration increased continuously until the run was terminated. At the end of Run 2, there was 12.5 g/L glycerol and 36 g/L ethanol in the second vessel. The high pH promoted by-product production instead of ethanol production. In Run 3, approximately 30% of the xylose was utilized. At the end of Run 3 there was 32 g/L ethanol in the second vessel, but glucose concentration also increased in the first vessel.

In Run 4 after all of the glucose was utilized and approximately 29 g/L ethanol produced, the glucose concentration in the first vessel started to increase (and the ethanol concentration decrease). Whereas in the PDU, all the glucose was consumed in the first fermenter. After approximately 250 hours, the system appeared to attain steady state with an ethanol concentration of 28 g/L in the second vessel. This corresponds to a process yield of 53.4% and a metabolic yield of 86.1%. Analysis of the steady state material indicated that 36% of the xylose was converted. Note that these results are very similar to those obtained in run 3 (24% total solids and a 36 hour residence time per vessel). This run also proved that the fermenters can be operated at a 21% solids concentration and 24 hour residence time without washout.

Table 6 compares xylose conversion from the chemostat runs to some PDU data generated during this run. The best match is Run 3 data with PDU data at 25% solids and a 72 hour residence time. The xylose conversions are somewhat similar at 31% and 37%, respectively. The problem with this comparison is that differences in pretreatment severity will affect xylose conversion, and as previously noted, pretreatment severity decreased throughout the run. The pretreated material for Runs 1—3 was taken at the end of Task 4 when pretreatment severity was high. This may explain why data from the chemostat runs generally appears poorer than PDU data. However, given this fact, the chemostat data does seem to provide a good indication of large scale performance.

Table 6. Comparison of Xylose Conversion From PDU and Chemostat Runs

	Solids Concentration (%)	Xylose Converted (%)	Residence Time (h)
Run 1	35	15	72
Run 2	35	29	72
Run 3	24	31	72
Run 4	21	36	48
Task 5, PDU	25	50	108
Task 5, PDU	25	37	72
Task 5, PDU	15	80	108
Task 5, PDU	15	70	72

A detailed report on the continuous bench-scale fermentation work that occurred before and during Task 5 is given in Appendix E.

4.3.3 Viscosity and Density Data

Viscosity and density were measured for hydrolyzate, fermentation, and centrifugation samples during Task 5 as shown in Table 7. The fermentation sample viscosity measurements were done using a Thomas-Stormer viscometer at temperatures of 30°C and 55°C and compared to three standard solutions curves of known viscosity. Density was determined by weighing a known volume of material. Other viscosity measurements were done with a Brookfield viscometer as noted in the table. Measurements on the hydrolyzate and cake samples may be unreliable as separation between the instrument spindle and the sample were noted. This would produce an erroneously low value.

Table 7. Density and Viscosity Data for Hydrolyzate, Fermentation, and Centrifugation Samples

Sample	Solids Concentration (%)	Temperature for Density Measurement (°C)	Density (g/cc)	Viscosity		
				30°C (cP)	55°C (cP)	100°C (cP)
Hydrolyzate ¹	29	20	1.149		3737 ²	5782 ²
Fermentation (25% solids)						
First Fermenter	NM	28	1.073	434	N/A	
Second Fermenter	16.4	29	1.073	188	N/A	
Third Fermenter	16.7	28	1.072	162	141	
Fermentation (15% solids)						
First Fermenter	NM	28	1.056	125	63	
Second Fermenter	9.6	28	1.052	120	31	
Third Fermenter	10.4	28	1.054	79	36	
Centrifugation (from 25% fermentation samples)						
Feed	15.3	28	1.082	140 ³		
Centrate	11.2	26	1.052	3 ³		
Cake ¹	23.9	20	1.086			2285 ²

¹ Samples too solid to measure a viscosity with the Stormer

² Viscosity measured with Brookfield viscometer by Hauser Laboratories

³ Viscosity measured with Brookfield viscometer by Bird Machine Co. at 23°C

NA- Not available, viscosity was far above upper viscosity standard of 140 cP

NM - Not measured

Newtonian or non-Newtonian flow characteristics could be determined by plotting the rate of flow versus various drive weights and comparing these curves to the known curves of plastics, pseudoplastics, inverted plastics and Newtonian materials. Because of the consistency of the fermentation broth samples, the data was inconclusive. Solids would settle out using the lighter drive weights (30g and 50g) resulting in unrepeatable data. The data using the heavier drive weights (70g, 90g and 110g), kept the material suspended, but only provided a partial picture of the curves needed to interpret the rheology of this material.

4.3.4 Fermentation Solids Separation and Recovery

4.3.4.1 Alfa Laval Testing

On May 14 and 15, Alfa Laval tested fermentation broth in their laboratory on a Sharples P-660 Decanter centrifuge. This unit should give comparable results to the Sharples P-3000 currently installed in the PDU. The goal was to find conditions that increased the solids concentration in the cake above the 25% solids achieved by the P-3000 during Task 4.

The P-660 was tested with three different conveyors (Kiwi, BD disc, and Plough) at feed rates ranging from 0.25—1.43 gpm. Pond depth was varied between 2.0—4.06. Cake solids concentrations were above 30% with both the Kiwi and Plough type conveyors at any feed rate with a pond depth setting of 2.0. Increasing the pond depth decreased the solids concentration to the 25%—30% range. Cake solids concentrations of only 22% were achieved with the BD disc conveyor.

The P-3000 has a helical type conveyor and this configuration was not tested by Alfa Laval. Therefore, these results were not useful for identifying conditions that would improve cake solids concentrations in the PDU with existing equipment.

4.3.4.2 Bird Machine Testing

Bird Machine Co. performed bench scale testing on fermentation broth in their laboratory and had a representative present during operation of the PDU centrifuge. Based on laboratory testing and observations in the PDU, they conclude that this material is more difficult to dewater than whole stillage from a dry corn mill operation and that dewatered solids in the 30%—35% range will not be obtainable in a production solid bowl centrifuge. Dewatered solids in the 20%—25% solids range can be obtained, but only with high gravitational levels (3000 g's) and reduced flow rates.

4.3.4.3 Solids Recovery

Table 8 shows material collected for animal feed testing during Tasks 4 and 5, along with total solids information and sulfate levels in the cake. A total of approximately 12.5 tons of cake were collected at an average solids concentration of 22.5%. Part of this material was not acceptable as an animal feed, because it was collected during the early part of the run in which the material was killed at 125°C instead of 80°C. The higher temperature produced a darker product which likely contains additional degradation products. Although not suitable for animal feed, it was useful as a test material for drying equipment development. The total collected excluding this material was 11 tons.

Data (solids concentrations and flows of feed, centrate, and cake) were taken twice during operation of the centrifuge to determine solids recovery. Insoluble solids recovery at the two points were 65% and 94%. The poor recovery at the first point corresponded to a 25% solids concentration in fermentation. The higher solids loading in the feed or difficulties with operation of the centrifuge during that run may be responsible the poor

Table 8. Product Collected During Tasks 4 and 5

Date	Lot No.	Number of Drums	Percent Total Solids			Sulfates (%)	Solids Collected (lb)	COMMENTS
			Feed	Cake	Centrate			
4-2-96	1	6	14.72	22.53	11.32	0.63	635	Dark Lot
4-3-96	2	8	15.03	21.7	11.06	0.73	816	Dark Lot
4-5-96	3	2	14.5	28	12	0.8	263	Dark Lot
4-6-96	4	6	14.66	21.13	11.63	0.7	596	Dark Lot
4-9-96	5	9	17.17	21.73	14.61	0.87	919	Reduced kill - product lighter
4-10-96	6	14	15.57	24.12	14.45	1	1587	
4-13-96	7	6	12.68	20.92	10.02	0.7	590	
4-17-96	8	11	16.58	24.25	15.46	0.73	1254	Slow going to drums
4-18-96	9	0	14.33	21.7	9.68	0.5		Initial separation
4-18-96	9	4	10.04	17.15	6.14	0.25	322	After one wash (1:1 ratio)
4-19-96	10	12	17.85	24.46	14.51	0.65	1380	Started with temp. @ 48 C
4-22-96	11	9	14.75	21.46	10.97	0.65	908	Started with temp. @ 48 C
4-26-96	12	8	17.39	24.27	15.49	0.88	913	Started with temp. @ 48 C
4-27-96	13	4	18.4	27	14.8	0.73	508	Used Bird Centrifuge
5-24-96	14	4	13.72	23.86	14	0.6	449	
5-24-96	15	8	10.98	21.42	5.99	0.5	805	
5-26-96	16	7	10.88	19.79	8.51	0.6	651	
5/28/96	17	6	10.93	20.37	7.97	0.5	574	
5/31/96	18	8	12.2	22.02	9.93	0.6	828	
6/1/96	19	10	14.02	23.55	8.1	0.6	1107	Feed temperature = 60 C
6/4/96	20	11	14.67	25.1	10.7	0.6	1298	
6/7/96	21	13	17.56	26.2	13.66	0.72	1601	
6/9/96	22	9	15.16	24.4	11.59	0.65	1032	
6/13/96	23	10	15.7	23.09	13.17	0.75	1085	
6/17/96	24	8	13.33	22.15		0.65	833	
6/18/96	25	9	11.42	21.31	8.27	0.55	901	
6/20/96	26	8	10.14	21.97	7.94	0.6	826	
6/21/96	27	8	10.72	20.45	6.37	0.45	769	
6/23/96	28	8	9.21	22.1	7.93	0.4	831	
6/25/96	29	6	8.6	22	6.2	0.5	620	
6/27/96	30	1.25	5.5					
Total/Average		233.25	13.76	22.67	10.77	0.64	24901	
							22591	Excluding lots 1-4

recovery.

4.3.5 Cross-Flow Filtration Work

A Niro skid-mounted filtration system capable of separations from micro to nano particle-size ranges as well as reverse osmosis was tested with broth from the third fermenter. The permeate was a candidate for recycling to increase the ethanol concentration in the fermenters. The unit, capable of up to 40 L/min feed rate, was fitted with a 0.2 micron polymer-coated ceramic membrane cartridge (U.S. Filter model 1P19) with a design flux (on water) of 1700 L/m²-h. Two Niro representatives performed the initial check-out on the unit and provided training.

Three tests were performed on fermentation broth containing 18% total solids. For the first test, broth was diluted to 20% of its original concentration with water and fed to the filtration system at a 15—20 psig differential pressure across the membrane (membrane pressure). This feed easily filtered and the retentate was concentrated back to the original feed solids level. The system was back pulsed every 15 seconds to clear the filter.

Undiluted fermentation broth was then fed to the unit at an unknown feed rate. The filter plugged as the membrane pressure exceeded 35 psig. The system was then flushed with water and flux across the filter was measured at 1300 L/m²-h.

To avoid plugging the filter again, membrane pressure was controlled and gradually increased by the operators in the third test. Feed rate was 35-40 L/min, roughly 20 times the flow rate from fermentation. The system operated smoothly for 2.5 hours with a membrane pressure of 20—25 psig and a flux of 100—150 L/m²-h, producing clear permeate. The permeate rate increased from 18 to 39 L/h during the test, possibly due to the increase in temperature of the recirculated "feed" (retentate and permeate mixed in the feed tank) from 68°F to 87°F. After an extensive CIP process with various agents including bleach, caustic, detergent, and nitric acid, the membrane flux on water was only 300 L/m²-h, severely reduced from the original 1700 L/m²-h. Several mechanical problems slowed down testing including a failed pump seal.

If the system were incorporated into the PDU fermentation train at its feed rate of 100 L/h, the permeate (or recycle) rate would be 1-2 L/h, too small to have a significant effect on ethanol concentration. However, the results obtained are more promising than anticipated and suggest further testing could be conducted with a larger pore size (0.5 or 1 micron) or different type filter.

4.3.6 Kinetic Modelling and Predictions

The kinetic model has been upgraded to include terms describing the effects of organic acids (lactic and acetic) on xylose utilization, HMF on glucose utilization, and furfural on cell mass production in a continuous train. Details of the work are in Appendix F. Data to determine these terms were generated from shake flask studies performed on pretreated corn fiber. HMF and furfural disappear early in the fermentation, so HMF and furfural disappearance expressions were added to the model. Acetic and lactic acid inhibit xylose utilization, so an organic acid inhibition term was added to the xylose utilization expression. An HMF inhibition term was added to the glucose utilization expression to account for a reduced rate in the presence of HMF and furfural. The kinetic parameters were modified to fit shake flask and Task 3 experimental data. Because the continuous model still overpredicted xylose utilization, a cell mass reduction term was added to the first fermenter in the continuous model. This term was found to be a function of furfural concentration entering the first fermenter.

Figures 13 and 14 show the measured and predicted concentrations of ethanol, xylose, and cellulose in each fermenter during the first and second mass balance points, respectively. Oligomeric glucose and xylose were converted to ethanol during the second point. These amounts were entered into the model as additional monomeric sugars, because conversion of oligomeric sugars has not been modeled. The measured xylose concentrations in the first and second 9000-L fermenters is lower than predicted in both cases. The discrepancy was also seen in the chemostat and may be caused by the extra utilization of xylose during and after glucose utilization. The cellulose concentration in the third 9000-L fermenter is lower than predicted in both cases. However, changes in pretreatment conditions since the constants were first determined, different mixing properties, or running in continuous mode, could increase cellulose conversion. If the predicted cellulose conversion were closer to the measured conversion, ethanol concentration would also be closer to the measured value.

The predicted ethanol concentration in third fermenter is 5.5% lower than the measured concentration for both mass balance points. The 5.5% error is within the 20% error specification.

4.3.7 Particle Size Analysis of Fermentation Broth Samples

Particle size analysis of fermentation broth samples were performed using a Coulter Model LS130 Particle Size Analyzer. Volume and number of particles distributions in each of the three 9000-L fermenters are shown in Figure 15. Each line represents an average of three different samples. On a volume or mass basis, most of the particles are greater than 10 μm with an average near 40 μm . There is little difference in the distribution except for a slight shift to smaller particle sizes with increasing residence time (down the fermentation train). The number plot shows that most of the mass is occupied by a few larger particles and also shows a shift to smaller particles sizes with increasing residence times.

4.3.8 Purdue Mass Spectrometer System For On-Line Analysis of Fermentation Products

Membrane Induction Mass Spectrometry (MIMS) was used for on-line analysis of fermentation products (primarily ethanol, but some work was accomplished identifying acetic and lactic acid and furfural). The work employed a Finnigan ITS-40 ion trap instrument, adopted to MIMS experiments by addition of an external membrane/jet separator interface. The broth from the first 9000-L fermenter was continuously circulated through a tangential stainless-steel filter that supplied a clean sample for the mass spectrometer. No significant plugging of the filter occurred during the approximate 40 hours of operation with a 15% solids, fermentation broth.

The filtrate was sampled using a flow injection system that allowed quantification using an external standard. Calibration experiments established that the system displayed a linear response to ethanol concentration from 1%—10% by volume. Subsequent experiments alternated injections of ethanol standard and the sample stream, using standard solutions to quantitate the response of the sample stream and reduce errors associated with long-term instrument drift. Measured ethanol concentrations were about 3% and were in agreement with off-line HPLC data.

5.0 Overall Mass Balances and Product Yields

Overall mass balances for the entire plant were calculated at the two points identified in Figure 6. One point was at 25% solids concentration in SSCF and the other was at 15%. A spreadsheet was developed that takes compositional information and flow rates and calculates all the major component flows for the entire plant (pretreatment through distillation and centrifugation). All relevant yields can then be calculated from this

information. The average feedstock composition shown in Table 2 was used.

The following samples were collected at each mass balance point. One fermentation sample from the third fermenter was collected at the time identified on the figure for complete compositional analysis. Three APR samples were collected from approximately 2—6 days before this time point. Each of these samples was completely analyzed and the average was assumed to be the composition of pretreated material entering the process during this period. Fermentation broth was then collected in the beer well over a period of 1—2 days and fed to distillation and finally collected in the fourth fermenter and subjected to the kill procedure. This material, collected during the period that the fermentation sample taken from, was then sampled for complete compositional analysis and identified as centrifuge feed. Since centrifugation was operated as a batch process, the following information was collected: initial feed weight, final cake weight, centrate weight by difference, and feed, centrate, and cake total and insoluble solids. Three cake and centrate samples were collected during the centrifugation process and combined to produce an average sample.

The printouts giving complete flow and conversion information are shown in Appendix H. A summary of yield information is given in Table 9. The pretreatment information shows that the pretreatment severity was probably greater during the first mass balance point as shown by the higher degradation product (HMF and furfural) yields and greater monomeric xylose production. However, the second point shows greater conversion of xylan to soluble xylose. The yields may be influenced by averaging of the pretreatment samples and the assumption of constant feedstock composition. The high pretreatment severity at the beginning of the run was noted earlier.

Approximately 40% of the cellulose was hydrolyzed in the fermenters, which was expected at the low enzyme loadings (5 IU/g cellulose) used in this run. Even though pretreatment severity was lower for the second point, the enzymatic digestibility of the cellulose is about the same. But, it would be advantageous to increase the pretreatment severity to increase the fraction of monomeric xylose as long as yields do not suffer because of increased inhibitor levels.

Cell mass measurements have shown that a relatively small amount of glucose (3%—4%) goes to cell mass in continuous operation. Batch fermentations on corn fiber hydrolyzate measured 5%—10% glucose going to cell mass or with pure sugars, sometimes as high as 15%. Only glucose is assumed to produce cells.

Xylose to ethanol and xylitol were significantly influenced by solids concentrations as expected from chemostat and shake flask results. Although 80% of the monomeric xylose was converted at the lower solids concentration (15%), the ethanol yield was only 54% because of significant xylitol formation. However, this data is suspect as will be discussed below.

C6 to ethanol yields were 67% and 78% and after accounting for glycerol and cell mass, the unconverted glucose was 22% and 8%, respectively for the first and second mass balance points. The unconverted C6 is primarily oligomeric glucose that has been observed in previous work and as reported in section 4.3.1.2. Examination of the material flows reveals that as expected the same amount of oligomeric glucose is unconverted at both solids concentrations. Thus, the large difference in unconverted glucose does not make sense. Examination of material flows also unexpectedly shows conversion of oligomeric xylose at the lower solids level. C6 to ethanol yield is calculated after subtracting ethanol produced by monomeric xylose. If oligomeric xylose is being converted to monomeric form and subsequently converted to ethanol, then the glucose to ethanol yield is too high because additional ethanol is produced from oligomeric xylose. Recalculating the C6 to ethanol yield accounting for loss of oligomeric xylose gives 65.5% for the second mass balance point and brings unconverted glucose to the same level for both solids concentrations. Additionally, the recalculated total soluble xylose to xylitol yield for the second point is now only 13% and

total soluble xylose to ethanol is 56%. By-product yields are still higher at the lower solids level but not at the magnitude first suspected. It is not known why 60% to the oligomeric xylose was apparently converted to monomeric form. The lower xylose concentration at the second point (3 g/L compared to 22 g/L) may have removed an inhibition to xylanase activity or because of the high xylose conversion an equilibrium forces more oligomers to monomers. Xylanase is known to be present in cellulase preparations. This is important and should be further investigated.

Table 9. Conversion (%) and Yield (%) Information for the Two Mass Balance Points

	First Point (6/6 10:00, run time 480 hours) 25% Solids Concentration Ethanol Conc. 37.4 g/L	Second Point (6/21 15:00, run time 845 hours) 15% Solids Concentration Ethanol Conc. 29.6 g/L
Pretreatment		
Fraction Cellulose Hydrolyzed	16.7	4.4
Starch to Total Soluble Glucose	99.1	99.8
Acetate to Acetic Acid	63.7	19.7
Xylan to Total Soluble Xylose	85.0	95.7
Xylan to Monomeric Xylose	67.2	47.9
Arabinan to Total Soluble Arabinose	76.1	88.2
Glucose to HMF	0.6	0.2
Xylose to Furfural	2.8	1.8
Fermentation		
Fraction Cellulose Hydrolyzed	44.3	41.3
Total Soluble C6 ¹ to Cell Mass	2.9	4.1
Total Soluble Glucose to Glycerol	7.5	9.9
Monomeric Xylose to Xylitol	7.5	26.3
Monomeric Xylose to Ethanol	26.2	53.2
Total Soluble C6 to Ethanol	67.1	79.5
Total Process Yield	46.9	55.1
Total Metabolic Yield	84.6	82.2

¹ C6 is glucose and galactose

Metabolic yields based on conversion of glucose, galactose, and xylose show that about 80%—85% of the sugars consumed are converted to ethanol. Process yields show that 47% and 55% of potential sugars (glucose, galactose, and xylose) are converted to ethanol. The form and percentage of potentially fermentable sugars (to ethanol) entering and leaving SSCF and conversion are shown in Table 10. If the amount entering fermentation is low (e.g., starch), the conversions are suspect due to experimental error and can be ignored. Cellulose, and oligomeric and monomeric glucose and xylose are the main sugars entering fermentation. The only sugar completely converted was monomeric glucose. Clearly cellulose, oligomeric glucose, and oligomeric and monomeric xylose are the important sugars left at the end of the fermentation. Cellulose may not be economically recoverable because of enzyme cost. But, converting additional oligomeric glucose and

both forms of xylose may be necessary to improve process economics.

Table 10. Form and Percentage of Sugars Entering, Leaving and Converted During SSCF

	First Point		% Converted	Second Point		% Converted
	% of Total			% of Total		
	In	Out	In	Out		
Starch	0.4	1.1	-14.3	0.5	1.6	0.0
Cellulose	16.4	20.6	44.2	17.5	31.7	41.9
Galactan	0.4	0.7	28.6	0.1	0.5	-200.0
Xylan	1.6	1.2	66.7	1.3	2.3	45.8
Oligomeric Glucose	13.4	24.6	18.4	22.7	29.7	58.0
Monomeric Glucose	38.3	0.8	99.1	26.6	0.7	99.2
Oligomeric Galactose	0.2	2.2	-300.0	2.0	4.5	27.8
Monomeric Galactose	3.5	6.1	22.4	2.5	3.6	53.3
Oligomeric Xylose	5.4	12.4	-2.2	13.4	17.0	59.5
Monomeric Xylose	20.3	30.4	33.6	13.4	8.5	79.7

Component and overall carbon closure information are shown in Table 11. The SSCF and overall carbon balance information were generated from the carbon balance spreadsheets used in previous task reports and also shown in Appendix F. The balance was only around SSCF and used the average pretreated material composition as the input to SSCF. Component closures on pretreated material are good except for galactan, which is expected to be less accurate because it is only a small fraction of the feedstock. The SSCF product yields do not approach 100 because of the poor carbon dioxide number, which is not near the value expected from stoichiometry (approximately 40). If this correction is made, the values are much nearer 100 (103 and 94 for the first and second points, respectively). The problem with determining carbon dioxide probably resides with the flow rate measurement, because of difficulty in measuring a highly oscillating flow, in addition to low flow rates. The overall carbon closure is also low because of carbon dioxide, but is also near 100% after correction to the stoichiometric value. Lignin balance for both points was also closed to within 15%.

6.0 Review of Run Specifications

The following is the list of criteria for success defined in the Task 5 run specification, and a short discussion of how each of these criteria were met.

1. Operate the APR and SSCF train at steady state and maintain ethanol concentration in the first tank at or above 45 g/L using the LNHST2 yeast. Utilize antibiotics or changes in process conditions to control contamination.

The APR and fermentation train were operated for a period of six weeks, in which periods of steady state were obtained following major changes in process conditions (i.e., solids concentration) and elimination of contamination using antibiotics. Maximum ethanol concentrations of 42--43 g/L were

achieved at a 25% solids concentration and 29—30 g/L at 15% solids concentration. Ethanol recycle was not used at either condition to maintain ethanol concentrations at or above 45 g/L.

Table 11. Component Mass Closure and Product Yield Information for the Two Mass Balance Points

	First Point	Second Point
Pretreatment Mass Closure (%)		
Glucan	99.9	105.9
Galactan	68.9	80.4
Xylan	95.1	104.0
Arabinan	84.3	93.4
SSCF Product Yields (g/100g C6+C5 consumed)		
Ethanol	42.8	42.3
Carbon Dioxide	15.5	13.2
Cell Mass	1.6	1.9
Glycerol	4.3	4.6
Acetic Acid	0.1	4.9
Lactic Acid	1.3	3.7
Xylitol	2.7	5.3
Total	68.2	75.7
Overall SSCF Carbon Closure (%)	89.4	87.3

- Run the APR at 120 lb/h and obtain 85% conversion of the available xylan in the feed with no more than 25 mg/g dry solids acetic acid and 3 mg/g dry solids furfural plus HMF in the pretreated feed.
85% of the xylan was converted to soluble xylose. Acetic acid concentrations were always below 25 mg/g TS, but combined furfural and HMF concentrations were greater than 3 mg/g TS except for the period from 80—250 hours and the period after 800 hours to the end of the run.
- Run three stages of SSCF at a total solids concentration of at least 25%.
Three stages of SSCF were operated at a solids concentration of 25% and 15%.
- Yeast growth in the first fermenter should be sufficient to provide yeast to the SSCF train without the use of continuous inoculation.
Yeast growth was sufficient in the first fermenter to provide yeast to the SSCF train once past the period of high pretreatment severity noted at the beginning of the run.
- Use ammonia or ammonia hydroxide to control pH in SSCF.
This was not done because bench scale testing during Task 4 proved that there was no advantage to using ammonia hydroxide over sodium hydroxide.
- Close carbon balances and component balances around pretreatment and SSCF to 100% within the 95% confidence limits. Use process off gas measurements to close the balances.

95% confidence limits were not calculated with Task 5 data as a new **mass** balance presentation **was** used. **However**, Task 4 results have shown that the data is within 100% at the **95%** confidence limits and the same results would be expected for Task 5 results. There **is** still the problem with the **obviously** low value measured for carbon dioxide.

7. Compare the performance of the kinetics model with Task 5 data and see if the ethanol production rate **is** within 20% of the predicted value.

New **terms** were added to the kinetic model to account for inhibition by organic acids, HMF, and furfural. The model predicted ethanol concentration in the third 9000-L fermenter to within **5.5%**.

7. Collect fermentation solids and wash so that sulfate levels in the wash water are no higher than 0.3 **wt. %**. Save solids for future testing **as** animal feed.

A total of 12.5 tons of *dry* solids was collected by the end of **Task 5**. The solids were not washed and sulfate levels in the cake were 0.5% —0.9%.

9. Operate the **PDU** with **3** operators during the day and **only 2** operators during evening and night shifts. The **PDU** was normally operated this run with **3** operators/shift. The extra coverage **was** provided to cover operation of the ultrafiltration unit and ethanol recycle system, and install the **APR** direct steam injection system. However, the ethanol recycle system was not used and the ultrafiltration unit **was** only tested for **a** few days.

7.0 Problems and Recommendations

Below is **a** list of some of significant technological problems encountered during this run and possible recommendations for improving understanding and economics of the process,

1) Critical to understanding fermentation performance is identifying and controlling pretreatment conditions. It has not been possible to achieve known and constant conditions in the APR.

The changing pH treatment severity has

made it difficult to compare bench fermentation data to PDU data. Effort is needed to achieve better control so that **performance** can be predicted and repeatable conditions can be routinely achieved.

2) Bench fermentation work has provided some of the necessary data needed to operate the **PDU**. However, lacking is arigorous investigation of the pertinent variables (e.g., nutrients, ethanol and other inhibitors, etc.) that may have a significant effect on fermentation performance.

3) Significant work **has** been accomplished building a **kinetic** model to predict fermentation performance. But **as** shown by the recent addition of terms to the model for effects of acids, HMF, and furfural, there may be **unknown** factors that are not included in the model. Additionally, there **is** a significant difference between batch and continuous performance that is not predicted by the model. Work **is** needed to improve and experimentally verify the predictive capability of the kinetic model.

fermentable form. It is also important to convert more of the **xylose** (oligomeric and monomeric) to ethanol. The conversion of oligomeric xylose during the latter half of the **Task 5** run is promising and should be

investigated **as** a way of converting additional xylose to ethanol. Higher yields may also be achieved by removing the inhibitors, particularly acetic acid.

5) A commercial plant will need to pump pretreated material to the fermenters. It is necessary to identify a suitable pump for this service.

8.0 Summary

LNHST2 was initially grown in the seed fermentation **train using** the 20-L, 160-L, and 1450-L fermenters and used to inoculate the first 9000-L fermenter. The cells did not grow or produce ethanol in the first fermenter because of the **high** pretreatment severity during the first week of the **run** that produced high inhibitor levels. Since the cells did not grow, they were washed out of the first fermenter and the first fermenter was reinoculated four days later. By this time, growth and ethanol production was occurring in the second and third fermenters. This lag in performance is due to high furfural levels. Bench scale work has shown that furfural must be reduced to low levels (< 0.1 g/L) before fermentation can begin. It is not known **if** furfural is being metabolized by the cells or chemically reacting.

The APR and fermentation equipment were successfully operated for the duration of the run (approximately **six** weeks). The APR was shut down numerous times.

These shutdowns were typically **less than** one hour and probably had no significant effect on steady state in the fermenters. It is still difficult to maintain constant pretreatment conditions in the APR. The most sensitive indicators of pretreatment severity was the inhibitor concentrations, which had a significant drop from the beginning **to** the end of the run. Monomeric xylose also showed a small but steady decline throughout the run. The changing pretreatment severity makes it difficult to compare PDU performance with bench fermentation data.

As in previous runs, contamination was present throughout the run. The quick use of Lactrol avoided high by-product (i.e., acetic and lactic acid) concentrations that could have severely disrupted the fermentation. However, the elevated levels of the acids **was** enough to inhibit xylose fermentation. The primary contaminant **was** *lactobacillus plantarum*, which **was** found in pretreated material. But it **is** not clear if this organism is surviving pretreatment or is infecting the feed downstream of the APR discharge. This **was** not the organism found in the **CSL** transfer line.

The process appeared to approach steady state twice during the run. **This** first occurred **at** approximately 600 hours at a 25% solids level in fermentation. All of the available glucose was consumed and about half of the xylose was consumed, although not all of it was converted to ethanol. The second point was near the end of the run (runtime 845 hours) when fermentation was at a 15% solids level. Approximately 80% of the xylose **was** consumed because of the lower inhibitor levels. Even at the second point, only 55% of the total available sugars were being converted to ethanol. Unconverted sugars are in the form of cellulose, oligomeric glucose, and monomeric and oligomeric xylose. Cellulose conversion depends on pretreatment severity and enzyme loading, and because of enzyme cost, cellulose may not be economically recoverable. But conversion of the remaining glucose and xylose to ethanol is necessary to improve process economics.

9.0 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, Kevin Enomoto (electrical problems) Rick Houston (SWAN), Kelly Ibsen,

Ed Jennings, Tim Johnston, John Lesko (SWAN), Bob Lyons, Sam McWilliams (SWAN), Tim Plummer, Dana Rice, Cindy Riley, Mark Ruth, Dan Schell, Larry Schwartz (SWAN), and Ian Thompson (DACS problems). Analytical support was provided by Larry Brown, Tina Ehrman, Fannie Posey Eddy, Jim Hora, Netta Ingle, Janet Pride, Ray Ruiz, and David Templeton. Raphael Nieves and Bill Adney provided the information on oligomeric sugars in fermentation broth and Tina Ehrman performed the disaccharide analysis. Raphael Nieves, Ed Jennings, and Cindy Riley performed the viscosity and density measurements. Tina Ehrman did the particle size analysis on the fermentation broth samples. Christos Hatzis provided the carbon balance spreadsheets that were subsequently modified for use with PDU data. This report was put together with written contributions from Nancy Combs, Kelly Ibsen, Ed Jennings, Rudy Johnson (Purdue University), Mark Ruth, Dan Schell, Larry Schwartz (SWAN), and Susan Toon.

Figure 1. PDU Task 5 Run History

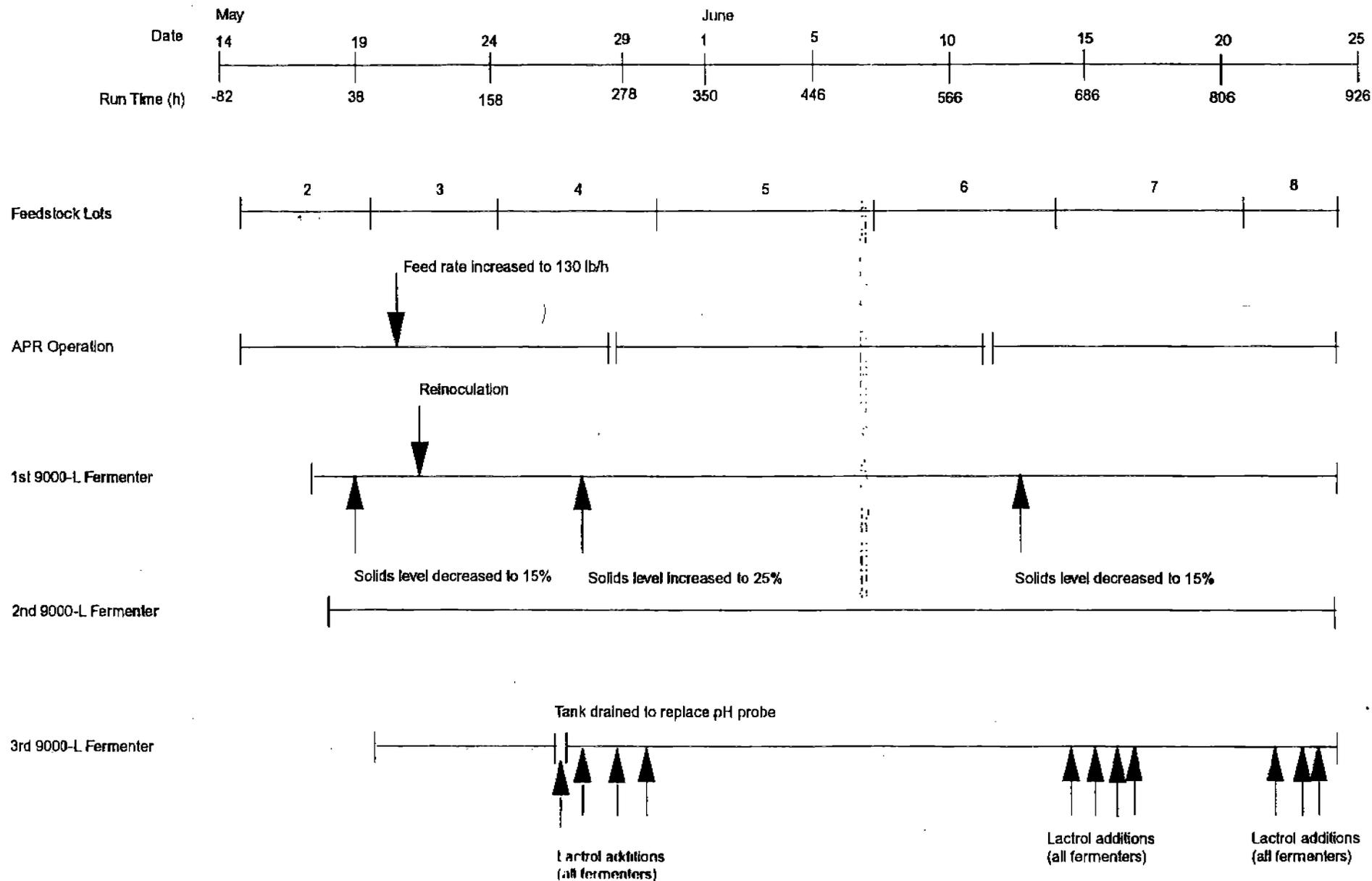
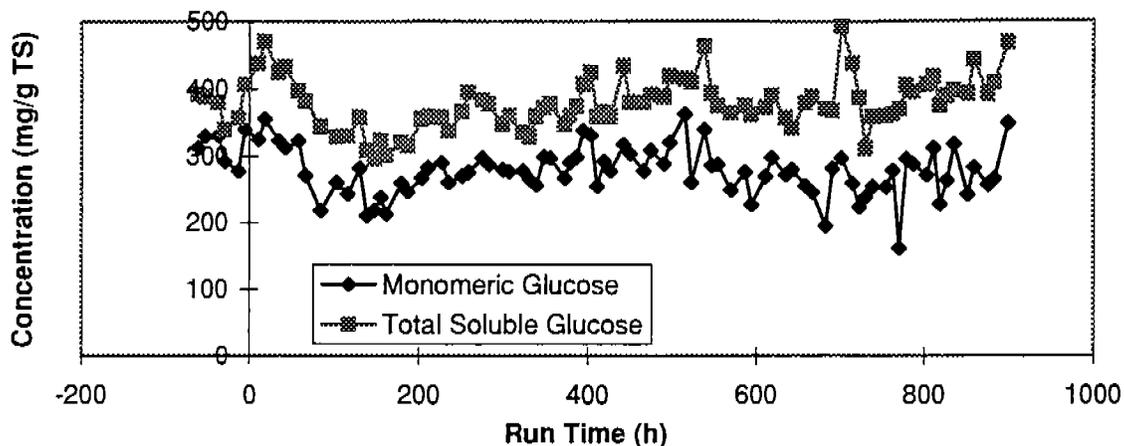
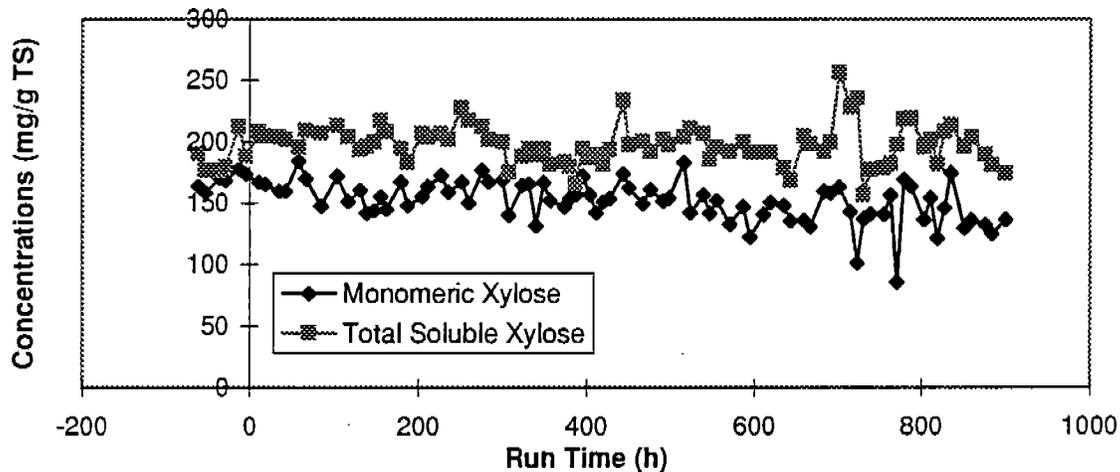


Figure 3. Component Concentrations in APR Samples

Glucose Concentrations in APR Samples



Xylose Concentrations in APR Samples



Acetic Acid, Furfural, and HMF in APR Samples

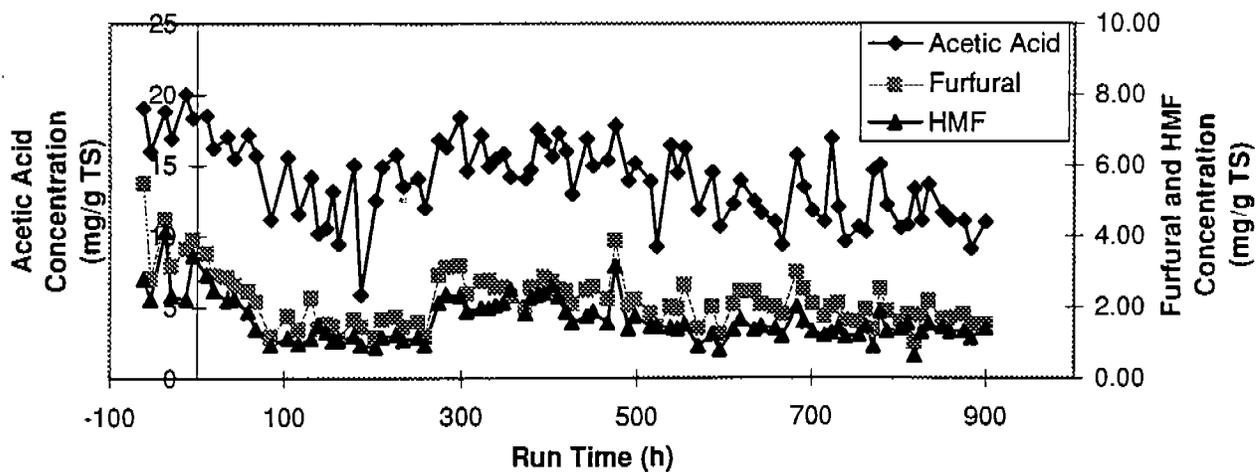


Figure 4. Glucose Concentrations in APR Samples and Feedstock Usage

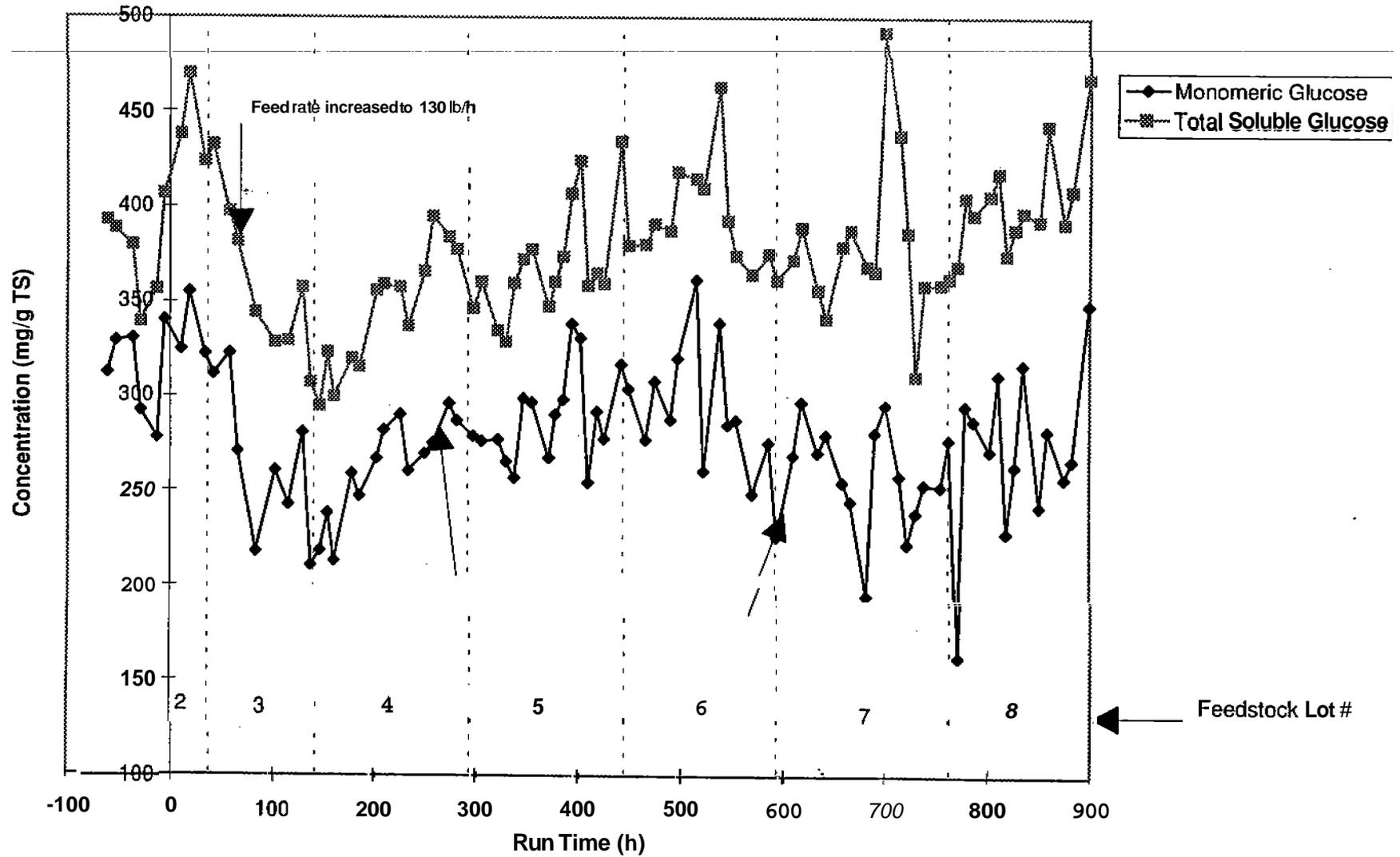


Figure 5. Ratio of Monomeric to Total Soluble Sugar for Glucose and Xylose

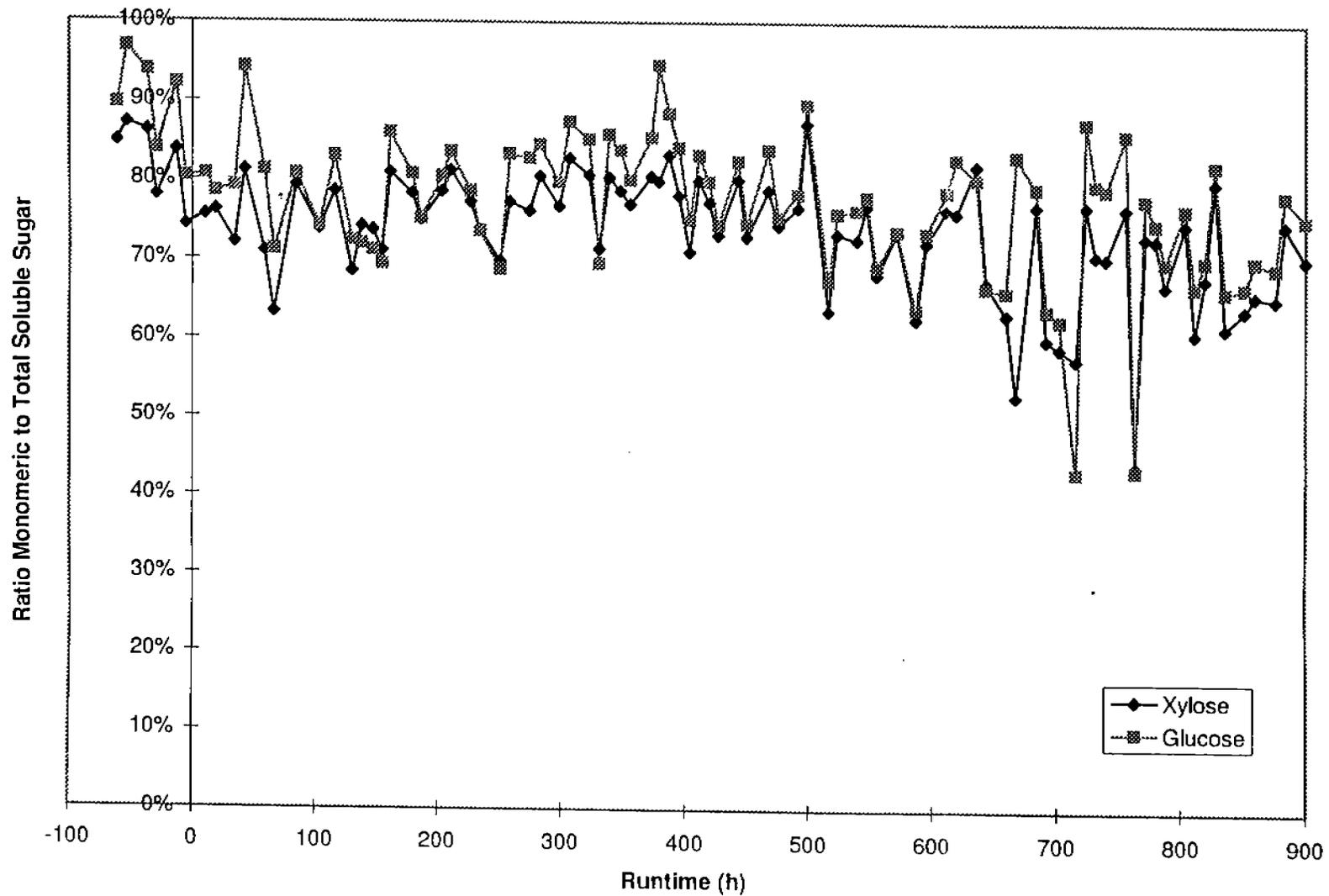


Figure 6. Total Soluble Glucose, Xylose, and Acetic Acid Yields

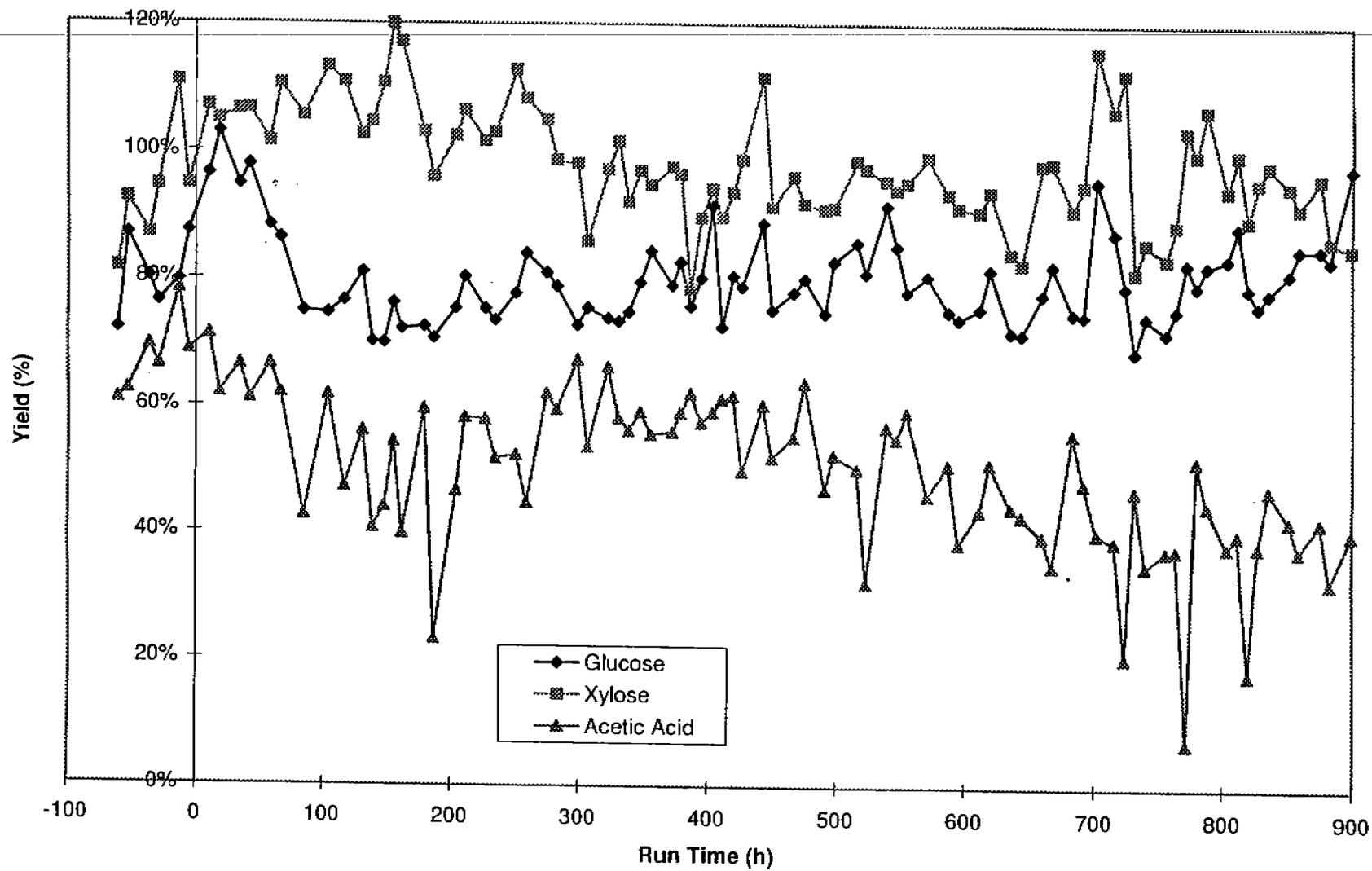
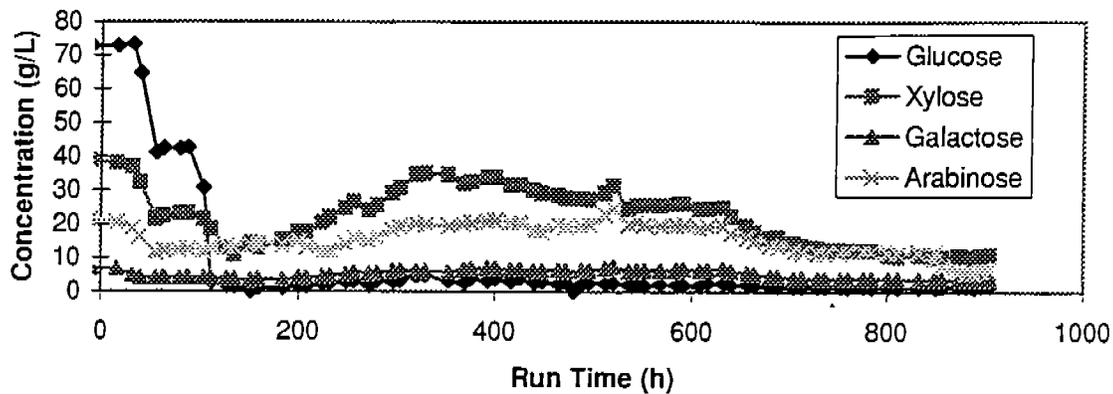
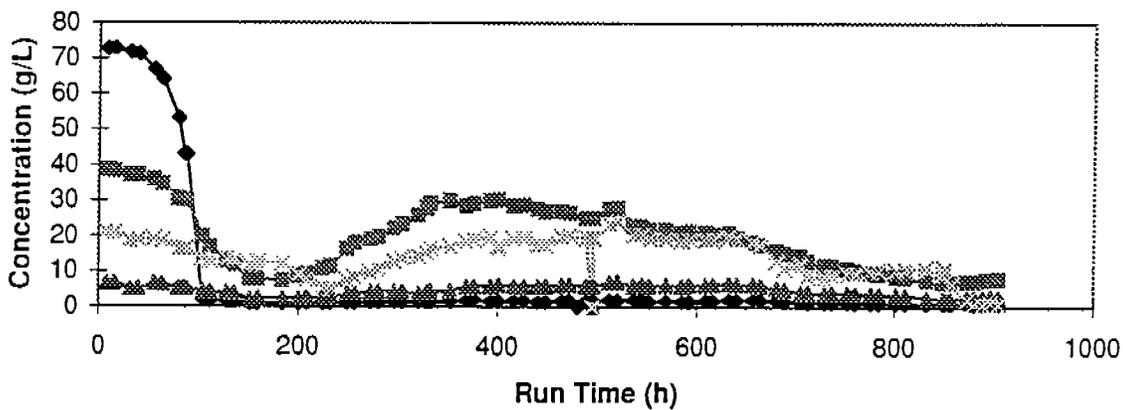


Figure 7. Monomeric Sugar Concentrations in the 9000-L Fermenters

First 9000-L Fermenter



Second 9000-L Fermenter



Third 9000-L Fermenter

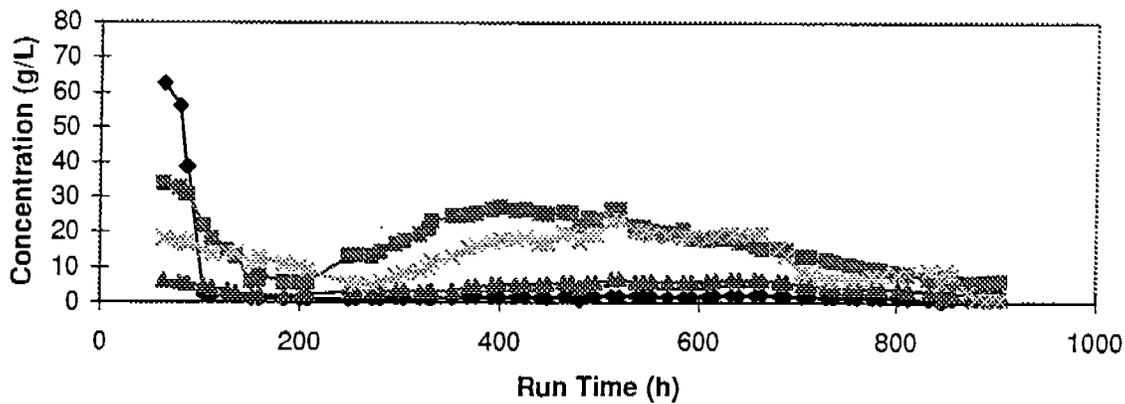
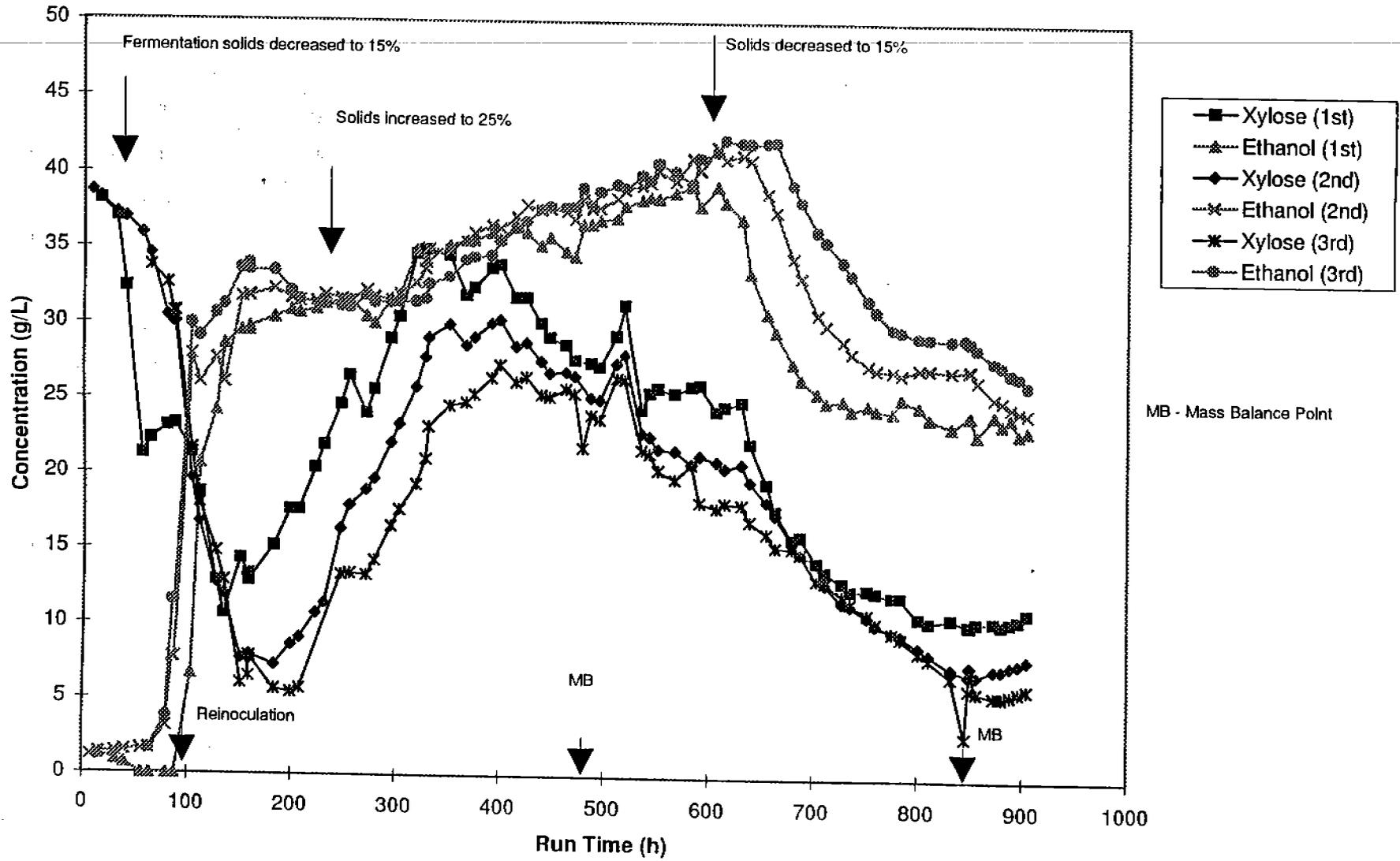


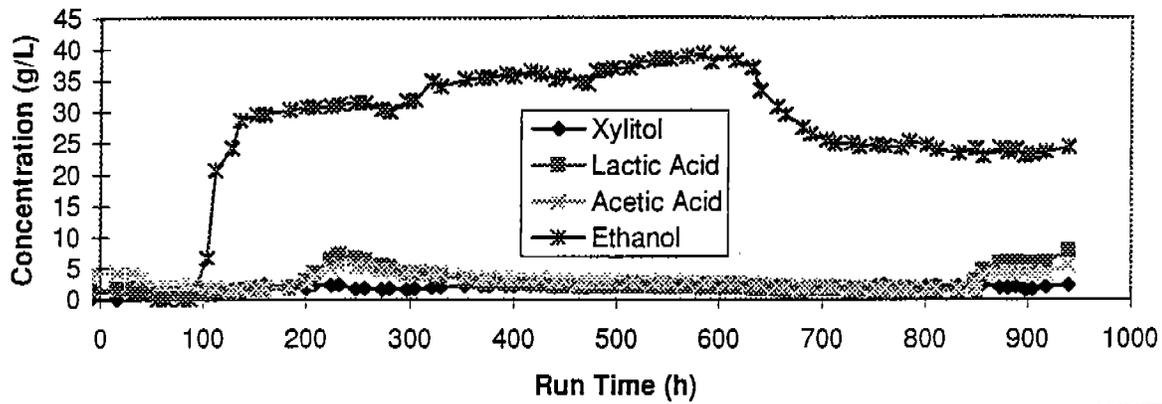
Figure 8. Ethanol and Xylose in the 9000-L Fermenters



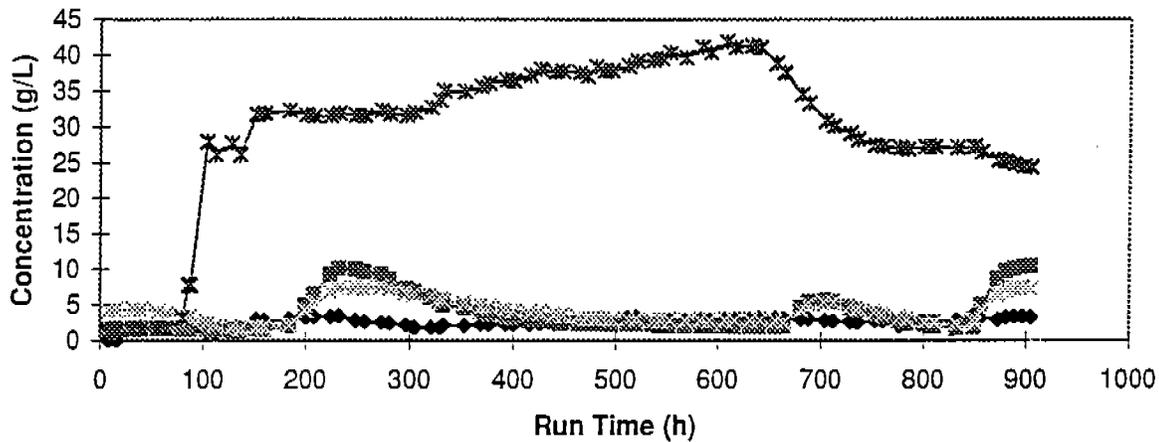
MB - Mass Balance Point

Figure 9. Product Concentrations in the 9000-L Fermenters

First 9000-L Fermenter



Second 9000-L Fermenter



Third 9000-L Fermenter

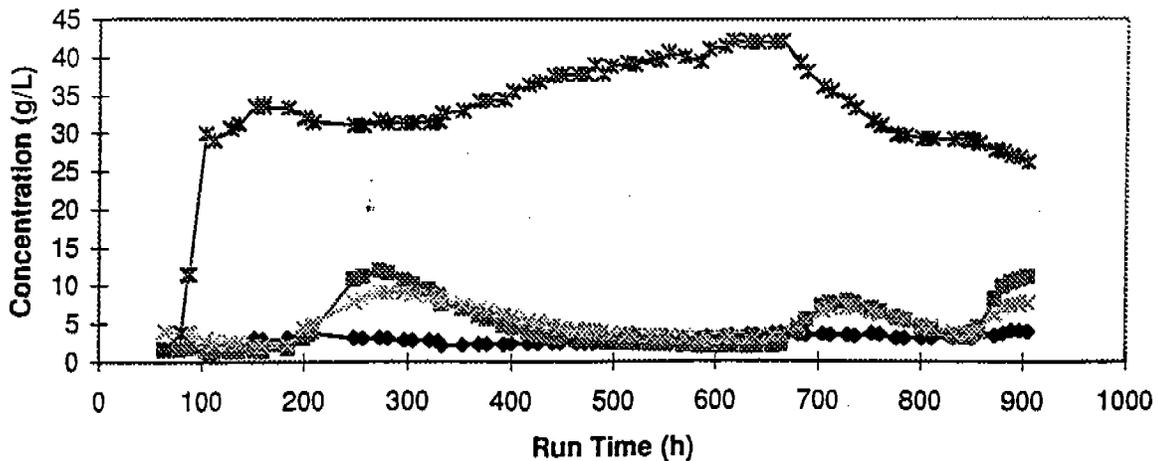


Figure 12. Sugar Consumption by LNHST2 on Pretreated Corn Fiber

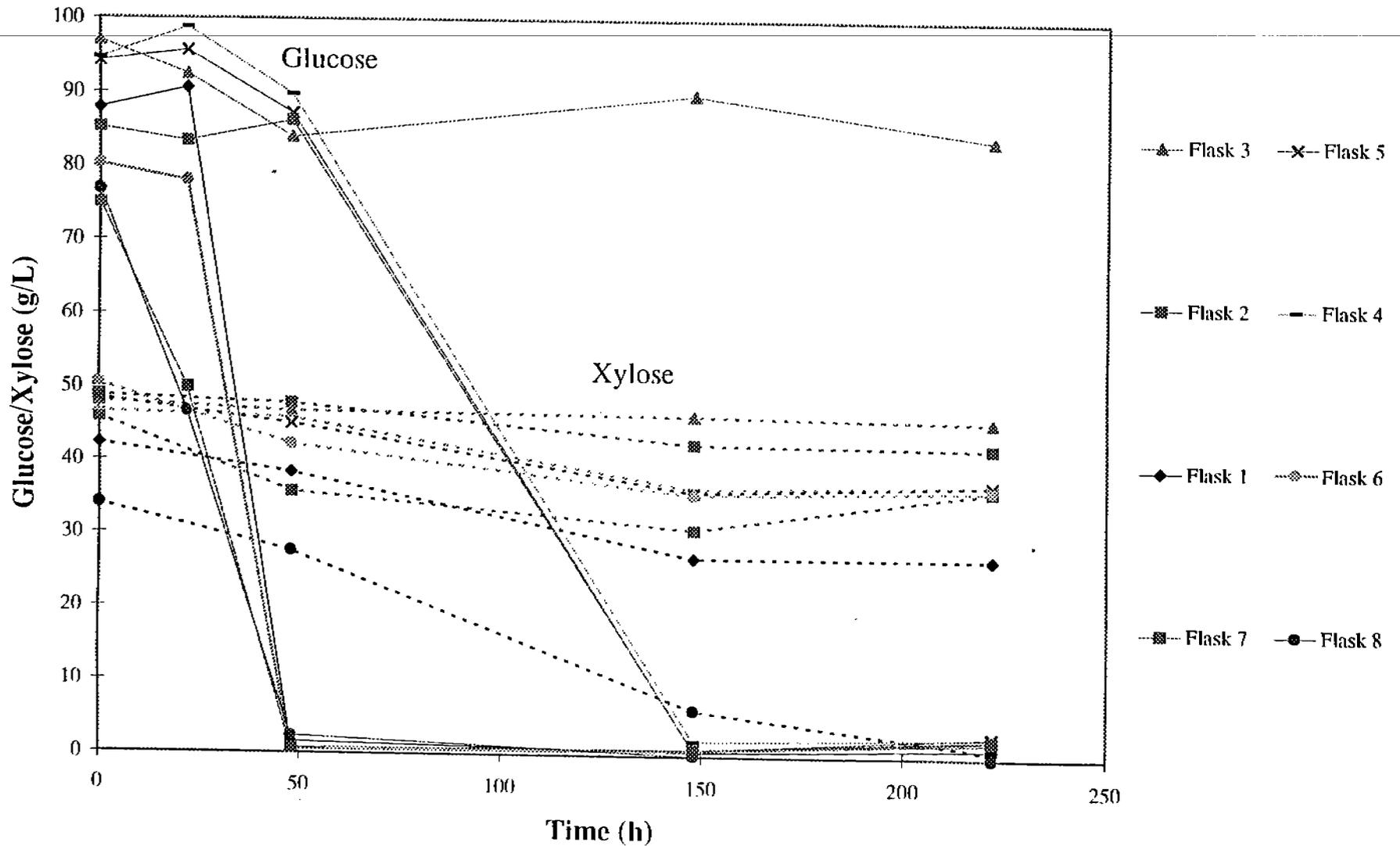


Figure 13. Concentrations at the First Mass Balance Point

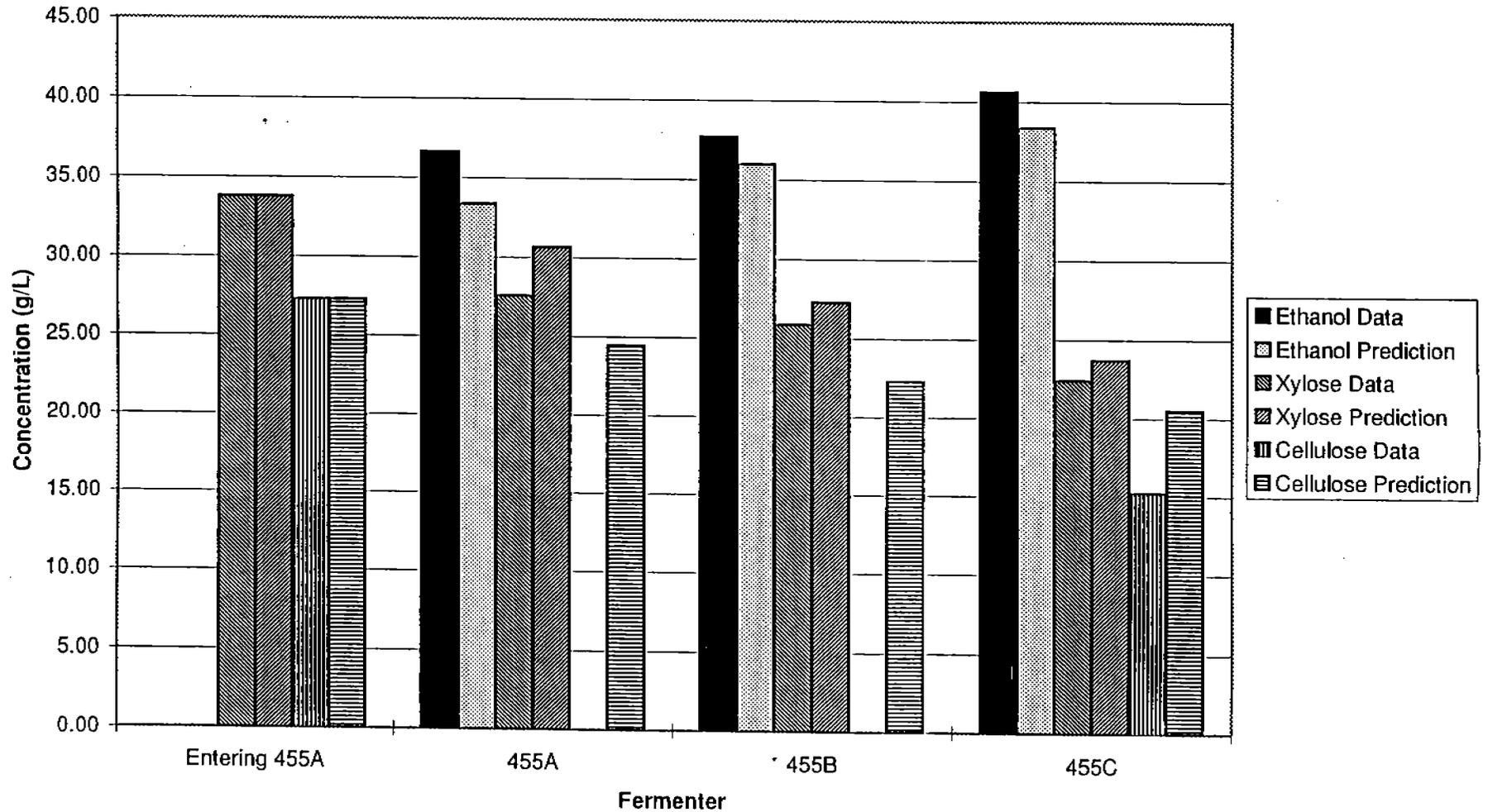


Figure 14. Concentrations at the Second Mass Balance Point

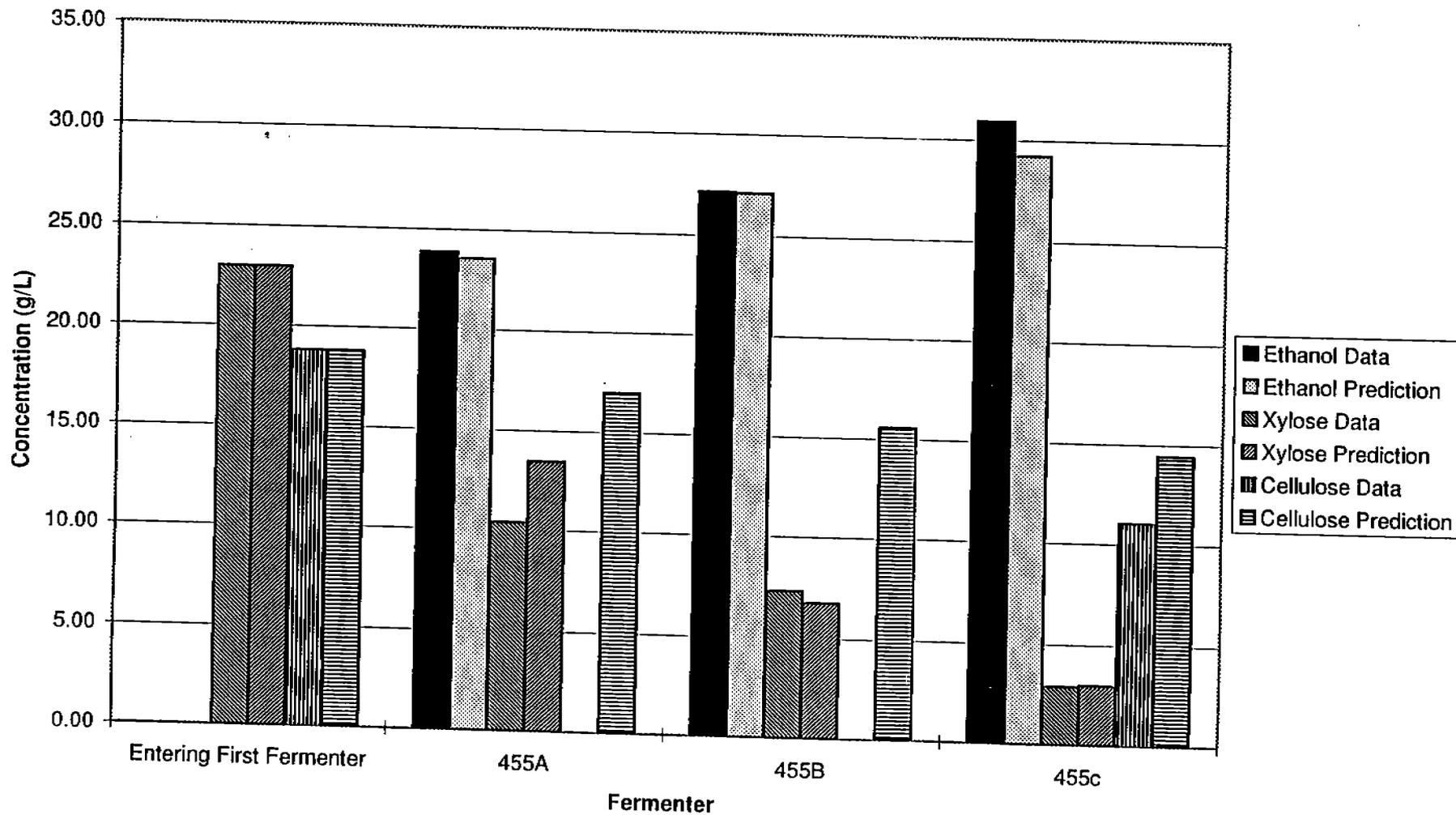
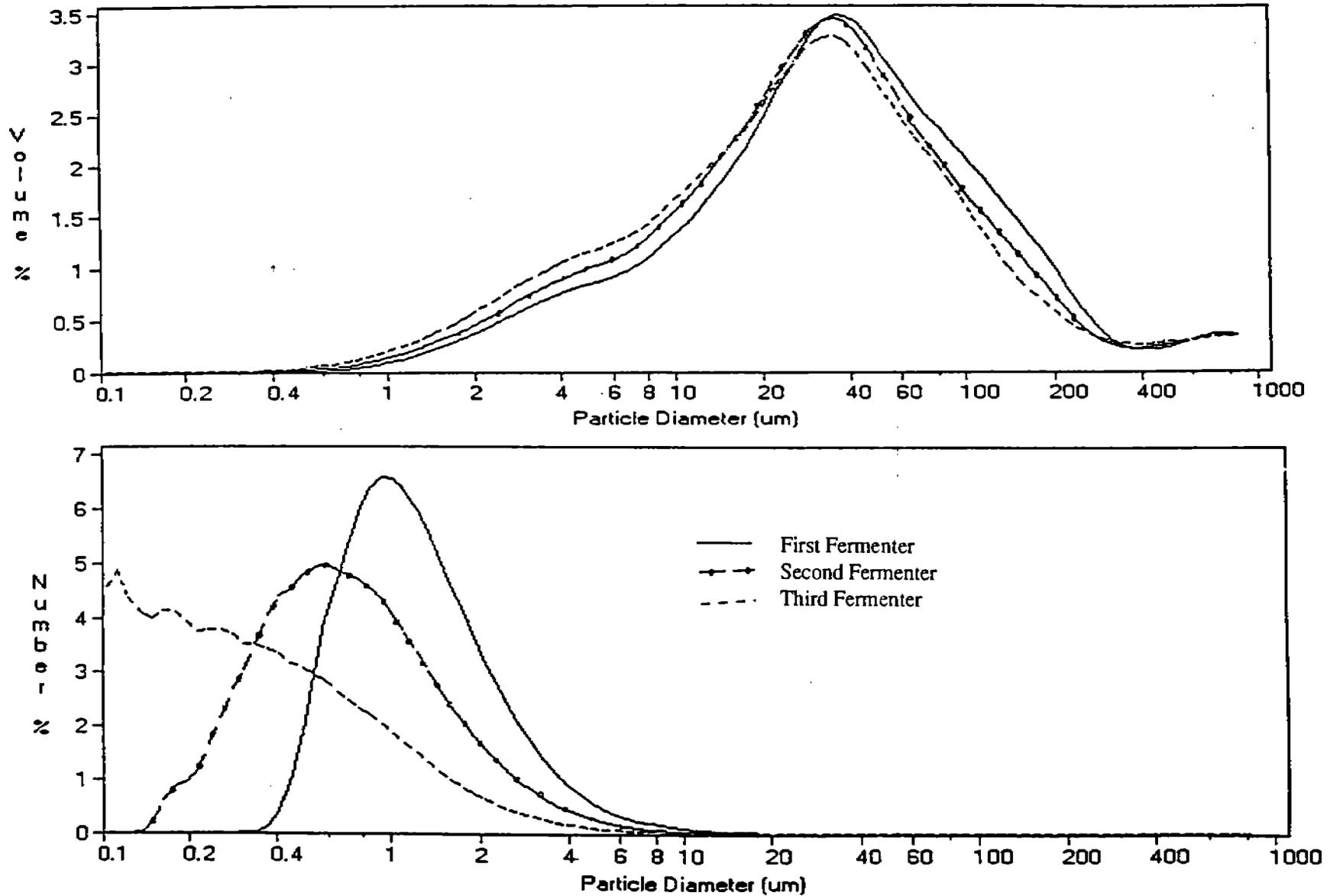


Figure 15. Volume and Number of Particle Size Distributions in Fermentation Broth in the Three 9000-L Fermenters.



Run start date 5/17/96
 Run Name: CRADA Task 5
 Run ID#: P90605CF

APR Data

Date	Time	Run time (h)	Tot. Solids Oven (%)	TDS Liquid (%)	Ins.Solids (%)	Sample Wt. (g)	HPLC (g/L)								
							Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol	Succinic	Lactic
14-May-96	21:00	-61.00	27.74%	0.00%	6.94%	0.00	93.05	48.74	8.19	25.53	0.00	0.00	0.92	0.00	2.08
15-May-96	5:00	-53.00	34.12%	0.00%	8.53%	0.00	122.77	59.08	10.13	31.34	0.00	0.00	1.00	0.00	2.06
15-May-96	21:30	-36.50	32.95%	0.00%	8.24%	0.00	118.61	61.16	9.98	31.57	0.00	13.61	3.10	1.29	1.65
16-May-96	5:00	-29.00	34.33%	0.00%	8.58%	0.00	109.75	63.28	10.69	33.10	0.00	0.00	2.53	1.17	2.15
16-May-96	21:00	-13.00	33.52%	0.00%	8.38%	0.00	101.64	65.04	11.73	34.35	0.00	14.33	2.66	1.34	1.84
17-May-96	5:00	-5.00	33.46%	0.00%	8.37%	0.00	124.27	63.44	10.65	32.37	0.00	15.06	3.10	1.35	1.69
17-May-96	21:00	11.00	33.10%	0.00%	8.27%	0.00	117.19	60.18	10.32	31.85	9.43	0.00	0.00	0.00	2.33
18-May-96	5:00	19.00	33.41%	0.00%	8.35%	0.00	129.42	60.22	10.23	31.36	9.98	0.00	0.00	0.00	1.66
18-May-96	21:00	35.00	34.46%	0.00%	8.62%	0.00	121.62	60.19	8.68	30.51	0.00	0.00	2.94	1.16	1.78
19-May-96	5:00	43.00	33.99%	0.00%	8.50%	0.00	115.82	59.26	8.66	30.31	0.00	0.00	3.26	1.07	0.00
19-May-96	21:00	59.00	33.67%	0.00%	8.42%	0.00	118.58	67.66	12.22	35.88	0.00	0.00	3.34	1.29	1.35
20-May-96	5:00	67.00	33.83%	0.00%	8.46%	0.00	100.03	62.73	11.69	34.73	0.00	0.00	2.83	1.08	1.78
20-May-96	23:00	85.00	34.87%	0.00%	8.72%	0.00	83.16	56.27	11.99	36.90	8.91	0.00	2.47	0.96	1.62
21-May-96	21:00	104.00	35.42%	0.00%	8.86%	0.00	101.19	66.88	12.90	44.60	0.00	0.00	2.40	1.83	2.60
22-May-96	6:45	116.75	36.12%	0.00%	9.03%	0.00	96.34	60.06	12.41	42.25	0.00	0.00	2.10	1.46	1.43
22-May-96	21:00	131.00	37.39%	0.00%	9.35%	0.00	115.83	66.30	13.39	37.83	0.00	0.00	2.87	1.24	2.02
23-May-96	5:00	139.00	35.04%	0.00%	8.76%	0.00	80.83	54.46	11.63	35.61	0.00	0.00	2.25	1.04	2.36
23-May-96	14:00	148.00	38.53%	0.00%	9.63%	0.00	93.12	61.17	12.39	37.94	8.77	0.00	2.70	0.96	1.99
23-May-96	21:30	155.50	36.04%	0.00%	9.01%	0.00	94.26	61.40	12.08	36.70	8.88	0.00	2.65	0.99	1.95
24-May-96	4:00	162.00	36.16%	0.00%	9.04%	0.00	84.69	57.43	11.80	36.53	8.04	0.00	2.21	0.79	1.45
24-May-96	21:30	179.50	35.53%	0.00%	8.88%	0.00	100.95	65.21	11.10	35.39	8.04	0.00	2.73	1.24	3.07
25-May-96	5:00	187.00	35.82%	0.00%	8.96%	0.00	97.20	58.19	10.36	31.77	7.34	0.00	2.42	1.00	2.97
25-May-96	22:00	204.00	33.35%	0.00%	8.34%	0.00	97.10	56.53	11.92	34.73	7.23	0.00	2.35	0.92	3.08
26-May-96	5:00	211.00	33.62%	0.00%	8.40%	0.00	103.57	60.05	11.76	34.52	3.59	0.00	2.46	0.89	2.11
26-May-96	21:00	227.00	33.33%	0.00%	8.33%	0.00	105.58	62.71	12.05	35.18	0.00	0.00	2.93	1.08	3.09
27-May-96	5:00	235.00	33.95%	0.00%	8.49%	0.00	96.59	58.89	11.80	34.38	0.00	0.00	2.68	0.91	2.44
27-May-96	21:00	251.00	33.17%	0.00%	8.29%	0.00	97.52	60.48	12.39	36.37	0.00	0.00	2.76	1.36	3.27
28-May-96	5:00	259.00	33.75%	0.00%	8.44%	0.00	101.51	55.19	11.67	33.84	0.00	0.00	2.40	1.04	2.32
28-May-96	21:00	275.00	31.32%	0.00%	7.83%	0.00	100.65	60.10	11.77	33.97	0.00	0.00	3.63	1.36	3.14
29-May-96	5:00	283.00	31.87%	0.00%	7.97%	0.00	99.41	57.94	11.04	32.33	0.00	0.00	2.87	1.26	2.89
29-May-96	21:00	299.00	32.57%	0.00%	8.14%	0.00	98.87	59.80	11.27	32.96	0.00	0.00	2.64	1.43	3.20
30-May-96	5:00	307.00	35.54%	0.00%	8.88%	0.00	107.77	54.52	10.43	30.48	0.00	0.00	2.68	1.22	2.54
30-May-96	21:00	323.00	33.90%	0.00%	8.47%	0.00	102.63	60.99	9.60	31.09	0.00	0.00	2.66	1.19	3.04
31-May-96	5:00	331.00	33.21%	0.00%	8.30%	0.00	96.11	60.04	9.45	30.95	0.00	0.00	2.68	1.12	3.12
31-May-96	13:00	339.00	31.40%	0.00%	7.85%	39.40	87.43	44.82	7.46	27.29	0.00	1.15	2.64	1.18	2.29
31-May-96	22:00	348.00	32.50%	0.00%	8.12%	0.00	105.74	58.93	8.55	29.45	0.00	0.00	2.95	1.11	2.54
1-Jun-96	6:00	356.00	35.05%	0.00%	8.76%	0.00	113.96	58.39	8.32	28.72	0.00	0.00	2.93	1.02	1.96
1-Jun-96	23:00	373.00	35.19%	0.00%	8.80%	0.00	103.14	56.67	11.17	33.38	0.00	0.00	89.93	0.99	2.29
2-Jun-96	5:00	379.00	35.81%	0.00%	8.95%	0.00	114.19	60.45	10.98	32.45	0.00	0.00	89.93	1.02	2.05

Run start date 5/17/96
 Run Name: CRADA Task 5
 Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)					Liquor Analysis (Total Sugars, g/L)				
			Glycerol	Acetic	EIOH	HMF	furfural	Glucose	Xylose	Galactose	Arabinose	Mannose
14-May-96	21:00	-61.00	0.96	5.67	0.55	0.84	1.64	116.96	56.70	11.68	34.64	0.00
15-May-96	5:00	-53.00	0.76	5.96	0.00	0.83	1.05	144.75	65.94	13.38	39.90	0.00
15-May-96	21:30	-36.50	1.03	6.76	0.00	1.48	1.61	136.18	63.20	13.25	38.52	0.00
16-May-96	5:00	-29.00	0.95	6.34	0.00	0.87	1.19	127.39	67.48	13.90	40.61	0.00
16-May-96	21:00	-13.00	0.78	7.34	0.00	0.82	1.33	130.37	77.62	15.83	46.51	0.00
17-May-96	5:00	-5.00	0.91	6.69	0.00	1.26	1.43	148.41	68.81	13.93	40.97	0.00
17-May-96	21:00	11.00	1.11	6.67	0.00	1.06	1.27	157.87	74.93	13.15	43.95	0.00
18-May-96	5:00	19.00	0.00	5.90	0.00	0.90	1.06	171.29	74.65	12.86	43.04	0.00
18-May-96	21:00	35.00	1.07	6.42	0.00	0.83	1.08	159.77	76.74	13.38	44.92	0.00
19-May-96	5:00	43.00	0.00	5.75	0.00	0.83	0.97	160.58	74.86	12.83	43.58	0.00
19-May-96	21:00	59.00	0.00	6.30	0.00	0.69	0.90	146.02	71.78	14.92	43.01	0.00
20-May-96	5:00	67.00	0.00	5.81	0.00	0.51	0.79	140.92	77.24	16.13	47.07	0.00
20-May-96	23:00	85.00	0.00	4.28	0.00	0.36	0.45	131.39	79.13	15.91	48.47	11.78
21-May-96	21:00	104.00	1.26	6.04	0.00	0.44	0.68	127.57	82.86	16.61	47.99	13.60
22-May-96	6:45	116.75	0.84	4.60	0.00	0.38	0.54	130.62	80.99	16.26	47.33	13.68
22-May-96	21:00	131.00	0.98	5.84	0.00	0.46	0.94	147.37	79.90	15.99	45.76	0.00
23-May-96	5:00	139.00	1.63	3.92	0.08	0.58	0.53	118.03	75.28	15.28	44.67	0.00
23-May-96	14:00	148.00	0.99	4.53	0.00	0.56	0.65	125.63	85.11	16.19	47.19	12.06
23-May-96	21:30	155.50	1.03	5.23	0.00	0.41	0.59	127.99	86.28	16.29	47.57	12.50
24-May-96	4:00	162.00	0.00	3.76	0.00	0.42	0.45	119.11	82.74	15.76	46.40	11.77
24-May-96	21:30	179.50	1.00	5.86	0.00	0.47	0.65	124.75	75.88	15.62	44.74	9.49
25-May-96	5:00	187.00	0.68	2.32	0.00	0.37	0.57	124.09	72.07	14.86	42.11	9.12
25-May-96	22:00	204.00	0.00	4.57	0.00	0.32	0.42	129.46	75.24	15.40	43.52	9.36
26-May-96	5:00	211.00	0.00	5.46	0.00	0.43	0.60	131.77	74.63	15.42	43.63	9.81
26-May-96	21:00	227.00	0.00	5.73	0.00	0.45	0.63	130.04	75.08	15.57	43.90	10.26
27-May-96	5:00	235.00	0.00	5.03	0.00	0.40	0.54	125.03	74.94	15.45	44.01	9.93
27-May-96	21:00	251.00	0.00	5.10	0.00	0.42	0.57	132.40	82.27	16.77	47.61	0.00
28-May-96	5:00	259.00	0.00	4.43	0.00	0.35	0.43	145.45	80.31	16.19	46.17	0.00
28-May-96	21:00	275.00	1.19	5.71	0.00	0.72	0.99	130.37	72.22	15.01	43.83	0.00
29-May-96	5:00	283.00	0.89	5.64	0.00	0.82	1.09	130.71	69.98	14.74	42.69	0.00
29-May-96	21:00	299.00	1.24	6.50	0.00	0.82	1.12	122.86	70.83	14.61	41.52	0.00
30-May-96	5:00	307.00	0.81	5.70	0.00	0.73	0.93	140.48	68.37	13.94	39.80	0.00
30-May-96	21:00	323.00	1.19	6.36	0.00	0.73	1.01	123.99	69.83	14.02	38.55	0.00
31-May-96	5:00	331.00	0.97	5.40	0.00	0.71	1.00	119.12	70.53	14.04	39.15	0.00
31-May-96	13:00	339.00	0.00	5.27	0.00	0.70	0.87	122.56	64.50	9.06	37.04	0.00
31-May-96	22:00	348.00	0.00	5.61	0.00	0.76	0.91	131.64	68.75	13.29	37.43	0.00
1-Jun-96	6:00	356.00	0.00	5.47	0.00	0.96	0.89	144.93	69.68	13.17	37.18	0.00
1-Jun-96	23:00	373.00	0.00	5.43	0.00	0.70	0.76	134.05	70.84	14.21	39.31	0.00
2-Jun-96	5:00	379.00	0.00	5.79	0.00	0.90	1.01	141.87	70.77	14.34	38.84	0.00

Run start date 5/17/96
 Run Name: CRADA Task 5
 Run ID#: P90605CF

APR Data

Date	Time	Run time (h)	Tot. Solids Oven (%)	TDS Liquid (%)	Ins.Solids (%)	Sample Wt. (g)	HPLC (g/L)								
							Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol	Succinic	Lactic
2-Jun-96	13:00	387.00	31.63%	0.00%	7.91%	29.15	102.49	53.79	9.30	33.57	0.00	0.84	3.06	1.06	2.22
2-Jun-96	21:00	395.00	31.97%	0.00%	7.99%	0.00	117.62	59.77	10.46	31.02	0.00	17.08	3.43	1.16	2.13
3-Jun-96	5:30	403.50	34.03%	0.00%	8.51%	0.00	123.10	58.37	10.08	29.79	0.00	16.74	3.32	1.05	1.92
3-Jun-96	13:00	411.00	31.69%	0.00%	7.92%	27.90	87.57	48.94	8.52	27.51	0.00	0.00	2.52	1.25	2.09
3-Jun-96	21:30	419.50	33.37%	0.00%	8.34%	0.00	106.30	54.98	10.22	31.12	0.00	0.00	3.06	1.06	2.27
4-Jun-96	4:45	426.75	33.05%	0.00%	8.26%	0.00	100.04	55.44	10.55	32.27	0.00	0.00	2.96	0.94	1.53
4-Jun-96	21:00	443.00	30.71%	0.00%	7.68%	0.00	105.51	57.87	10.75	36.33	0.00	0.00	3.07	1.07	2.10
5-Jun-96	4:00	450.00	31.77%	0.00%	7.94%	0.00	104.86	56.12	10.12	35.03	0.00	0.00	3.12	0.98	1.22
5-Jun-96	21:00	467.00	31.81%	0.00%	7.95%	0.00	95.72	51.62	9.34	31.54	0.00	0.00	2.90	1.01	2.33
6-Jun-96	5:30	475.50	34.75%	0.00%	8.69%	0.00	117.19	61.21	10.78	34.24	0.00	0.00	3.78	1.34	2.46
6-Jun-96	21:00	491.00	30.80%	0.00%	7.70%	0.00	96.04	50.65	9.24	30.92	2.38	0.00	2.76	0.85	2.07
7-Jun-96	4:00	498.00	31.32%	0.00%	7.83%	0.00	108.82	52.49	9.42	30.37	2.53	0.00	3.16	0.97	2.12
7-Jun-96	22:00	516.00	31.99%	0.00%	8.00%	0.00	125.81	63.58	11.43	37.90	3.01	0.00	0.00	0.00	1.99
8-Jun-96	5:00	523.00	31.33%	0.00%	7.83%	0.00	88.55	48.23	9.94	38.65	2.17	0.00	0.00	0.00	2.09
8-Jun-96	21:00	539.00	31.28%	0.00%	7.82%	0.00	115.02	53.14	9.51	31.29	2.59	0.00	2.72	1.09	1.40
9-Jun-96	5:00	547.00	33.74%	0.00%	8.44%	0.00	105.03	52.15	9.30	30.95	2.71	0.00	2.45	0.92	0.94
9-Jun-96	13:00	555.00	32.59%	0.00%	8.15%	0.00	101.97	54.00	10.75	32.34	2.66	0.00	2.52	1.23	1.78
10-Jun-96	5:00	571.00	32.98%	0.00%	8.25%	0.00	89.29	47.75	10.87	33.13	2.21	0.00	2.28	0.95	1.21
10-Jun-96	21:00	587.00	31.72%	0.00%	7.93%	0.00	94.88	50.64	10.55	31.66	2.36	0.00	2.38	1.13	2.20
11-Jun-96	5:00	595.00	32.39%	0.00%	8.10%	0.00	79.68	43.02	10.16	31.09	2.00	0.00	2.08	0.87	1.53
11-Jun-96	21:00	611.00	31.97%	0.00%	7.99%	0.00	93.34	48.79	10.25	30.63	0.00	0.00	2.43	1.14	1.95
12-Jun-96	5:00	619.00	28.64%	0.00%	7.16%	0.00	91.61	46.38	8.80	26.65	0.00	0.00	2.42	0.95	1.65
12-Jun-96	21:00	635.00	28.38%	0.00%	7.10%	0.00	82.57	45.13	9.74	28.75	3.03	0.00	2.18	1.03	1.56
13-Jun-96	5:00	643.00	29.78%	0.00%	7.45%	0.00	89.97	43.49	9.17	26.96	2.63	0.00	2.35	0.93	1.22
13-Jun-96	21:00	659.00	28.76%	0.00%	7.19%	0.00	78.87	42.09	10.39	30.32	3.29	0.00	2.18	1.03	2.16
14-Jun-96	5:00	667.00	28.43%	0.00%	7.11%	0.00	74.83	40.02	9.92	28.81	2.70	0.00	2.17	0.86	1.89
14-Jun-96	21:00	683.00	28.47%	0.00%	7.12%	0.00	59.66	48.88	9.17	28.38	6.04	0.00	2.36	1.18	1.99
15-Jun-96	5:00	691.00	28.66%	0.00%	7.17%	0.00	86.69	48.66	9.00	28.98	2.33	0.00	2.22	1.00	1.26
15-Jun-96	15:30	701.50	28.55%	0.00%	7.14%	0.00	90.80	50.09	10.83	32.17	2.97	0.00	2.16	0.98	1.39
16-Jun-96	5:00	715.00	32.70%	0.00%	8.18%	0.00	91.79	50.74	11.62	32.55	3.00	0.00	2.42	1.05	1.39
16-Jun-96	13:00	723.00	31.13%	0.00%	7.78%	169.48	74.97	34.17	8.07	25.58	0.00	3.64	0.91	0.00	2.90
16-Jun-96	21:00	731.00	34.60%	0.00%	8.65%	0.00	90.31	51.87	11.38	33.72	0.00	0.00	2.51	1.23	3.39
17-Jun-96	5:00	739.00	31.65%	0.00%	7.91%	0.00	87.09	48.47	11.31	33.37	0.00	0.00	2.29	1.04	2.90
17-Jun-96	21:00	755.00	32.10%	0.00%	8.03%	0.00	88.03	49.09	11.80	34.08	0.00	0.00	3.56	0.00	2.37
18-Jun-96	5:00	763.00	32.14%	0.00%	8.04%	0.00	96.82	54.59	11.96	34.45	0.00	0.00	1.12	0.00	1.36
18-Jun-96	13:00	771.00	32.84%	0.00%	8.21%	169.53	58.00	30.60	6.45	27.04	0.00	3.19	0.81	0.00	2.34
18-Jun-96	21:00	779.00	31.16%	0.00%	7.79%	0.00	99.67	57.16	12.80	36.87	10.78	0.00	3.03	1.37	2.11
19-Jun-96	5:00	787.00	31.37%	0.00%	7.84%	0.00	97.70	55.61	12.76	36.34	3.00	0.00	2.87	1.13	1.95
19-Jun-96	21:00	803.00	32.66%	0.00%	8.16%	0.00	96.37	48.55	11.99	34.11	0.00	0.00	2.53	1.16	2.77

Run start date 5/17/96
 Run Name: CRADA Task 5
 Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)					Liquor Analysis (Total Sugars, g/L)				
			Glycerol	Acetic	EtOH	HMF	furfural	Glucose	Xylose	Galactose	Arabinose	Mannose
2-Jun-96	13:00	387.00	0.00	6.01	0.00	0.81	0.88	128.37	56.85	9.37	33.17	0.00
2-Jun-96	21:00	395.00	1.15	5.80	0.00	0.83	0.99	141.37	67.60	13.67	39.55	8.88
3-Jun-96	5:30	403.50	0.00	5.82	0.00	0.98	1.02	157.65	69.41	14.05	40.52	9.67
3-Jun-96	13:00	411.00	0.00	5.95	0.00	0.79	0.86	123.34	65.24	8.80	37.77	0.00
3-Jun-96	21:30	419.50	0.00	5.83	0.00	0.68	0.90	132.90	66.11	13.13	38.65	0.00
4-Jun-96	4:45	426.75	0.00	4.68	0.00	0.57	0.76	129.52	69.46	13.71	40.69	0.00
4-Jun-96	21:00	443.00	0.00	5.62	0.00	0.58	0.83	144.36	77.80	15.46	45.23	0.00
5-Jun-96	4:00	450.00	0.00	5.18	0.00	0.65	0.89	130.97	68.10	13.49	39.52	0.00
5-Jun-96	21:00	467.00	1.17	5.32	0.00	0.54	0.77	131.30	69.24	13.74	41.92	10.05
6-Jun-96	5:30	475.50	1.30	6.78	0.00	1.21	1.48	148.69	72.98	14.79	44.22	11.09
6-Jun-96	21:00	491.00	0.82	4.65	0.00	0.46	0.69	129.21	67.29	13.48	40.50	3.86
7-Jun-96	4:00	498.00	0.63	5.15	0.00	0.59	0.75	142.17	67.06	13.53	39.91	3.87
7-Jun-96	22:00	516.00	0.00	4.83	0.00	0.51	0.63	144.17	70.94	13.82	41.97	4.14
8-Jun-96	5:00	523.00	0.00	3.16	0.00	0.50	0.48	139.27	71.67	13.89	44.29	4.19
8-Jun-96	21:00	539.00	0.00	5.57	0.00	0.49	0.68	157.08	70.09	14.17	42.24	4.20
9-Jun-96	5:00	547.00	0.00	5.34	0.00	0.51	0.72	144.73	68.41	13.79	41.42	4.30
9-Jun-96	13:00	555.00	0.00	5.76	0.00	0.54	0.94	132.82	69.31	14.03	40.81	4.18
10-Jun-96	5:00	571.00	0.00	4.27	0.00	0.33	0.50	130.98	69.00	13.35	41.92	3.87
10-Jun-96	21:00	587.00	0.00	5.00	0.00	0.43	0.69	129.25	68.80	13.74	40.98	4.27
11-Jun-96	5:00	595.00	0.00	3.79	0.00	0.28	0.44	127.38	67.38	13.46	41.15	4.50
11-Jun-96	21:00	611.00	0.00	4.28	0.00	0.48	0.72	129.33	66.50	13.32	39.56	4.52
12-Jun-96	5:00	619.00	0.00	4.30	0.00	0.51	0.75	119.94	59.02	11.91	35.21	3.67
12-Jun-96	21:00	635.00	0.00	3.82	0.00	0.42	0.74	108.86	54.57	12.15	34.69	3.65
13-Jun-96	5:00	643.00	0.00	3.77	0.00	0.47	0.67	109.84	54.24	12.15	34.68	3.64
13-Jun-96	21:00	659.00	0.00	3.42	0.00	0.43	0.62	117.40	63.31	13.76	39.17	3.88
14-Jun-96	5:00	667.00	0.00	2.88	0.00	0.36	0.56	118.61	60.67	13.12	37.51	3.46
14-Jun-96	21:00	683.00	0.00	4.82	0.00	0.62	0.92	112.96	58.81	12.22	35.67	3.35
15-Jun-96	5:00	691.00	0.00	4.16	0.00	0.49	0.78	112.97	61.56	12.65	37.37	3.77
15-Jun-96	15:30	701.50	0.00	3.63	0.00	0.40	0.65	151.41	78.68	15.63	46.40	0.00
16-Jun-96	5:00	715.00	0.00	3.96	0.00	0.43	0.62	155.79	81.32	16.29	47.92	0.00
16-Jun-96	13:00	723.00	0.00	5.72	0.00	0.44	0.68	130.33	79.43	11.56	41.65	0.00
16-Jun-96	21:00	731.00	0.00	4.58	0.00	0.54	0.80	117.68	59.42	13.47	38.07	4.42
17-Jun-96	5:00	739.00	0.00	3.32	0.00	0.40	0.56	123.30	61.00	13.67	39.23	4.31
17-Jun-96	21:00	755.00	0.00	3.73	0.00	0.42	0.56	125.28	62.23	13.31	39.53	3.91
18-Jun-96	5:00	763.00	0.00	3.61	0.00	0.51	0.67	126.56	63.62	14.56	40.71	4.42
18-Jun-96	13:00	771.00	0.00	5.24	0.00	0.32	0.49	132.00	70.66	12.08	43.57	0.00
18-Jun-96	21:00	779.00	0.15	5.10	0.00	0.63	0.85	136.75	73.71	14.93	44.28	4.38
19-Jun-96	5:00	787.00	0.00	4.16	0.00	0.45	0.64	134.59	74.64	15.78	45.18	4.60
19-Jun-96	21:00	803.00	0.00	3.76	0.00	0.50	0.56	144.26	69.64	15.19	42.49	0.00

Run start date 5/17/96
 Run Name: CRADA Task 5
 Run ID#: P90605CF

APR Data

Date	Time	Run time (h)	Tot. Solids Oven (%)	TDS Liquid (%)	Ins.Solids (%)	Sample Wt. (g)	HPLC (g/L)									
							Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol	Succinic	Lactic	
20-Jun-96	5:00	811.00	33.07%	0.00%	8.27%	0.00	112.13	55.54	12.55	35.01	2.87	0.00	2.79	1.15	1.92	
20-Jun-96	13:00	819.00	31.63%	0.00%	7.91%	171.37	78.24	41.57	5.30	29.83	0.00	3.58	0.70	0.00	1.59	
20-Jun-96	21:00	827.00	31.77%	0.00%	7.94%	0.00	90.69	50.24	12.12	35.65	0.00	0.00	2.45	1.16	2.06	
21-Jun-96	5:00	835.00	31.78%	0.00%	7.94%	0.00	109.34	60.34	12.52	36.50	0.00	0.00	2.73	1.16	1.59	
21-Jun-96	21:00	851.00	30.22%	0.00%	7.56%	0.00	79.01	42.35	10.28	29.54	8.10	0.00	0.00	0.00	5.76	
22-Jun-96	5:00	859.00	29.02%	0.00%	7.25%	0.00	88.21	42.42	9.85	28.10	8.25	0.00	0.00	0.00	3.37	
22-Jun-96	21:30	875.50	30.93%	0.00%	7.73%	0.00	86.03	44.36	10.58	30.36	3.06	0.00	2.14	0.94	4.77	
23-Jun-96	5:00	883.00	31.70%	0.00%	7.92%	0.00	91.59	42.98	10.26	28.99	2.79	0.00	2.35	6.94	3.85	
23-Jun-96	21:30	899.50	31.99%	0.00%	8.00%	0.00	121.22	47.41	11.19	29.88	0.00	0.00	2.69	1.15	3.97	
24-Jun-96	5:00	907.00	29.47%	0.00%	7.37%	0.00	87.38	44.17	10.92	30.74	2.74	0.00	2.19	1.00	2.82	
APR Testing with Steam Injection																
11-Jul-96	8:00	1325.00	33.77%	0.00%	8.44%	0.00	111.40	44.13	10.32	27.58	0.00	0.00	3.55	TRACE	1.56	
11-Jul-96	16:00	1326.42	32.49%	0.00%	8.12%	0.00	74.20	36.82	9.62	29.50	0.00	0.00	2.51	TRACE	2.71	
12-Jul-96	0:00	1311.00	29.88%	0.00%	7.47%	0.00	106.04	39.27	8.55	25.95	0.00	0.00	1.06	0.00	3.75	
12-Jul-96	8:00	1331.00	31.64%	0.00%	7.91%	0.00	51.93	24.52	7.37	26.97	0.00	0.00	1.79	0.00	3.70	
12-Jul-96	16:00	1343.00	32.59%	0.00%	8.15%	0.00	73.99	32.45	8.78	28.53	0.00	0.00	2.39	0.00	1.42	
13-Jul-96	0:00	1350.75	30.87%	0.00%	7.72%	0.00	86.96	39.57	10.05	29.22	0.00	0.00	2.59	0.00	2.58	

Run start date 5/17/96
 Run Name: CRADA Task 5
 Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)					Liquor Analysis (Total Sugars, g/L)				
			Glycerol	Acetic	EtOH	HMF	furfural	Glucose	Xylose	Galactose	Arabinose	Mannose
20-Jun-96	5:00	811.00	0.00	3.90	0.00	0.56	0.64	150.48	72.66	15.71	43.57	0.00
20-Jun-96	13:00	819.00	0.00	4.59	0.00	0.22	0.35	128.63	62.42	11.99	41.28	0.00
20-Jun-96	21:00	827.00	0.00	3.84	0.00	0.45	0.61	133.95	71.85	15.12	43.81	0.00
21-Jun-96	5:00	835.00	0.00	4.73	0.00	0.53	0.75	137.00	73.64	15.39	44.23	0.00
21-Jun-96	21:00	851.00	1.71	3.82	0.00	0.47	0.54	128.30	64.10	14.16	40.27	4.50
22-Jun-96	5:00	859.00	0.86	3.49	0.00	0.41	0.53	138.35	63.70	14.23	39.96	4.52
22-Jun-96	21:30	875.50	0.00	3.71	0.00	0.45	0.60	131.12	63.42	14.05	39.92	4.79
23-Jun-96	5:00	883.00	0.00	3.13	0.00	0.39	0.52	140.53	62.20	13.64	38.96	4.41
23-Jun-96	21:30	899.50	0.00	3.83	0.00	0.49	0.52	162.65	60.62	13.60	36.87	3.84
24-Jun-96	5:00	907.00	1.01	3.05	0.00	0.41	0.48	124.42	58.76	12.67	36.47	3.75
APR Testing with Steam Injection												
11-Jul-96	8:00	1325.00	0.00	4.53	0.00	0.68	0.68					
11-Jul-96	16:00	1326.42	0.00	3.86	0.00	0.38	0.41					
12-Jul-96	0:00	1311.00	0.00	4.34	0.00	0.52	0.38					
12-Jul-96	8:00	1331.00	0.83	3.22	0.00	0.00	0.00					
12-Jul-96	16:00	1343.00	0.00	3.56	0.00	0.29	0.00					
13-Jul-96	0:00	1350.75	0.68	4.16	0.00	0.33	0.00					

Run start date 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455A

Date	Time	Run time (h)	O.D.	Cell Mass	YSI Gluc	YSI EtOH	YSI Lactate	HPLC (g/L)						
			600 nm	counts/mL	(g/L)	(g/L)	(g/L)	pH	Glucose	Xylose	Gal.	Arab.		
17-May-96	0:00	-10.00												
17-May-96	10:00	0.00		1.35E+06	76.20	0.89	0.92	4.98	72.87	38.88	6.85	21.14		
17-May-96	18:00	8.00			73.40	1.96	0.73	5.01						
18-May-96	2:00	16.00			63.90	2.08	1.40	4.98	72.71	38.14	6.72	20.75		
18-May-96	10:00	24.00			69.40	1.55	0.68	4.88						
18-May-96	18:00	32.00			71.00	1.24	0.74	4.97	73.30	37.00	5.07	18.67		
19-May-96	2:00	40.00			58.90	1.04	0.58	4.80	64.66	32.34	4.29	16.23		
19-May-96	9:00	47.00			38.40	0.68	0.38	4.87						
19-May-96	18:00	56.00			40.90	0.49	0.37	4.91	41.14	21.27	4.08	11.47		
20-May-96	2:00	64.00			39.70	0.28	0.36	4.71	42.51	22.28	4.28	12.05		
20-May-96	10:00	72.00			43.10	0.51	0.37	4.99						
20-May-96	18:00	80.00			42.00	0.28		4.92	42.39	23.12	4.05	12.54		
21-May-96	2:00	88.00			38.50	0.18	0.40	4.85	42.63	23.26	4.19	12.82		
21-May-96	10:50	96.83			37.30	0.80	0.44	4.86						
21-May-96	14:40	100.67			38.80									
21-May-96	18:00	104.00			33.20	15.30		4.93	30.81	21.35	4.02	11.93		
22-May-96	2:00	112.00			1.60	15.90		4.92	2.74	18.72	4.04	12.68		
22-May-96	10:20	120.33			1.03	24.15	0.49	4.88						
22-May-96	18:00	128.00			1.28	20.60	0.53	4.93	1.50	12.88	3.92	13.32		
23-May-96	2:00	136.00			1.13	27.50	0.49	5.08	1.40	10.68	3.70	13.05		
23-May-96	11:15	145.25		1.36E+08	1.34	31.50	0.73	4.90						
23-May-96	18:00	152.00			0.96	23.00	0.63	4.93	0.00	14.32	3.24	13.57		
24-May-96	1:30	159.50							0.96	13.30	3.40	13.44		
24-May-96	2:00	160.00			0.50	20.80	0.39	4.85	1.09	12.83	3.87	14.63		
24-May-96	11:30	169.50		1.22E+08	0.80	36.05	0.58	4.82						
24-May-96	18:00	176.00			0.84	33.85	0.64	4.94						
25-May-96	2:00	184.00			0.74	27.65	0.75	4.92	0.96	15.18	3.54	13.89		
25-May-96	10:00	192.00		9.70E+07	0.95	32.40	1.06	4.92						
25-May-96	18:00	200.00			0.98	32.00	1.78	4.91	1.45	17.61	4.19	14.85		

Run start date 17-May-96
 Run Name: CRADA Task
 Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)									
			Man.	Cellb.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
17-May-96	0:00	-10.00	7.20	14.73	0.00	0.00	1.47	0.52	4.06	1.03	0.47	0.75
17-May-96	10:00	0.00										
17-May-96	18:00	8.00										
18-May-96	2:00	16.00	7.52	14.74	0.00	0.00	1.66	0.71	4.26	1.28	0.46	0.64
18-May-96	10:00	24.00										
18-May-96	18:00	32.00	0.00	13.58	1.94	0.69	1.70	0.64	4.02	0.91	0.58	0.68
19-May-96	2:00	40.00	0.00	11.92	1.69	0.62	1.55	0.64	3.51	0.69	0.50	0.62
19-May-96	9:00	47.00										
19-May-96	18:00	56.00	0.00	9.22	1.02	0.37	0.77	0.00	2.01	0.00	0.27	0.34
20-May-96	2:00	64.00	0.00	7.44	1.11	0.42	0.91	0.00	2.08	0.00	0.26	0.33
20-May-96	10:00	72.00										
20-May-96	18:00	80.00	4.66	7.16	1.10	0.33	0.83	0.00	2.05	0.00	0.23	0.00
21-May-96	2:00	88.00	4.68	7.44	1.37	0.50	1.14	0.00	2.40	0.00	0.25	0.00
21-May-96	10:50	96.83										
21-May-96	14:40	100.67										
21-May-96	18:00	104.00	2.90	8.49	0.83	0.57	1.16	0.84	2.08	6.64	0.00	0.00
22-May-96	2:00	112.00	0.00	8.61	0.85	0.63	1.33	2.70	1.85	20.68	0.00	0.00
22-May-96	10:20	120.33										
22-May-96	18:00	128.00	0.00	7.31	1.42	0.47	1.17	2.83	1.44	24.19	0.00	0.00
23-May-96	2:00	136.00	0.00	5.72	1.65	0.59	1.30	3.17	1.88	28.64	0.00	0.00
23-May-96	11:15	145.25										
23-May-96	18:00	152.00	0.00	7.74	1.96	0.59	1.53	2.39	1.79	29.52	0.00	0.00
24-May-96	1:30	159.50	0.00	7.65	2.01	0.66	1.53	2.68	1.87	29.51	0.00	0.00
24-May-96	2:00	160.00	0.00	8.33	2.42	0.62	1.65	3.17	1.97	29.75	0.00	0.00
24-May-96	11:30	169.50										
24-May-96	18:00	176.00										
25-May-96	2:00	184.00	0.00	8.19	2.04	0.68	1.90	2.65	2.08	30.40	0.00	0.00
25-May-96	10:00	192.00										
25-May-96	18:00	200.00	0.00	8.86	1.82	0.49	2.98	2.31	2.73	30.89	0.00	0.00

Run start date 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455A

Date	Time	Run time (h)	O.D.	Cell Mass	YSI Gluc	YSI ETOH	YSI Lactate	HPLC (g/L)				
			600 nm	counts/mL	(g/L)	(g/L)	(g/L)	pH	Glucose	Xylose	Gal.	Arab.
26-May-96	2:00	208.00			1.16	24.90	2.68	4.92	1.46	17.62	4.10	13.32
26-May-96	10:00	216.00			1.09	21.60	3.45	4.86				
26-May-96	18:00	224.00			1.57	30.38	4.71	4.79	2.31	20.41	4.37	11.91
27-May-96	2:00	232.00			1.24	34.20	5.19	4.95	2.41	21.93	4.57	12.00
27-May-96	10:00	240.00			2.20	21.70	5.45	4.66				
27-May-96	18:00	248.00			2.05	29.10	4.62	4.77	2.91	24.65	5.29	14.14
28-May-96	2:00	256.00			2.06	32.60	4.32	4.94	3.13	26.52	5.77	15.66
28-May-96	10:00	264.00			0.77	32.90	3.82	4.81				
28-May-96	18:00	272.00			0.33	30.75	3.88	4.83	1.59	24.05	5.37	14.93
29-May-96	2:00	280.00			1.74	29.95	3.40	4.98	3.36	25.64	5.67	16.02
29-May-96	10:00	288.00		6.75E+07	1.07	20.80	2.82	4.67				
29-May-96	18:00	296.00			1.29	31.50	2.74	4.81	2.75	29.01	6.20	18.44
30-May-96	2:00	304.00			1.61	33.85	2.44	4.98	3.22	30.50	6.43	19.23
30-May-96	18:00	320.00			2.51	33.65	2.24	4.84	4.67	34.65	6.25	19.95
31-May-96	3:00	329.00			2.58	35.50	2.00	4.99	4.75	34.93	6.23	19.92
31-May-96	10:00	336.00		7.25E+07	2.01	34.15	1.79	4.74				
31-May-96	18:00	344.00			1.49	31.15	1.73	4.84				
1-Jun-96	2:00	352.00			1.22	34.50	1.38	4.97	3.13	34.55	5.77	19.51
1-Jun-96	10:00	360.00			1.29	34.35	1.38	4.91				
1-Jun-96	18:00	368.00			0.43	38.40	1.25	5.00	2.43	31.90	6.58	20.11
2-Jun-96	2:00	376.00			1.79	35.10	1.15	4.95	3.95	32.41	6.83	20.46
2-Jun-96	10:00	384.00			1.42	32.20	1.05	4.92				
2-Jun-96	18:00	392.00			1.47	33.40	1.01	5.01				
2-Jun-96	18:20	392.33							3.15	33.73	6.93	21.06
3-Jun-96	2:20	400.33			1.55	33.10	0.97	4.79	3.81	33.94	7.05	21.11
3-Jun-96	10:00	408.00		6.60E+07	1.34	29.70	0.89	4.80				
3-Jun-96	18:00	416.00			0.86	20.60	0.89	4.80	2.80	31.77	6.55	20.31
4-Jun-96	3:45	425.75			1.93	33.60	0.90	4.96	3.96	31.78	6.60	20.43
4-Jun-96	10:00	432.00		8.85E+07	1.03	30.10	0.82	5.05				
4-Jun-96	18:00	440.00			0.86	28.60	0.83	4.82	2.58	30.10	6.23	18.27

Run start date 17-May-96
 Run Name: CRADA Task
 Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)									
			Man.	Cello.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
26-May-96	2:00	208.00	0.00	9.19	2.41	0.58	4.29	2.62	3.64	30.73	0.00	0.00
26-May-96	10:00	216.00										
26-May-96	18:00	224.00	0.00	9.52	2.31	0.48	6.44	2.34	5.00	30.94	0.00	0.00
27-May-96	2:00	232.00	0.00	10.03	2.42	0.59	7.26	2.67	5.57	31.31	0.00	0.00
27-May-96	10:00	240.00										
27-May-96	18:00	248.00	0.00	11.19	1.69	0.65	6.70	2.53	5.30	31.50	0.00	0.00
28-May-96	2:00	256.00	0.00	12.08	1.75	0.79	6.34	2.95	5.18	31.53	0.00	0.00
28-May-96	10:00	264.00										
28-May-96	18:00	272.00	0.00	10.74	1.59	0.57	5.47	2.73	4.64	30.49	0.00	0.00
29-May-96	2:00	280.00	0.00	10.87	1.72	0.72	5.21	2.87	4.66	30.01	0.00	0.00
29-May-96	10:00	288.00										
29-May-96	18:00	296.00	0.00	10.89	1.61	0.70	4.25	3.00	4.29	31.65	0.00	0.00
30-May-96	2:00	304.00	0.00	11.13	1.74	0.86	4.18	3.45	4.37	31.92	0.00	0.00
30-May-96	18:00	320.00	0.00	12.81	1.87	0.73	3.92	3.88	4.32	34.90	0.00	0.00
31-May-96	3:00	329.00	0.00	12.67	1.93	0.83	3.78	4.16	4.28	34.11	0.00	0.00
31-May-96	10:00	336.00										
31-May-96	18:00	344.00										
1-Jun-96	2:00	352.00	0.00	12.99	2.14	0.80	3.23	4.26	3.75	35.24	0.00	0.00
1-Jun-96	10:00	360.00										
1-Jun-96	18:00	368.00	0.00	11.49	2.17	0.65	2.72	3.94	3.37	35.53	0.00	0.00
2-Jun-96	2:00	376.00	0.00	12.01	2.21	0.73	2.79	3.98	3.51	35.59	0.00	0.00
2-Jun-96	10:00	384.00										
2-Jun-96	18:00	392.00										
2-Jun-96	18:20	392.33	0.00	12.79	2.16	0.65	2.48	3.77	3.28	36.08	0.00	0.00
3-Jun-96	2:20	400.33	0.00	12.74	2.21	0.72	2.53	3.94	3.40	35.68	0.00	0.00
3-Jun-96	10:00	408.00										
3-Jun-96	18:00	416.00	0.00	12.00	2.16	0.64	2.34	3.72	3.25	36.49	0.00	0.00
4-Jun-96	3:45	425.75	0.00	12.38	2.24	0.70	2.40	3.89	3.42	36.09	0.00	0.00
4-Jun-96	10:00	432.00										
4-Jun-96	18:00	440.00	0.00	12.52	2.10	0.60	2.11	3.67	2.97	35.21	0.00	0.00

Run start date 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455A

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass counts/mL	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	HPLC (g/L)				
								pH	Glucose	Xylose	Gal.	Arab.
5-Jun-96	2:00	448.00			1.29	33.60	0.78	4.97	3.11	29.15	6.19	17.89
5-Jun-96	11:00	457.00		8.05E+07	0.29	30.25	0.81	4.98				
5-Jun-96	18:00	464.00			0.32	22.20	0.71	4.99	2.12	28.66	6.46	19.83
6-Jun-96	2:00	472.00			1.30	34.20	0.75	5.03	2.87	27.65	6.23	19.29
6-Jun-96	10:00	480.00		9.20E+07	0.82	34.80	0.69	4.99	0.00			
6-Jun-96	18:00	488.00			0.76	27.80	0.81	4.96	2.25	27.47	6.33	19.64
7-Jun-96	2:00	496.00			1.14	37.30	0.78	5.01	2.65	27.22	6.29	19.73
7-Jun-96	10:00	504.00		8.20E+07	0.78	36.65	0.82	5.03				
7-Jun-96	18:00	512.00			0.21	33.78	0.34	4.89	2.32	29.28	6.97	22.70
8-Jun-96	2:15	520.25			0.90	35.65	0.75	5.12	2.78	31.30	7.52	24.41
8-Jun-96	10:30	528.50			0.76	39.40	0.80	4.97				
8-Jun-96	18:00	536.00			0.23	32.75	0.79	4.94	1.93	24.42	6.17	19.84
9-Jun-96	2:00	544.00			0.70	19.80	0.73	5.02	2.26	25.53	6.37	20.52
9-Jun-96	10:00	552.00			0.56	39.80	0.75	4.98	1.99	25.82	6.54	19.73
9-Jun-96	18:00	560.00			0.51	40.52	0.87	4.78				
10-Jun-96	2:00	568.00			0.62	34.30	0.70	4.99	2.35	25.50	6.39	19.40
10-Jun-96	10:00	576.00		1.03E+08	0.47	36.20	0.68	5.00				
10-Jun-96	18:00	584.00			0.46	32.15	0.65	4.90	2.08	25.95	6.59	19.80
11-Jun-96	2:00	592.00			0.57	28.40	0.61	5.02	2.20	26.08	6.66	19.91
11-Jun-96	10:00	600.00		1.18E+08	0.24	33.58	0.65	4.84				
11-Jun-96	18:00	608.00			0.17	34.15	0.65	4.85	1.89	24.34	6.43	19.08
12-Jun-96	2:00	616.00			0.79	20.50	0.66	5.04	2.64	24.65	6.57	19.38
12-Jun-96	10:00	624.00			0.58	35.60	0.68	4.86				
12-Jun-96	18:00	632.00			0.559	33.00	0.67	4.86	2.49	24.93	6.86	19.67
13-Jun-96	2:00	640.00			0.51	30.70	0.57	4.97	2.14	22.22	6.13	17.52
13-Jun-96	10:30	648.50		7.70E+07	0.48	34.40	0.57	4.99				
13-Jun-96	18:00	656.00			0.41	28.90	0.51	4.88	2.12	19.54	5.57	16.12
14-Jun-96	2:00	664.00			0.33	21.90	0.50	5.00	1.88	17.78	5.15	14.97
14-Jun-96	10:00	672.00		6.20E+07	0.37	35.20	0.51	4.99				
14-Jun-96	18:15	680.25			0.22	27.60	0.56	5.04	1.55	15.90	4.69	13.64

Run start date 17-May-96
 Run Name: CRADA Task
 Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)									
			Man.	Cello.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
5-Jun-96	2:00	448.00	0.00	12.24	2.19	0.67	2.23	3.88	3.12	35.76	0.00	0.00
5-Jun-96	11:00	457.00										
5-Jun-96	18:00	464.00	0.00	7.78	2.07	0.59	2.00	3.59	2.74	34.84	0.00	0.00
6-Jun-96	2:00	472.00	0.00	7.55	2.11	0.65	2.15	3.71	2.94	34.52	0.00	0.00
6-Jun-96	10:00	480.00			2.15	0.64	2.18	3.81	2.99	36.63	0.00	0.00
6-Jun-96	18:00	488.00	0.00	7.72	2.13	0.60	2.10	3.61	2.89	36.65	0.00	0.00
7-Jun-96	2:00	496.00	0.00	7.74	2.17	0.66	2.22	3.74	3.00	36.95	0.00	0.00
7-Jun-96	10:00	504.00										
7-Jun-96	18:00	512.00	0.00	8.68	2.78	0.00	2.17	3.70	2.88	37.10	0.00	0.00
8-Jun-96	2:15	520.25	0.00	9.47	2.85	0.00	2.20	3.70	2.92	37.94	0.00	0.00
8-Jun-96	10:30	528.50										
8-Jun-96	18:00	536.00	0.00	7.81	2.06	0.67	2.08	3.45	2.68	38.32	0.00	0.00
9-Jun-96	2:00	544.00	0.00	7.98	2.15	0.79	2.27	3.80	3.04	38.53	0.00	0.00
9-Jun-96	10:00	552.00	0.00	8.39	2.37	0.75	1.93	3.22	2.82	38.42	0.00	0.00
9-Jun-96	18:00	560.00										
10-Jun-96	2:00	568.00	0.00	8.32	2.44	0.80	2.04	3.26	2.97	38.84	0.00	0.00
10-Jun-96	10:00	576.00										
10-Jun-96	18:00	584.00	0.00	8.45	2.40	0.75	1.89	2.91	2.83	39.27	0.00	0.00
11-Jun-96	2:00	592.00	0.00	8.63	2.38	0.78	1.94	2.91	2.89	37.95	0.00	0.00
11-Jun-96	10:00	600.00										
11-Jun-96	18:00	608.00	0.00	8.40	2.49	0.71	1.84	2.79	2.76	39.26	0.00	0.00
12-Jun-96	2:00	616.00	0.00	8.49	2.50	0.75	1.88	2.84	2.80	38.20	0.00	0.00
12-Jun-96	10:00	624.00										
12-Jun-96	18:00	632.00	0.00	8.24	2.33	0.68	1.81	2.76	2.58	37.08	0.00	0.00
13-Jun-96	2:00	640.00	0.00	7.38	2.17	0.67	1.71	2.75	2.44	33.51	0.00	0.00
13-Jun-96	10:30	648.50										
13-Jun-96	18:00	656.00	16.12	6.70	2.12	0.62	1.51	2.64	2.23	30.87	0.00	0.00
14-Jun-96	2:00	664.00	0.00	6.31	2.10	0.63	1.49	2.73	2.15	29.67	0.00	0.00
14-Jun-96	10:00	672.00										
14-Jun-96	18:15	680.25	0.00	5.45	1.53	0.52	1.50	2.50	2.00	27.56	0.00	0.00

Run start date 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455A

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass counts/mL	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	HPLC (g/L)				
								pH	Glucose	Xylose	Gal.	Arab.
15-Jun-96	2:00	688.00			0.37	27.25	0.64	4.81	1.58	16.10	4.62	13.32
15-Jun-96	11:15	697.25			0.40	25.40	0.66	4.86				
15-Jun-96	18:00	704.00			0.31	28.55	0.69	4.90	1.38	14.36	4.02	12.03
16-Jun-96	2:00	712.00			0.42	22.95	0.63	4.91	1.42	13.73	3.96	11.74
16-Jun-96	10:00	720.00			0.29	26.55	0.59	4.94				
16-Jun-96	18:15	728.25			0.40	22.25	0.62	4.91	1.40	13.00	3.87	11.53
17-Jun-96	2:00	736.00			0.26	20.00	0.60	4.94	1.29	12.47	3.76	11.21
17-Jun-96	10:00	744.00		7.10E+07	0.26	16.25	0.68	4.93				
17-Jun-96	18:00	752.00			0.31	28.10	0.62	4.93	1.27	12.53	3.92	11.71
18-Jun-96	2:00	760.00			0.16	18.20	0.58	4.86	1.14	12.36	3.93	11.83
18-Jun-96	10:00	768.00		1.47E+08	0.19	14.80	0.59	5.01				
18-Jun-96	18:00	776.00			0.31	22.30	0.56	5.02	1.31	12.07	3.90	11.84
19-Jun-96	2:00	784.00			0.23	24.87	0.50	5.11	1.22	12.07	3.92	11.95
19-Jun-96	10:00	792.00		9.05E+07	0.22	26.40	0.50	5.06				
19-Jun-96	19:00	801.00			0.37	24.45	0.54	4.94	1.39	10.70	3.80	11.48
20-Jun-96	2:00	808.00			0.23	25.45	0.54	4.86				
20-Jun-96	5:00	811.00							1.20	10.45	3.75	11.60
20-Jun-96	10:00	816.00		8.65E+07	0.24	19.25	0.58	5.00				
20-Jun-96	19:30	825.50			0.31	23.95	0.50	4.95				
21-Jun-96	2:00	832.00			0.33	28.25	0.50	4.99	1.20	10.67	3.71	11.67
21-Jun-96	10:00	840.00		7.70E+07	0.26	23.60	0.59	5.00				
21-Jun-96	18:20	848.33							1.35	10.28	3.60	9.56
21-Jun-96	18:30	848.50			0.42	24.00	1.48	4.81				
21-Jun-96	19:30	849.50							1.22	10.22	3.68	11.63
22-Jun-96	2:00	856.00			0.70	25.55	2.80	5.02	1.59	10.44	3.65	7.78
22-Jun-96	10:00	864.00			0.33	20.80	3.18	5.01				
22-Jun-96	18:20	872.33			0.67	22.80	3.29	4.92	1.49	10.51	3.63	5.89
23-Jun-96	2:00	880.00			0.57	23.85	3.52	4.94	1.45	10.32	3.57	5.62
23-Jun-96	10:00	888.00			0.45	22.55	3.43	4.86	1.11	10.47	3.60	5.71
23-Jun-96	18:00	896.00			0.63	22.15	3.42	4.90	1.70	10.62	3.73	6.12

Run start date 17-May-96
 Run Name: CRADA Task
 Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)									
			Man.	Cello.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
15-Jun-96	2:00	688.00	0.00	5.29	1.92	0.57	1.67	2.62	2.16	26.49	0.00	0.00
15-Jun-96	11:15	697.25										
15-Jun-96	18:00	704.00	0.00	4.49	1.93	0.54	1.70	2.79	2.18	25.58	0.00	0.00
16-Jun-96	2:00	712.00	0.72	4.36	1.90	0.54	1.66	2.85	2.13	24.97	0.00	0.00
16-Jun-96	10:00	720.00										
16-Jun-96	18:15	728.25	0.00	4.46	1.82	0.49	1.48	2.71	1.92	25.08	0.00	0.00
17-Jun-96	2:00	736.00	0.00	4.31	1.87	0.55	1.57	2.92	2.00	24.45	0.00	0.00
17-Jun-96	10:00	744.00										
17-Jun-96	18:00	752.00	0.00	4.18	2.18	0.00	1.38	2.68	1.76	24.81	0.00	0.00
18-Jun-96	2:00	760.00	0.00	4.19	2.40	0.00	1.46	2.84	1.87	24.50	0.00	0.00
18-Jun-96	10:00	768.00										
18-Jun-96	18:00	776.00	0.00	4.15	1.82	0.52	1.33	2.71	1.75	24.34	0.00	0.00
19-Jun-96	2:00	784.00	0.00	4.27	1.89	0.59	1.40	2.93	1.81	25.25	0.00	0.00
19-Jun-96	10:00	792.00										
19-Jun-96	19:00	801.00	0.00	4.29	2.11	0.50	1.29	2.95	1.64	24.75	0.00	0.00
20-Jun-96	2:00	808.00										
20-Jun-96	5:00	811.00	0.00	4.51	2.18	0.57	1.43	3.21	1.75	23.96	0.00	0.00
20-Jun-96	10:00	816.00										
20-Jun-96	19:30	825.50										
21-Jun-96	2:00	832.00	0.00	4.29	2.10	0.57	1.30	3.23	1.79	23.40	0.00	0.00
21-Jun-96	10:00	840.00										
21-Jun-96	18:20	848.33	0.00	7.27	2.52	0.00	2.97	3.20	2.77	24.12	0.00	0.00
21-Jun-96	18:30	848.50										
21-Jun-96	19:30	849.50	0.00	4.33	2.07	0.49	1.20	3.02	1.60	24.13	0.00	0.00
22-Jun-96	2:00	856.00	0.00	7.47	2.47	0.00	4.68	3.32	3.74	22.88	0.00	0.00
22-Jun-96	10:00	864.00										
22-Jun-96	18:20	872.33	0.00	4.41	1.89	0.48	5.79	3.19	4.31	24.20	0.00	0.00
23-Jun-96	2:00	880.00	0.00	4.43	1.83	0.54	6.02	3.29	4.41	23.60	0.00	0.00
23-Jun-96	10:00	888.00	0.00	4.57	2.10	0.27	6.06	3.32	4.35	24.02	0.00	0.00
23-Jun-96	18:00	896.00	0.00	4.74	1.56	0.48	5.86	3.06	4.13	22.97	0.00	0.00

Run start date 17-May-96 Time 4.17E-01
Run Name: CRADA Task 5
Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455A

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass counts/mL	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	pH	HPLC (g/L)			
									Glucose	Xylose	Gal.	Arab.
24-Jun-96	2:00	904.00			0.72	24.80	3.48	4.92	1.75	11.07	3.81	6.43
24-Jun-96	15:00	917.00							0.69	11.24	3.72	5.80
25-Jun-96	13:00	939.00							0.92	9.16	3.59	2.94

Run start date 17-May-96
Run Name: CRADA Task
Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)									
			Man.	Cello.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
24-Jun-96	2:00	904.00	0.00	4.93	1.68	0.30	5.81	3.08	4.08	23.21	0.00	0.00
24-Jun-96	15:00	917.00	0.00	4.85	2.01	0.00	6.02	3.15	4.30	23.62	0.00	0.00
25-Jun-96	13:00	939.00	0.00	4.55	2.30	0.00	7.78	3.12	5.49	24.28	0.00	0.00

Run start data 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455B

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass counts/mL	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	HPLC (g/L)				
								pH	Glucose	Xylose	Gal.	Arab.
17-May-96	18:00	8.00							72.76	38.70	6.83	21.04
18-May-96	2:00	16.00			64.50	1.99	1.40	4.95	72.85	38.27	6.76	20.87
18-May-96	10:00	24.00			67.50	1.88	0.66	4.90				
18-May-96	18:00	32.00			67.60	1.77	0.68	4.81	71.82	37.23	5.19	18.92
19-May-96	2:00	40.00			65.60	2.00	0.67	4.80	71.27	36.94	5.13	18.76
19-May-96	9:00	47.00			71.40	2.05		4.86				
19-May-96	18:00	56.00			64.50	1.22	0.59	4.97	66.90	35.92	6.64	19.32
20-May-96	2:00	64.00			58.50	2.45	0.55	4.80	64.08	34.56	6.43	18.60
20-May-96	10:00	72.00			55.20	2.32	0.51	4.97				
20-May-96	18:00	80.00			51.80	1.13		5.04	53.01	30.48	5.25	16.31
21-May-96	0:00	86.00							43.07	30.05	5.22	16.26
21-May-96	2:00	88.00			37.30	7.00	0.50	4.94	43.07	30.05	5.22	16.26
21-May-96	10:50	96.83			6.13	18.94	0.48	4.92				
21-May-96	18:00	104.00			1.89	16.10		4.99	1.98	19.68	4.41	13.62
22-May-96	2:00	112.00			0.24	24.10		5.03	1.62	16.79	4.14	12.66
22-May-96	10:20	120.33			0.99	27.80	0.47	4.98				
22-May-96	18:00	128.00			0.26	22.18		5.01	1.63	12.74	3.95	13.49
23-May-96	2:00	136.00			0.95	25.73	0.55	4.99	1.26	11.96	3.92	13.33
23-May-96	11:15	145.25		1.08E+08	1.08	31.30	0.49	4.94				
23-May-96	18:00	152.00			1.02	26.00	0.52	4.96	0.91	7.73	2.06	11.86
24-May-96	1:30	159.50							0.98	7.60	2.05	11.88
24-May-96	2:00	160.00			0.54	22.70	0.33	4.89	1.09	7.92	2.53	12.91
24-May-96	11:30	169.50		1.68E+08	1.00	36.10	0.53	4.80				
24-May-96	18:00	176.00			1.14	34.30	0.62	4.88				
25-May-96	2:00	184.00			0.96	30.30	1.00	4.98	0.97	7.26	2.38	12.06
25-May-96	10:00	192.00		1.37E+08	0.90	38.70	1.94	4.97				
25-May-96	18:00	200.00			0.96	32.30	3.48	4.95	0.98	8.61	2.46	9.16
26-May-96	2:00	208.00			0.86	28.50	4.60	4.90	1.03	9.07	2.50	7.58
26-May-96	10:00	216.00			0.81	31.50	5.48	4.84				
26-May-96	18:00	224.00			0.85	32.05	6.95	4.79	0.96	10.71	2.70	5.54

Run start data

17-May-96

Run Name:

CRADA Task :

Run ID#:

P90605CF

Date	Time	Run time (h)	HPLC (g/L)									
			Man.	Cello.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
17-May-96	18:00	8.00	7.41	15.21	0.00	0.00	1.59	0.67	4.19	1.20	0.46	0.66
18-May-96	2:00	16.00	7.65	11.08	0.00	0.00	1.66	0.74	4.28	1.36	0.45	0.62
18-May-96	10:00	24.00										
18-May-96	18:00	32.00	0.00	10.62	1.86	0.70	1.70	0.81	4.35	1.47	0.55	0.63
19-May-96	2:00	40.00	0.00	0.00	1.89	0.71	1.73	0.85	4.40	1.53	0.53	0.61
19-May-96	9:00	47.00										
19-May-96	18:00	56.00	0.00	16.21	1.60	0.91	1.61	0.82	4.10	1.61	0.38	0.36
20-May-96	2:00	64.00	0.00	12.47	1.60	0.78	1.54	0.78	3.85	1.68	0.34	0.00
20-May-96	10:00	72.00										
20-May-96	18:00	80.00	7.10	10.90	1.57	0.56	1.46	0.89	3.41	3.17	0.00	0.00
21-May-96	0:00	86.00	0.00	10.95	1.58	0.58	1.51	1.40	3.25	7.76	0.00	0.00
21-May-96	2:00	88.00	0.00	10.95	1.58	0.58	1.51	1.40	3.25	7.76	0.00	0.00
21-May-96	10:50	96.83										
21-May-96	18:00	104.00	0.00	8.96	1.18	0.85	1.48	3.70	2.57	27.91	0.00	0.00
22-May-96	2:00	112.00	0.00	7.83	1.11	0.77	1.37	3.11	2.17	26.06	0.00	0.00
22-May-96	10:20	120.33										
22-May-96	18:00	128.00	0.00	6.23	1.56	0.58	1.30	3.18	1.97	27.69	0.00	0.00
23-May-96	2:00	136.00	0.00	7.13	1.76	0.63	1.37	3.06	1.66	26.07	0.00	0.00
23-May-96	11:15	145.25										
23-May-96	18:00	152.00	0.00	6.40	2.88	0.74	1.62	3.01	1.91	31.74	0.00	0.00
24-May-96	1:30	159.50	0.00	6.42	2.82	0.74	1.49	3.08	1.85	31.85	0.00	0.00
24-May-96	2:00	160.00	0.00	6.84	2.69	0.61	1.47	3.22	1.89	31.77	0.00	0.00
24-May-96	11:30	169.50										
24-May-96	18:00	176.00										
25-May-96	2:00	184.00	0.00	6.33	3.02	0.76	2.11	2.94	2.23	32.29	0.00	0.00
25-May-96	10:00	192.00										
25-May-96	18:00	200.00	0.00	7.01	3.28	0.68	4.95	2.83	4.17	31.67	0.00	0.00
26-May-96	2:00	208.00	0.00	7.36	3.35	0.70	6.47	2.81	5.17	31.47	0.00	0.00
26-May-96	10:00	216.00										
26-May-96	18:00	224.00	0.00	7.67	3.41	0.68	9.17	2.83	6.89	31.49	0.00	0.00

Run start data 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455B

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass counts/mL	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	HPLC (g/L)				
								pH	Glucose	Xylose	Gal.	Arab.
27-May-96	2:00	232.00			0.65	35.80	7.96	4.94	1.13	11.41	2.82	4.98
27-May-96	10:00	240.00			0.95	31.10	7.33	4.66				
27-May-96	18:00	248.00			0.86	31.70	7.53	4.72	1.14	16.36	3.68	7.72
28-May-96	2:00	256.00			0.90	34.45	7.36	4.92	1.09	17.92	4.01	8.68
28-May-96	10:00	264.00			0.21	35.20	6.95	4.78				
28-May-96	18:00	272.00			0.22	32.13	6.93	4.76	1.30	18.95	4.29	9.85
29-May-96	2:00	280.00			0.26	32.65	6.38	4.97	1.31	19.70	4.38	10.62
29-May-96	10:00	288.00		8.15E+07	0.16	30.90	5.63	4.66				
29-May-96	18:00	296.00			0.21	32.70	5.42	4.77	1.46	22.12	4.42	12.86
30-May-96	2:00	304.00			0.26	34.60	4.82	4.98	1.44	23.35	4.39	13.83
30-May-96	18:00	320.00			0.24	32.10	4.22	4.83	1.46	25.80	3.71	14.03
31-May-96	3:00	329.00			0.34	37.50	3.68	4.97	1.36	27.78	4.16	15.29
31-May-96	6:00	332.00							1.51	29.09	4.40	16.34
31-May-96	10:00	336.00		6.70E+07	0.25	32.95	3.28	4.77				
31-May-96	18:00	344.00			0.26	34.75	3.14	4.86				
1-Jun-96	2:00	352.00			0.28	36.15	2.72	4.99	1.59	29.94	4.66	17.04
1-Jun-96	10:00	360.00			0.23	33.97	2.51	4.81				
1-Jun-96	18:00	368.00			0.16	32.40	2.21	4.97	1.83	28.58	5.68	18.59
2-Jun-96	2:00	376.00			0.27	37.80	2.06	4.99	2.17	29.13	5.83	19.06
2-Jun-96	10:00	384.00			0.24	35.40	1.84	4.92				
2-Jun-96	18:00	392.00			0.21	33.10	1.58	4.98				
2-Jun-96	18:20	392.33							1.57	30.05	5.94	19.69
3-Jun-96	2:20	400.33			0.23	36.40	1.60	4.83	1.65	30.27	6.20	16.91
3-Jun-96	10:00	408.00		7.00E+07	0.19	33.35	1.39	4.74				
3-Jun-96	18:00	416.00			0.25	34.70	1.36	4.96	1.89	28.51	5.81	19.45
4-Jun-96	3:45	425.75			0.23	35.60	1.23	5.01	1.79	28.80	5.89	19.64
4-Jun-96	10:00	432.00		7.80E+07	0.24	35.10	1.11	5.09				
4-Jun-96	18:00	440.00			0.20	34.80	1.00	4.78	1.47	27.56	5.77	17.82
5-Jun-96	2:00	448.00			0.97	35.70	0.96	4.99	1.40	26.79	5.78	17.51
5-Jun-96	11:00	457.00		8.60E+07	0.23	36.40	0.96	5.01				

Run start data 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455B

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass counts/mL	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	HPLC (g/L)				
								pH	Glucose	Xylose	Gal.	Arab.
5-Jun-96	18:00	464.00			0.20	36.60	0.86	5.01	1.92	26.87	6.14	19.86
6-Jun-96	2:00	472.00			0.22	35.10	0.83	4.94	1.88	26.58	6.17	19.94
6-Jun-96	10:00	480.00		6.60E+07	0.25	38.50	0.87	5.01	0.00			
6-Jun-96	18:00	488.00			0.19	36.50	0.79	4.98	1.81	25.16	6.02	19.54
7-Jun-96	2:00	496.00			0.16	36.50	0.80	4.97	1.76	25.02	6.08	19.67
7-Jun-96	10:00	504.00		1.02E+08	0.17	39.00	0.82	5.08				
7-Jun-96	18:00	512.00			0.12	33.20	0.46	4.89	1.96	27.47	6.89	23.26
8-Jun-96	2:15	520.25			0.15	36.65	0.77	5.13	1.93	28.05	7.15	24.03
8-Jun-96	10:30	528.50			0.26	37.95	0.80	4.95				
8-Jun-96	18:00	536.00			0.20	34.45	0.86	4.91	1.98	22.87	6.11	20.43
9-Jun-96	2:00	544.00			0.13	37.15	0.75	5.00	1.95	22.65	6.13	20.41
9-Jun-96	10:00	552.00			0.24	42.05	0.78	4.99	1.59	21.82	5.95	19.48
9-Jun-96	18:00	560.00			0.19	42.63	0.75	4.76				
10-Jun-96	2:00	568.00			0.15	42.90	0.77	5.01	1.79	21.72	6.13	19.29
10-Jun-96	2:10	568.17							1.79	21.72	6.13	19.29
10-Jun-96	10:00	576.00		1.12E+08	0.23	38.40	0.73	5.02				
10-Jun-96	18:00	584.00			0.16	34.45	0.69	4.95	1.67	20.85	6.01	18.69
11-Jun-96	2:00	592.00			0.15	36.40	0.66	5.09	1.65	21.38	6.14	19.10
11-Jun-96	10:00	600.00		1.24E+08	0.15	35.30	0.67	4.89				
11-Jun-96	18:00	608.00			0.17	36.30	0.65	4.94	1.91	21.02	6.25	19.05
12-Jun-96	2:00	616.00			0.16	36.40	0.65	5.09	1.78	20.57	6.24	19.03
12-Jun-96	10:00	624.00			0.15	38.70	0.65	4.95				
12-Jun-96	18:00	632.00			0.12	37.65	0.66	4.94	2.20	20.86	6.78	19.81
13-Jun-96	2:00	640.00			0.08	39.10	0.64	5.00	1.98	19.67	6.37	19.23
13-Jun-96	10:30	648.50		8.25E+07	0.29	42.05	0.69	5.08				
13-Jun-96	18:00	656.00			0.12	34.80	0.65	5.06	2.16	18.38	6.35	18.54
14-Jun-96	2:00	664.00			0.16	36.60	0.71	5.16	1.97	17.48	6.00	17.53
14-Jun-96	10:00	672.00		9.45E+07	0.17	40.50	1.10	5.07				
14-Jun-96	18:15	680.25			0.17	32.68	1.98	5.10	1.81	15.76	5.35	12.82
15-Jun-96	2:00	688.00			0.09	32.70	2.40	4.92	1.65	14.94	5.02	10.72

Run start data

17-May-96

Run Name:

CRADA Task :

Run ID#:

P90605CF

Date	Time	Run time (h)	HPLC (g/L)									
			Man.	Cello.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
5-Jun-96	18:00	464.00	0.00	9.84	2.38	0.70	2.32	3.89	3.29	37.50	0.00	0.00
6-Jun-96	2:00	472.00	0.00	10.02	2.33	0.69	2.27	3.85	3.13	36.99	0.00	0.00
6-Jun-96	10:00	480.00			2.36	0.69	2.25	3.85	3.14	38.36	0.00	0.00
6-Jun-96	18:00	488.00	0.00	7.27	2.35	0.68	2.24	3.82	3.07	37.71	0.00	0.00
7-Jun-96	2:00	496.00	0.00	7.41	2.36	0.68	2.20	3.79	2.98	37.77	0.00	0.00
7-Jun-96	10:00	504.00										
7-Jun-96	18:00	512.00	0.00	8.40	3.21	0.00	2.23	3.85	3.00	38.43	0.00	0.00
8-Jun-96	2:15	520.25	0.00	8.83	3.26	0.00	2.24	3.87	2.99	39.04	0.00	0.00
8-Jun-96	10:30	528.50										
8-Jun-96	18:00	536.00	0.00	7.38	2.32	0.77	2.18	3.72	2.85	39.29	0.00	0.00
9-Jun-96	2:00	544.00	0.00	7.52	2.31	0.76	2.13	3.67	2.82	39.51	0.00	0.00
9-Jun-96	10:00	552.00	0.00	7.34	2.80	0.85	2.10	3.62	2.88	40.24	0.00	0.00
9-Jun-96	18:00	560.00										
10-Jun-96	2:00	568.00	0.00	12.15	2.78	0.86	2.05	3.51	2.81	39.61	0.00	0.00
10-Jun-96	2:10	568.17	0.00	12.15								
10-Jun-96	10:00	576.00										
10-Jun-96	18:00	584.00	0.00	7.61	2.91	0.87	2.05	3.46	2.91	41.12	0.00	0.00
11-Jun-96	2:00	592.00	0.00	7.95	2.85	0.85	1.97	3.26	2.85	40.33	0.00	0.00
11-Jun-96	10:00	600.00										
11-Jun-96	18:00	608.00	0.00	8.01	3.03	0.84	1.97	3.26	2.96	41.84	0.00	0.00
12-Jun-96	2:00	616.00	0.00	8.02	3.03	0.82	1.91	3.14	2.89	41.04	0.00	0.00
12-Jun-96	10:00	624.00										
12-Jun-96	18:00	632.00	0.00	7.98	3.04	0.83	1.93	3.11	2.92	41.31	0.00	0.00
13-Jun-96	2:00	640.00	0.00	7.92	3.11	0.84	1.89	3.05	2.89	41.03	0.00	0.00
13-Jun-96	10:30	648.50										
13-Jun-96	18:00	656.00	0.00	7.31	3.07	0.85	1.94	3.03	2.76	38.80	0.00	0.00
14-Jun-96	2:00	664.00	0.00	7.97	3.05	0.82	2.10	3.01	2.68	37.57	0.00	0.00
14-Jun-96	10:00	672.00										
14-Jun-96	18:15	680.25	0.00	6.30	2.99	0.77	4.53	2.88	4.36	34.55	0.00	0.00
15-Jun-96	2:00	688.00	0.00	5.90	2.98	0.75	5.43	2.86	5.05	33.25	0.00	0.00

Run start data 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455B

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass counts/mL	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	HPLC (g/L)					
								pH	Glucose	Xylose	Gal.	Arab.	
15-Jun-96	11:15	697.25			0.09	30.78	2.88	4.93					
15-Jun-96	18:00	704.00			0.09	33.63	2.90	5.00	1.38	14.36	4.02	12.05	
16-Jun-96	2:00	712.00			0.09	28.95	2.73	4.99	1.33	12.89	4.25	8.53	
16-Jun-96	10:00	720.00			0.15	31.95	2.64	5.02					
16-Jun-96	18:15	728.25			0.12	29.58	2.34	5.00	1.20	11.70	4.01	8.40	
17-Jun-96	2:00	736.00			0.13	27.88	2.13	5.02	1.20	11.50	4.02	8.79	
17-Jun-96	10:00	744.00		7.45E+07	0.13	26.20	1.90	5.05					
17-Jun-96	18:00	752.00			0.15	32.00	1.56	5.06	1.24	10.79	4.00	9.46	
18-Jun-96	2:00	760.00			0.07	26.10	1.55	5.00	1.11	10.22	3.83	9.45	
18-Jun-96	10:00	768.00		8.60E+07	0.15	24.80	1.39	5.16					
18-Jun-96	18:00	776.00			0.18	27.50	1.31	5.18	1.18	9.78	3.75	10.13	
19-Jun-96	2:00	784.00			0.10	27.52	1.11	5.09	1.11	9.58	3.68	10.35	
19-Jun-96	10:00	792.00		8.60E+07	0.12	27.80	1.03	5.18					
19-Jun-96	19:00	801.00			0.18	27.68	0.95	5.13	1.15	8.78	3.41	10.65	
20-Jun-96	2:00	808.00			0.09	28.15	0.88	5.04					
20-Jun-96	5:00	811.00							1.09	8.32	3.26	10.65	
20-Jun-96	10:00	816.00		8.50E+07	0.14	27.80	0.82	5.16					
20-Jun-96	19:30	825.50			0.13	27.68	0.78	5.16					
21-Jun-96	2:00	832.00			0.11	31.15	0.79	5.18	0.91	7.43	2.73	10.92	
21-Jun-96	10:00	840.00		8.05E+07	0.10	26.40	0.80	5.18					
21-Jun-96	18:20	848.33							1.04	6.97	2.37	9.50	
21-Jun-96	18:30	848.50			0.10	27.90	1.38	5.07					
21-Jun-96	19:30	849.50							1.00	7.57	2.91	10.91	
22-Jun-96	2:00	856.00			0.07	28.70	2.98	5.12	0.91	6.95	2.35	6.62	
22-Jun-96	10:00	864.00			0.09	27.10	4.39	5.10					
22-Jun-96	18:20	872.33			0.13	25.18	5.36	4.99	1.02	7.34	2.69	2.21	
23-Jun-96	2:00	880.00			0.07	26.40	6.32	5.02	0.91	7.36	2.70	1.01	
23-Jun-96	10:00	888.00			0.14	26.25	6.25	4.92	0.86	7.60	2.79	0.87	
23-Jun-96	18:00	896.00			0.12	23.60	6.40	4.96	1.01	7.76	2.89	1.01	
24-Jun-96	2:00	904.00			0.05	25.75	6.86	5.02	0.96	8.01	3.02	1.03	

Run start data

17-May-96

Run Name:

CRADA Task :

Run ID#:

P90605CF

Date	Time	Run time (h)	HPLC (g/L)										
			Man.	Cello.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural	
15-Jun-96	11:15	697.25											
15-Jun-96	18:00	704.00	0.00	4.40	2.81	0.69	5.56	2.79	5.31	30.80	0.00	0.00	
16-Jun-96	2:00	712.00	0.66	4.77	2.76	0.68	5.36	2.82	5.24	30.07	0.00	0.00	
16-Jun-96	10:00	720.00											
16-Jun-96	18:15	728.25	0.00	4.58	2.66	0.67	4.65	2.84	4.83	29.06	0.00	0.00	
17-Jun-96	2:00	736.00	0.00	4.55	2.53	0.66	4.23	2.85	4.53	28.27	0.00	0.00	
17-Jun-96	10:00	744.00											
17-Jun-96	18:00	752.00	0.00	4.23	2.84	0.00	3.54	2.85	3.97	27.45	0.00	0.00	
18-Jun-96	2:00	760.00	0.00	3.86	2.85	0.00	3.22	2.88	3.68	27.19	0.00	0.00	
18-Jun-96	10:00	768.00											
18-Jun-96	18:00	776.00	0.00	3.72	2.06	0.63	2.68	2.87	3.23	27.04	0.00	0.00	
19-Jun-96	2:00	784.00	0.00	3.71	2.48	0.63	2.48	2.88	3.04	26.85	0.00	0.00	
19-Jun-96	10:00	792.00											
19-Jun-96	19:00	801.00	0.00	3.68	2.60	0.62	2.15	2.98	2.77	27.22	0.00	0.00	
20-Jun-96	2:00	808.00											
20-Jun-96	5:00	811.00	0.00	3.67	2.65	0.62	2.02	2.98	2.61	27.23	0.00	0.00	
20-Jun-96	10:00	816.00											
20-Jun-96	19:30	825.50											
21-Jun-96	2:00	832.00	0.00	3.70	2.86	0.61	1.77	3.13	2.35	27.09	0.00	0.00	
21-Jun-96	10:00	840.00											
21-Jun-96	18:20	848.33	0.00	6.19	3.42	0.00	2.84	3.22	2.93	27.15	0.00	0.00	
21-Jun-96	18:30	848.50											
21-Jun-96	19:30	849.50	0.00	3.68	2.77	0.62	1.83	3.12	2.43	27.21	0.00	0.00	
22-Jun-96	2:00	856.00	0.00	6.29	3.40	0.00	5.04	3.21	4.26	26.50	0.00	0.00	
22-Jun-96	10:00	864.00											
22-Jun-96	18:20	872.33	0.00	3.61	2.87	0.62	8.70	3.31	6.48	25.35	0.00	0.00	
23-Jun-96	2:00	880.00	0.00	3.65	3.32	0.83	9.74	3.30	7.10	25.14	0.00	0.00	
23-Jun-96	10:00	888.00	0.00	3.77	3.34	0.35	10.15	3.26	7.22	24.75	0.00	0.00	
23-Jun-96	18:00	896.00	0.00	3.81	3.36	0.27	10.27	3.28	7.31	24.46	0.00	0.00	
24-Jun-96	2:00	904.00	0.00	3.97	3.26	0.32	10.50	3.25	7.32	24.29	0.00	0.00	

Run start date 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455C

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass counts/mL	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	pH	Total Solids	
									Oven (%)	TDS (%)
5/20/96	2:00	64.00			58.6	2.31	0.55	4.81	0.00%	0.00%
5/20/96	10:00	72.00			57.5	2.38	0.57	4.95		
20-May-96	18:00	80.00			50.4	1.16		4.95	0.00%	0.00%
21-May-96	0:00	86.00							0.00%	0.00%
21-May-96	2:00	88.00			34.9	11.2	0.54	5.52	0.00%	0.00%
21-May-96	10:50	96.83			1.37	22.8	0.53	5.08	0.00%	0.00%
21-May-96	18:00	104.00			3.92			5.1	0.00%	0.00%
22-May-96	2:00	112.00			0.26	24.75		5.05	0.00%	0.00%
22-May-96	10:20	120.33			1.04	30.4	0.48	5.01		
22-May-96	18:00	128.00			0.27	24.2		5.01	0.00%	0.00%
23-May-96	2:00	136.00			1.15	30.63	0.47	5.06	0.00%	0.00%
23-May-96	11:15	145.25		1.52E+08	1.13	33.25	0.49	4.93	0.00%	0.00%
23-May-96	18:00	152.00			1.2	26.4	0.49	4.96	0.00%	0.00%
24-May-96	1:30	159.50							0.00%	0.00%
24-May-96	2:00	160.00			0.57	29.15	0.31	4.96	0.00%	0.00%
24-May-96	11:30	169.50		1.67E+08	1.18	37.2	0.47	4.81		
24-May-96	18:00	176.00			1.21	35.55	0.52	4.93		
25-May-96	2:00	184.00			1.14	34.35	0.76	4.76	0.00%	0.00%
25-May-96	10:00	192.00		1.55E+08	0.97	35.7	1.4	4.61		
25-May-96	18:00	200.00			1.04	30.1	2	6.13	0.00%	0.00%
26-May-96	2:00	208.00			0.83	31.3	2.6	5.76	0.00%	0.00%
26-May-96	10:00	216.00			0.79	34	3.69	5.24		
27-May-96	10:00	240.00			0.78	32.9	8.41	4.62		
27-May-96	18:00	248.00			0.87	31.7	8.16	4.78	0.00%	0.00%
28-May-96	2:00	256.00			0.85	32	8.8	5.01	0.00%	0.00%
28-May-96	10:00	264.00			0.14	33.8	9.01	4.85		
28-May-96	18:00	272.00			0.15	30.83	9.08	4.8	0.00%	0.00%
29-May-96	2:00	280.00			0.14	33.1	9.02	5.03	0.00%	0.00%
29-May-96	10:00	288.00		1.15E+08	0.12	32.9	8.27	4.73		

Run start date 17-May-96
 Run Name: CRADA Task !
 Run ID#: P90605CF

Date	Time	Run time (h)	Liquor Analysis (Total Sugars, g/L)				
			Glucose	Xylose	Galactose	Arabinose	Mannose
5/20/96	2:00	64.00	0.00	0.00	0.00	0.00	0.00
5/20/96	10:00	72.00					
20-May-96	18:00	80.00	0.00	0.00	0.00	0.00	0.00
21-May-96	0:00	86.00	0.00	0.00	0.00	0.00	0.00
21-May-96	2:00	88.00	0.00	0.00	0.00	0.00	0.00
21-May-96	10:50	96.83					
21-May-96	18:00	104.00	0.00	0.00	0.00	0.00	0.00
22-May-96	2:00	112.00	0.00	0.00	0.00	0.00	0.00
22-May-96	10:20	120.33					
22-May-96	18:00	128.00	0.00	0.00	0.00	0.00	0.00
23-May-96	2:00	136.00	0.00	0.00	0.00	0.00	0.00
23-May-96	11:15	145.25					
23-May-96	18:00	152.00	0.00	0.00	0.00	0.00	0.00
24-May-96	1:30	159.50	0.00	0.00	0.00	0.00	0.00
24-May-96	2:00	160.00	0.00	0.00	0.00	0.00	0.00
24-May-96	11:30	169.50					
24-May-96	18:00	176.00					
25-May-96	2:00	184.00	0.00	0.00	0.00	0.00	0.00
25-May-96	10:00	192.00					
25-May-96	18:00	200.00	0.00	0.00	0.00	0.00	0.00
26-May-96	2:00	208.00	0.00	0.00	0.00	0.00	0.00
26-May-96	10:00	216.00					
27-May-96	10:00	240.00					
27-May-96	18:00	248.00	0.00	0.00	0.00	0.00	0.00
28-May-96	2:00	256.00	0.00	0.00	0.00	0.00	0.00
28-May-96	10:00	264.00					
28-May-96	18:00	272.00	0.00	0.00	0.00	0.00	0.00
29-May-96	2:00	280.00	13.69	21.22	4.82	8.07	0.00
29-May-96	10:00	288.00					

Run start date 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455C

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass counts/mL	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	pH	Total Solids	
									Oven (%)	TDS (%)
29-May-96	18:00	296.00			0.14	31.98	8.38	4.75	0.00%	0.00%
30-May-96	2:00	304.00			0.17	34.55	7.4	5.04	0.00%	0.00%
30-May-96	18:00	320.00			0.13	31.38	6.84	4.84	0.00%	0.00%
31-May-96	3:00	329.00			0.18	34.25	6.26	5.02	0.00%	0.00%
31-May-96	6:00	332.00							0.00%	0.00%
31-May-96	10:00	336.00		7.60E+07	0.14	32.23	5.66	4.79		
31-May-96	18:00	344.00			0.18	33.1	5.5	4.87		
1-Jun-96	2:00	352.00			0.2	35.45	4.92	5	0.00%	0.00%
1-Jun-96	10:00	360.00			0.17	29.55	4.52	4.86		
1-Jun-96	18:00	368.00			0.2	32.1	4	4.97	0.00%	0.00%
2-Jun-96	2:00	376.00			0.19	36	3.76	4.99	0.00%	0.00%
2-Jun-96	10:00	384.00			0.23	33.6	3.35	4.94		
2-Jun-96	18:00	392.00			0.21	32.4	2.88	4.95		
2-Jun-96	18:20	392.33							0.00%	0.00%
3-Jun-96	2:20	400.33			0.18	34.7	2.78	4.81	0.00%	0.00%
3-Jun-96	10:00	408.00		7.60E+07	0.18	33.45	2.42	4.76		
3-Jun-96	18:00	416.00			0.24	36.6	2.19	4.92	0.00%	0.00%
4-Jun-96	3:45	425.75			0.2	34.7	2.1	4.96	0.00%	0.00%
4-Jun-96	10:00	432.00		6.70E+07	0.2	35.3	1.74	5.01		
4-Jun-96	18:00	440.00			0.18	38.3	1.62	4.75	0.00%	0.00%
5-Jun-96	2:00	448.00			0.18	35.4	1.49	4.93	0.00%	0.00%
5-Jun-96	11:00	457.00		8.30E+07	0.24	37.9	1.4	4.99		
5-Jun-96	18:00	464.00			0.23	40.2	1.21	4.95	0.00%	0.00%
6-Jun-96	2:00	472.00			0.19	36.8	1.15	4.83	0.00%	0.00%
6-Jun-96	10:00	480.00		8.35E+07	0.23	37.55	1.16	4.97	16.73%	0.00%
6-Jun-96	18:00	488.00			0.23	35.8	1.13	4.94	0.00%	0.00%
7-Jun-96	2:00	496.00			0.15	37.8	0.99	4.91	0.00%	0.00%
7-Jun-96	10:00	504.00		1.02E+08	0.16	39.35	0.96	5.01		
7-Jun-96	18:00	512.00			0.23	34.8	0.97	5.12	0.00%	0.00%
8-Jun-96	2:15	520.25			0.14	36.83	0.87	5.08	0.00%	0.00%

Run start date

17-May-96

Run Name:

CRADA Task 1

Run ID#:

P90605CF

Date	Time	Run time (h)	Washed Solids		Sample Wt. (g)	HPLC (g/L)						
			Weight (g)	TS (%)		Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol
29-May-96	18:00	296.00	0.00	0.00%	0.00	1.20	16.56	3.72	7.57	0.00	6.73	2.81
30-May-96	2:00	304.00	0.00	0.00%	0.00	1.17	17.65	3.74	8.53	0.00	6.98	2.78
30-May-96	18:00	320.00	0.00	0.00%	0.00	1.24	19.36	3.10	8.80	0.00	8.98	2.81
31-May-96	3:00	329.00	0.00	0.00%	0.00	1.23	21.03	3.20	10.12	0.00	9.32	2.72
31-May-96	6:00	332.00	0.00	0.00%	0.00	1.30	23.18	3.31	11.83	0.00	9.72	2.17
31-May-96	10:00	336.00										
31-May-96	18:00	344.00										
1-Jun-96	2:00	352.00	0.00	0.00%	0.00	1.33	24.57	3.51	12.91	0.00	10.14	2.19
1-Jun-96	10:00	360.00										
1-Jun-96	18:00	368.00	0.00	0.00%	0.00	1.68	24.81	4.66	15.43	0.00	9.58	2.31
2-Jun-96	2:00	376.00	0.00	0.00%	0.00	1.64	25.34	4.83	15.97	0.00	9.75	2.33
2-Jun-96	10:00	384.00										
2-Jun-96	18:00	392.00										
2-Jun-96	18:20	392.33	0.00	0.00%	0.00	1.53	26.43	5.15	17.14	0.00	10.26	2.33
3-Jun-96	2:20	400.33	0.00	0.00%	0.00	1.51	27.30	5.38	17.84	0.00	10.66	2.35
3-Jun-96	10:00	408.00										
3-Jun-96	18:00	416.00	0.00	0.00%	0.00	1.65	26.15	5.18	18.00	0.00	10.23	2.37
4-Jun-96	3:45	425.75	0.00	0.00%	0.00	1.59	26.49	5.38	18.48	0.00	10.39	2.41
4-Jun-96	10:00	432.00										
4-Jun-96	18:00	440.00	0.00	0.00%	0.00	1.40	25.34	5.20	16.85	0.00	10.14	2.47
5-Jun-96	2:00	448.00	0.00	0.00%	0.00	1.37	25.18	5.32	16.95	0.00	10.38	2.43
5-Jun-96	11:00	457.00										
5-Jun-96	18:00	464.00	0.00	0.00%	0.00	1.88	25.75	5.86	19.72	0.00	6.65	2.46
6-Jun-96	2:00	472.00	0.00	0.00%	0.00	1.83	25.42	5.86	19.67	0.00	6.64	2.47
6-Jun-96	10:00	480.00	101.30	17.07%	393.27	0.55	21.84	4.38	17.47	0.00	2.38	2.51
6-Jun-96	18:00	488.00	0.00	0.00%	0.00	1.79	24.01	5.74	19.24	0.00	6.48	2.54
7-Jun-96	2:00	496.00	0.00	0.00%	0.00	1.72	23.73	5.79	19.30	0.00	6.55	2.53
7-Jun-96	10:00	504.00										
7-Jun-96	18:00	512.00	0.00	0.00%	0.00	1.99	26.50	6.75	23.40	0.00	7.56	3.41
8-Jun-96	2:15	520.25	0.00	0.00%	0.00	1.91	26.41	6.86	23.71	0.00	7.75	3.39

Run start date 17-May-96
 Run Name: CRADA Task I
 Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)							GC Ethanol	Acid Sol. Lignin (g/L)
			Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural		
29-May-96	18:00	296.00	0.87	10.68	3.01	9.11	31.42	0.00	0.00	0.00	0.00
30-May-96	2:00	304.00	0.86	10.16	3.07	8.93	31.44	0.00	0.00	0.00	0.00
30-May-96	18:00	320.00	0.75	9.43	3.16	8.70	31.50	0.00	0.00	0.00	0.00
31-May-96	3:00	329.00	0.76	8.63	3.28	8.33	31.64	0.00	0.00	0.00	0.00
31-May-96	6:00	332.00	0.76	7.57	3.46	7.84	32.66	0.00	0.00	0.00	0.00
31-May-96	10:00	336.00									
31-May-96	18:00	344.00									
1-Jun-96	2:00	352.00	0.76	6.97	3.60	7.50	33.11	0.00	0.00	0.00	0.00
1-Jun-96	10:00	360.00									
1-Jun-96	18:00	368.00	0.73	6.00	3.72	6.99	34.24	0.00	0.00	0.00	0.00
2-Jun-96	2:00	376.00	0.74	5.60	3.80	6.76	34.44	0.00	0.00	0.00	0.00
2-Jun-96	10:00	384.00									
2-Jun-96	18:00	392.00									
2-Jun-96	18:20	392.33	0.72	4.72	3.84	6.11	34.55	0.00	0.00	0.00	0.00
3-Jun-96	2:20	400.33	0.73	4.42	3.91	5.90	35.55	0.00	0.00	0.00	0.00
3-Jun-96	10:00	408.00									
3-Jun-96	18:00	416.00	0.73	3.90	3.96	5.48	36.38	0.00	0.00	0.00	0.00
4-Jun-96	3:45	425.75	0.74	3.61	3.96	5.16	36.83	0.00	0.00	0.00	0.00
4-Jun-96	10:00	432.00									
4-Jun-96	18:00	440.00	0.74	3.25	3.96	4.75	37.64	0.00	0.00	0.00	0.00
5-Jun-96	2:00	448.00	0.73	3.06	3.92	4.51	37.79	0.00	0.00	0.00	0.00
5-Jun-96	11:00	457.00									
5-Jun-96	18:00	464.00	0.74	2.79	3.95	4.15	37.78	0.00	0.00	0.00	0.00
6-Jun-96	2:00	472.00	0.73	2.70	3.94	4.01	37.84	0.00	0.00	0.00	0.00
6-Jun-96	10:00	480.00	0.82	2.64	3.93	3.96	39.05	0.00	0.00	37.37	4.36
6-Jun-96	18:00	488.00	0.79	2.67	4.12	3.89	37.98	0.00	0.00	0.00	0.00
7-Jun-96	2:00	496.00	0.73	2.48	3.92	3.67	38.84	0.00	0.00	0.00	0.00
7-Jun-96	10:00	504.00									
7-Jun-96	18:00	512.00	0.00	2.43	3.99	3.55	39.32	0.00	0.00	0.00	0.00
8-Jun-96	2:15	520.25	0.00	2.36	3.91	3.44	39.10	0.00	0.00	0.00	0.00

Run start date 17-May-96
 Run Name: CRADA Task I
 Run ID#: P90605CF

Date	Time	Run time (h)	Liquor Analysis (Total Sugars, g/L)				
			Glucose	Xylose	Galactose	Arabinose	Mannose
29-May-96	18:00	296.00	0.00	0.00	0.00	0.00	0.00
30-May-96	2:00	304.00	0.00	0.00	0.00	0.00	0.00
30-May-96	18:00	320.00	0.00	0.00	0.00	0.00	0.00
31-May-96	3:00	329.00	15.39	27.38	5.11	13.17	0.00
31-May-96	6:00	332.00	0.00	0.00	0.00	0.00	0.00
31-May-96	10:00	336.00					
31-May-96	18:00	344.00					
1-Jun-96	2:00	352.00	0.00	0.00	0.00	0.00	0.00
1-Jun-96	10:00	360.00					
1-Jun-96	18:00	368.00	0.00	0.00	0.00	0.00	0.00
2-Jun-96	2:00	376.00	0.00	0.00	0.00	0.00	0.00
2-Jun-96	10:00	384.00					
2-Jun-96	18:00	392.00					
2-Jun-96	18:20	392.33	0.00	0.00	0.00	0.00	0.00
3-Jun-96	2:20	400.33	0.00	0.00	0.00	0.00	0.00
3-Jun-96	10:00	408.00					
3-Jun-96	18:00	416.00	0.00	0.00	0.00	0.00	0.00
4-Jun-96	3:45	425.75	0.00	0.00	0.00	0.00	0.00
4-Jun-96	10:00	432.00					
4-Jun-96	18:00	440.00	0.00	0.00	0.00	0.00	0.00
5-Jun-96	2:00	448.00	0.00	0.00	0.00	0.00	0.00
5-Jun-96	11:00	457.00					
5-Jun-96	18:00	464.00	0.00	0.00	0.00	0.00	0.00
6-Jun-96	2:00	472.00	0.00	0.00	0.00	0.00	0.00
6-Jun-96	10:00	480.00	18.18	30.73	5.90	22.17	0.00
6-Jun-96	18:00	488.00	0.00	0.00	0.00	0.00	0.00
7-Jun-96	2:00	496.00	0.00	0.00	0.00	0.00	0.00
7-Jun-96	10:00	504.00					
7-Jun-96	18:00	512.00	0.00	0.00	0.00	0.00	0.00
8-Jun-96	2:15	520.25	0.00	0.00	0.00	0.00	0.00

Run start date 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455C

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass counts/mL	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	Total Solids	
								pH	Oven (%)
8-Jun-96	10:30	528.50			0.25	37.9	0.88	4.92	
8-Jun-96	18:00	536.00			0.2	34.67	0.93	4.82	0.00% 0.00%
9-Jun-96	2:00	544.00			0.2	38.25	0.81	4.94	0.00% 0.00%
9-Jun-96	10:00	552.00			0.23	42.35	0.87	4.94	0.00% 0.00%
9-Jun-96	18:00	560.00			0.19	42.45	0.78	4.71	
10-Jun-96	2:00	568.00			0.13	46.4	0.75	4.93	0.00% 0.00%
10-Jun-96	10:00	576.00		1.08E+08	0.26	38.2	0.79	4.97	
10-Jun-96	18:00	584.00			0.165	35.05	0.713	4.94	0.00% 0.00%
11-Jun-96	2:00	592.00			0.13	37.5	0.71	4.97	0.00% 0.00%
11-Jun-96	10:00	600.00		9.30E+07	0.137	35.93	0.711	4.84	
11-Jun-96	18:00	608.00			0.17	37.95	0.697	4.84	0.00% 0.00%
12-Jun-96	2:00	616.00			0.13	40.2	0.67	4.97	0.00% 0.00%
12-Jun-96	10:00	624.00			0.14	40.15	0.68	4.83	
12-Jun-96	18:00	632.00			0.12	37.3	0.667	4.82	0.00% 0.00%
13-Jun-96	2:00	640.00			0.08	40	0.66	4.87	0.00% 0.00%
13-Jun-96	10:30	648.50		1.10E+08	0.29	42.25	0.73	4.95	
13-Jun-96	18:00	656.00			0.12	38.2	0.7	4.89	0.00% 0.00%
14-Jun-96	2:00	664.00			0.18	41	0.76	4.98	0.00% 0.00%
14-Jun-96	10:00	672.00		1.03E+08	0.17	43.05	1	4.98	
14-Jun-96	18:15	680.25			0.18	33.23	1.54	5.03	0.00% 0.00%
15-Jun-96	2:00	688.00			0.1	35.2	2.4	4.82	0.00% 0.00%
15-Jun-96	11:15	697.25			0.1	35.88	3.42	4.88	
15-Jun-96	18:00	704.00			0.09	38.35	3.84	4.9	0.00% 0.00%
16-Jun-96	2:00	712.00			0.09	35.25	3.96	4.9	0.00% 0.00%
16-Jun-96	10:00	720.00			0.15	36.15	4.3	4.91	
16-Jun-96	18:15	728.25			0.13	33.55	4.2	4.88	0.00% 0.00%
17-Jun-96	2:00	736.00			0.12	32.25	4.22	4.93	0.00% 0.00%
17-Jun-96	10:00	744.00		8.15E+07	0.14	33.9	3.92	4.9	
17-Jun-96	18:00	752.00			0.18	37	3.76	4.86	
18-Jun-96	2:00	760.00			0.62	30.35	3.46	4.99	

Run start date

17-May-96

Run Name:

CRADA Task 1

Run ID#:

P90605CF

Date	Time	Run time (h)	Washed Solids		Sample Wt. (g)	HPLC (g/L)							
			Weight (g)	TS (%)		Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol	
8-Jun-96	10:30	528.50											
8-Jun-96	18:00	536.00	0.00	0.00%	0.00	1.98	21.75	5.92	20.36	0.00	6.43	2.50	
9-Jun-96	2:00	544.00	0.00	0.00%	0.00	1.96	21.52	5.98	20.43	0.00	6.66	2.48	
9-Jun-96	10:00	552.00	0.00	0.00%	0.00	1.76	20.42	5.99	19.41	0.00	11.82	3.03	
9-Jun-96	18:00	560.00											
10-Jun-96	2:00	568.00	0.00	0.00%	0.00	1.73	19.81	5.93	19.21	0.00	6.75	3.00	
10-Jun-96	10:00	576.00											
10-Jun-96	18:00	584.00	0.00	0.00%	0.00	1.64	20.81	5.96	18.70	0.00	7.47	2.83	
11-Jun-96	2:00	592.00	0.00	0.00%	0.00	1.62	18.30	5.81	18.52	0.00	6.53	3.13	
11-Jun-96	10:00	600.00											
11-Jun-96	18:00	608.00	0.00	0.00%	0.00	1.83	17.91	5.92	18.47	0.00	6.85	3.26	
12-Jun-96	2:00	616.00	0.00	0.00%	0.00	1.83	18.26	6.18	19.19	0.00	7.12	3.33	
12-Jun-96	10:00	624.00											
12-Jun-96	18:00	632.00	0.00	0.00%	0.00	2.18	18.14	6.64	19.57	0.00	7.19	3.37	
13-Jun-96	2:00	640.00	0.00	0.00%	0.00	1.92	17.07	6.37	19.21	0.00	6.86	3.42	
13-Jun-96	10:30	648.50											
13-Jun-96	18:00	656.00	0.00	0.00%	0.00	2.28	16.27	6.67	19.74	0.00	6.63	3.58	
14-Jun-96	2:00	664.00	0.00	0.00%	0.00	2.12	15.35	6.47	19.61	0.00	6.38	3.69	
14-Jun-96	10:00	672.00											
14-Jun-96	18:15	680.25	0.00	0.00%	0.00	2.03	15.30	6.10	15.69	0.00	6.31	3.58	
15-Jun-96	2:00	688.00	0.00	0.00%	0.00	1.91	14.94	5.83	13.29	0.00	6.19	3.50	
15-Jun-96	11:15	697.25											
15-Jun-96	18:00	704.00	0.00	0.00%	0.00	1.66	13.15	5.02	8.92	0.00	5.22	3.54	
16-Jun-96	2:00	712.00	0.00	0.00%	0.00	1.58	13.03	4.91	8.00	0.00	5.13	3.52	
16-Jun-96	10:00	720.00											
16-Jun-96	18:15	728.25	0.00	0.00%	0.00	1.48	12.17	4.67	6.67	0.00	4.93	3.45	
17-Jun-96	2:00	736.00	0.00	0.00%	0.00	1.37	11.68	4.50	6.36	0.00	4.77	3.34	
17-Jun-96	10:00	744.00											
17-Jun-96	18:00	752.00				1.40	10.94	4.42	6.40	0.00	4.26	3.65	
18-Jun-96	2:00	760.00				1.26	10.34	4.21	6.59	0.00	4.01	3.58	

Run start date 17-May-96
 Run Name: CRADA Task !
 Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)							GC	Acid Sol. Lignin
			Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural	Ethanol	(g/L)
8-Jun-96	10:30	528.50									
8-Jun-96	18:00	536.00	0.84	2.29	3.90	3.32	39.95	0.00	0.00	0.00	0.00
9-Jun-96	2:00	544.00	0.83	2.24	3.86	3.22	39.62	0.00	0.00	0.00	0.00
9-Jun-96	10:00	552.00	0.95	2.22	3.85	3.23	40.75	0.00	0.00	0.00	0.00
9-Jun-96	18:00	560.00									
10-Jun-96	2:00	568.00	0.92	2.13	3.71	3.12	40.19	0.00	0.00	0.00	0.00
10-Jun-96	10:00	576.00									
10-Jun-96	18:00	584.00	0.86	1.98	3.34	2.83	39.44	0.00	0.00	0.00	0.00
11-Jun-96	2:00	592.00	0.94	2.05	3.62	3.04	41.09	0.00	0.00	0.00	0.00
11-Jun-96	10:00	600.00									
11-Jun-96	18:00	608.00	0.92	2.01	3.58	3.07	41.53	0.00	0.00	0.00	0.00
12-Jun-96	2:00	616.00	0.94	2.02	3.56	3.13	42.30	0.00	0.00	0.00	0.00
12-Jun-96	10:00	624.00									
12-Jun-96	18:00	632.00	0.92	1.96	3.41	3.10	42.10	0.00	0.00	0.00	0.00
13-Jun-96	2:00	640.00	0.94	1.96	3.39	3.15	42.06	0.00	0.00	0.00	0.00
13-Jun-96	10:30	648.50									
13-Jun-96	18:00	656.00	1.61	2.06	3.43	3.35	42.09	0.00	0.00	0.00	0.00
14-Jun-96	2:00	664.00	1.03	2.18	3.43	3.53	42.16	0.00	0.00	0.00	0.00
14-Jun-96	10:00	672.00									
14-Jun-96	18:15	680.25	0.95	3.98	3.22	4.50	39.38	0.00	0.00	0.00	0.00
15-Jun-96	2:00	688.00	0.93	5.36	3.15	5.41	38.23	0.00	0.00	0.00	0.00
15-Jun-96	11:15	697.25									
15-Jun-96	18:00	704.00	0.88	7.27	3.13	6.67	36.27	0.00	0.00	0.00	0.00
16-Jun-96	2:00	712.00	0.87	7.67	3.15	6.97	35.60	0.00	0.00	0.00	0.00
16-Jun-96	10:00	720.00									
16-Jun-96	18:15	728.25	0.86	7.93	3.13	7.24	34.28	0.00	0.00	0.00	0.00
17-Jun-96	2:00	736.00	0.84	7.69	3.07	7.09	33.42	0.00	0.00	0.00	0.00
17-Jun-96	10:00	744.00									
17-Jun-96	18:00	752.00	0.00	7.01	3.06	6.61	31.74	0.00	0.00		
18-Jun-96	2:00	760.00	0.00	6.56	3.05	6.30	31.02	0.00	0.00		

Run start date

17-May-96

Run Name:

CRADA Task 1

Run ID#:

P90605CF

Date	Time	Run time (h)	Liquor Analysis (Total Sugars, g/L)				
			Glucose	Xylose	Galactose	Arabinose	Mannose
8-Jun-96	10:30	528.50					
8-Jun-96	18:00	536.00	0.00	0.00	0.00	0.00	0.00
9-Jun-96	2:00	544.00	0.00	0.00	0.00	0.00	0.00
9-Jun-96	10:00	552.00	0.00	0.00	0.00	0.00	0.00
9-Jun-96	18:00	560.00					
10-Jun-96	2:00	568.00	0.00	0.00	0.00	0.00	0.00
10-Jun-96	10:00	576.00					
10-Jun-96	18:00	584.00	0.00	0.00	0.00	0.00	0.00
11-Jun-96	2:00	592.00	0.00	0.00	0.00	0.00	0.00
11-Jun-96	10:00	600.00					
11-Jun-96	18:00	608.00	0.00	0.00	0.00	0.00	0.00
12-Jun-96	2:00	616.00	0.00	0.00	0.00	0.00	0.00
12-Jun-96	10:00	624.00					
12-Jun-96	18:00	632.00	0.00	0.00	0.00	0.00	0.00
13-Jun-96	2:00	640.00	0.00	0.00	0.00	0.00	0.00
13-Jun-96	10:30	648.50					
13-Jun-96	18:00	656.00	0.00	0.00	0.00	0.00	0.00
14-Jun-96	2:00	664.00	0.00	0.00	0.00	0.00	0.00
14-Jun-96	10:00	672.00					
14-Jun-96	18:15	680.25	0.00	0.00	0.00	0.00	0.00
15-Jun-96	2:00	688.00	0.00	0.00	0.00	0.00	0.00
15-Jun-96	11:15	697.25					
15-Jun-96	18:00	704.00	0.00	0.00	0.00	0.00	0.00
16-Jun-96	2:00	712.00	0.00	0.00	0.00	0.00	0.00
16-Jun-96	10:00	720.00					
16-Jun-96	18:15	728.25	0.00	0.00	0.00	0.00	0.00
17-Jun-96	2:00	736.00	0.00	0.00	0.00	0.00	0.00
17-Jun-96	10:00	744.00					
17-Jun-96	18:00	752.00					
18-Jun-96	2:00	760.00					

Run start date 17-May-96 Time 4.17E-01
 Run Name: CRADA Task 5
 Run ID#: P90605CF

PDU Analytical Results
Vessel: V-455C

Date	Time	Run time (h)	O.D.	Cell Mass	YSI Gluc	YSI EtOH	YSI Lactate	Total Solids		
			600 nm	counts/mL	(g/L)	(g/L)	(g/L)	pH	Oven (%)	TDS (%)
18-Jun-96	10:00	768.00		8.40E+07	0.13	30.8	3.16	5.11		
18-Jun-96	18:00	776.00			0.17	29.35	3.16	5.12		
19-Jun-96	2:00	784.00			0.11	29.6	2.74	5.02		
19-Jun-96	10:00	792.00		9.00E+07	0.12	30	2.51	5.08		
19-Jun-96	19:00	801.00			0.21	29.78	2.33	5.06	0.00%	0.00%
20-Jun-96	2:00	808.00			0.09	30.52	2.14	4.96		
20-Jun-96	5:00	811.00							0.00%	0.00%
20-Jun-96	10:00	816.00		7.40E+07	0.15	30	1.89	5.08		
20-Jun-96	19:30	825.50			0.16	28.93	1.83	5.05		
21-Jun-96	2:00	832.00			0.09	32.55	1.68	5.1	0.00%	0.00%
21-Jun-96	10:00	840.00		7.45E+07	0.11	27.4	1.47	5.11		
21-Jun-96	15:00	845.00							10.38%	0.00%
21-Jun-96	18:20	848.33							0.00%	0.00%
21-Jun-96	18:30	848.50			0.12	29.33	1.74	5.05		
21-Jun-96	19:30	849.50							0.00%	0.00%
22-Jun-96	2:00	856.00			0.06	31.55	2.4	5.06	0.00%	0.00%
22-Jun-96	10:00	864.00			0.12	29.6	3.66	5.08		
22-Jun-96	18:20	872.33			0.12	27.13	5.06	5.02	0.00%	0.00%
23-Jun-96	2:00	880.00			0.05	28.85	6.32	5.05	0.00%	0.00%
23-Jun-96	10:00	888.00			0.14	25.42	6.63	5.02	0.00%	0.00%
23-Jun-96	18:00	896.00			0.09	25.95	6.57	4.99	0.00%	0.00%

Run start date

17-May-96

Run Name:

CRADA Task 1

Run ID#:

P90605CF

Date	Time	Run time (h)	Washed Solids		Sample Wt. (g)	HPLC (g/L)								
			Weight (g)	TS (%)		Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol		
18-Jun-96	10:00	768.00												
18-Jun-96	18:00	776.00				1.32	9.70	4.13	7.33	0.00	3.84	3.05		
19-Jun-96	2:00	784.00				1.21	9.39	4.02	7.59	0.00	3.78	3.03		
19-Jun-96	10:00	792.00												
19-Jun-96	19:00	801.00	0.00	0.00%	0.00	1.23	8.43	3.83	7.99	0.00	3.51	3.02		
20-Jun-96	2:00	808.00												
20-Jun-96	5:00	811.00	0.00	0.00%	0.00	1.15	8.02	3.70	8.47	0.00	3.55	3.03		
20-Jun-96	10:00	816.00												
20-Jun-96	19:30	825.50												
21-Jun-96	2:00	832.00	0.00	0.00%	0.00	0.98	6.82	3.27	9.26	0.00	3.33	3.17		
21-Jun-96	10:00	840.00												
21-Jun-96	15:00	845.00	67.34	21.78%	400.61	0.23	2.90	1.23	5.65	0.00	1.02	3.77		
21-Jun-96	18:20	848.33	0.00	0.00%	0.00	1.02	6.04	2.80	9.21	0.00	5.89	3.71		
21-Jun-96	18:30	848.50												
21-Jun-96	19:30	849.50	0.00	0.00%	0.00	1.07	7.17	3.44	9.07	0.00	3.34	3.10		
22-Jun-96	2:00	856.00	0.00	0.00%	0.00	0.94	5.84	2.61	7.88	0.00	5.86	3.79		
22-Jun-96	10:00	864.00												
22-Jun-96	18:20	872.33	0.00	0.00%	0.00	0.96	5.59	2.51	3.08	0.00	3.05	3.38		
23-Jun-96	2:00	880.00	0.00	0.00%	0.00	0.89	5.55	2.50	1.14	0.00	3.06	3.69		
23-Jun-96	10:00	888.00	0.00	0.00%	0.00	0.84	5.68	2.53	0.82	0.00	3.14	4.11		
23-Jun-96	18:00	896.00	0.00	0.00%	0.00	1.09	5.88	2.61	0.89	0.00	3.18	4.00		

Run start date 17-May-96
 Run Name: CRADA Task !
 Run ID#: P90605CF

Date	Time	Run time (h)	HPLC (g/L)							GC Ethanol	Acid Sol. Lignin (g/L)
			Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural		
18-Jun-96	10:00	768.00									
18-Jun-96	18:00	776.00	0.78	5.71	3.08	5.68	29.85	0.00	0.00		
19-Jun-96	2:00	784.00	0.76	5.32	3.05	5.37	29.71	0.00	0.00		
19-Jun-96	10:00	792.00									
19-Jun-96	19:00	801.00	0.73	4.51	3.08	4.76	29.33	0.00	0.00	0.00	0.00
20-Jun-96	2:00	808.00									
20-Jun-96	5:00	811.00	0.73	4.18	3.10	4.50	29.24	0.00	0.00	0.00	0.00
20-Jun-96	10:00	816.00									
20-Jun-96	19:30	825.50									
21-Jun-96	2:00	832.00	0.73	3.33	3.21	3.76	29.14	0.00	0.00	0.00	0.00
21-Jun-96	10:00	840.00									
21-Jun-96	15:00	845.00	0.00	3.19	3.29	3.59	29.21	0.00	0.00	29.57	4.66
21-Jun-96	18:20	848.33	0.00	3.32	3.24	3.54	29.03	0.00	0.00	0.00	0.00
21-Jun-96	18:30	848.50									
21-Jun-96	19:30	849.50	0.73	3.52	3.16	3.93	29.01	0.00	0.00	0.00	0.00
22-Jun-96	2:00	856.00	0.00	4.43	3.29	4.07	28.59	0.00	0.00	0.00	0.00
22-Jun-96	10:00	864.00									
22-Jun-96	18:20	872.33	0.74	8.19	3.30	6.23	27.72	0.00	0.00	0.00	0.00
23-Jun-96	2:00	880.00	0.37	9.74	3.33	7.19	27.47	0.00	0.00	0.00	0.00
23-Jun-96	10:00	888.00	0.50	10.39	3.36	7.43	26.98	0.00	0.00	0.00	0.00
23-Jun-96	18:00	896.00	0.00	10.71	3.34	7.53	26.74	0.00	0.00	0.00	0.00

Run start date

17-May-96

Run Name:

CRADA Task :

Run ID#:

P90605CF

Date	Time	Run time (h)	Liquor Analysis (Total Sugars, g/L)				
			Glucose	Xylose	Galactose	Arabinose	Mannose
18-Jun-96	10:00	768.00					
18-Jun-96	18:00	776.00					
19-Jun-96	2:00	784.00					
19-Jun-96	10:00	792.00					
19-Jun-96	19:00	801.00	0.00	0.00	0.00	0.00	0.00
20-Jun-96	2:00	808.00					
20-Jun-96	5:00	811.00	0.00	0.00	0.00	0.00	0.00
20-Jun-96	10:00	816.00					
20-Jun-96	19:30	825.50					
21-Jun-96	2:00	832.00	0.00	0.00	0.00	0.00	0.00
21-Jun-96	10:00	840.00					
21-Jun-96	15:00	845.00	10.38	8.70	2.79	9.92	0.00
21-Jun-96	18:20	848.33	0.00	0.00	0.00	0.00	0.00
21-Jun-96	18:30	848.50					
21-Jun-96	19:30	849.50	0.00	0.00	0.00	0.00	0.00
22-Jun-96	2:00	856.00	0.00	0.00	0.00	0.00	0.00
22-Jun-96	10:00	864.00					
22-Jun-96	18:20	872.33	0.00	0.00	0.00	0.00	0.00
23-Jun-96	2:00	880.00	0.00	0.00	0.00	0.00	0.00
23-Jun-96	10:00	888.00	0.00	0.00	0.00	0.00	0.00
23-Jun-96	18:00	896.00	0.00	0.00	0.00	0.00	0.00

Appendix C

Effect of Hydrolyzate Dilution on Fermentation Performance of LNHST2

C.1 Objective

A shake flask study was performed to examine the fermentation performance of two hydrolyzates (APR-330 [Task 4, 4/29/96, 21:00, run time 1040.5 hours] and APR-392 [Task 5, 6/5/96, 04:00, run time 450 hours]) at solids concentrations of 25%, 18%, and 12%. Fermentation performance of hydrolyzate with and without solids present was also examined at the same effective solids concentration.

C.2 Materials and Methods

C.2.1 Inoculum Preparation

Inoculum was generated in two stages in a shaking incubator at 30°C and 150 rpm. The first stage, consisting of 50 mL of YEPD (1% w/v yeast extract, 2% w/v peptone and 2% w/v glucose, pH 5) in a 250 mL baffled Erlenmeyer flask, was inoculated with 1 mL of LNHST2 from a frozen seed stock. After a 12 hour incubation, a 10% v/v inoculum was transferred to a second stage consisting of 135 mL CSL medium (1% w/v CSL, 2% w/v glucose, pH 5) in a 500 mL baffled Erlenmeyer flask. The second stage was incubated for only 5.5 hours by which time a majority of the glucose was consumed (exponential growth stage). A 10% v/v inoculum from the second stage was used as inoculum for the hydrolyzate shake flasks.

C.2.2 Preparation of Liquor Hydrolyzate

This study was carried out in shake flasks using the liquid fraction of the hydrolyzate separated from a mixture of pretreated corn fiber and corn screenings (APR-330 and APR-392). Liquor from APR-330 was obtained for a laboratory two-stage continuous study and was separated from the solids using the PDU basket centrifuge and stored refrigerated. APR-392 liquor was separated from the solids by vacuum filtration the day the experiment was started. The pH of the liquor fractions was adjusted to 5.0 with sodium hydroxide (NaOH) pellets. After pH adjustment, the liquor was centrifuged at 8000 rpm for 30 minutes and sterilized by filtering through 0.45 μm and 0.2 μm filters.

C.2.3 Preparation of Whole Slurry

The pH of the whole slurry from APR-392 was adjusted to pH 5.0 with 14.5 M NaOH. An appropriate amount of material was weighed into sterilized flasks to achieve a solids concentration of 25%, 18%, and 12%. Tetracycline (10 $\mu\text{g}/\text{mL}$) was added to each flask to control contamination at the start of the experiment.

C.2.4 Flask set-up

Solids levels of 25%, 18%, and 12%, were tested with each material (APR-330 liquor, APR-392 liquor and APR-392 whole slurry). Each flask contained an initial level of 150 mL in a 250 mL baffled Erlenmeyer flask to minimize aeration. One percent corn steep liquor (CSL) was used as a nutrient source. The temperature was maintained at 30°C and mixing was controlled at 150 rpm. The pH of each flask was monitored and adjusted to pH 5 with 12.5 M NaOH as needed.

C.3 Results and Discussion

C.3.1 APR-330 Liquor

At an effective solids loading of 25% with APR-330 liquor, glucose utilization was observed after a lag phase of 96 hours (see Figure C-1). Once glucose consumption began, 95% of the glucose was utilized within 24 hours. Minimal xylose utilization (12.8%) was observed in 120 hours (see Table C-1). By 360 hours, no further xylose conversion occurred. At the lower solids level of 18%, the glucose lag phase was less than five hours, and virtually 100% of the glucose was consumed within 24 hours. After 120 hours, 84.7% of the xylose was utilized. No appreciable lag phase was observed at 12% solids, and 97% of the xylose was utilized in 120 hours. The rate of glucose utilization appears to be similar in each flask after the lag phase (see Figure C-1). The rate of xylose utilization appears to be similar at the lower solids levels.

Table C-1. Sugar Conversions and Ethanol Yields
(based on 120 hour time point)

APR Sample	Solids Concentration	Glucose Conversion	Xylose Conversion	Ethanol Process Yield	Ethanol Metabolic Yield
330	25%	93.00	12.78	58.39	87.31
330	18%	96.83	84.68	86.58	91.23
330	12%	97.44	97.09	87.48	89.89
392	25%	97.28	66.13	70.61	81.60
392	18%	97.72	92.59	84.83	88.41
392	12%	97.81	95.29	83.80	86.45
392	25% whole	97.08	42.43	71.57	91.55
392	12% whole	97.67	95.16	78.71	81.31

C.3.2 APR-392 Liquor

At an effective solids loading of 25% with APR-392 liquor, glucose and xylose utilization began after a lag phase of only 12 hours. A lag phase was not observed (only lag phases of greater than 5 hours can be observed due to the sampling times) at the lower solids loadings with this liquor. At all three solid loadings, the rate of glucose utilization appears the same after the lag phase (see Figure C-2). Similarly, the rate of xylose utilization appears nearly identical after the lag phase. After 120 hours of incubation, 66.1%, 92.6%, and 95.3% of the available xylose was consumed in the 25%, 18%, and 12% solids level flasks, respectively (see Table C-1).

C.3.3 Liquor and Whole Slurry Comparison

The major difference between the two hydrolyzates tested in this study was the longer lag phase observed for the APR-330 liquor at the 25% solids level (see Figure C-3). As the solids level decreased to 12% similar results were obtained with both material (see Figure C-4).

A set of flasks containing whole slurry was ran in parallel with liquor at the same effective solids level. The 18% solids flask broke at the beginning of the experiment. The whole slurry showed similar results to the liquor at the same effective solids level. Thus, results from the liquor can be used to predict results from whole slurries (see Figures C-3 and C-4).

C.3.4 HMF and Furfural Depletion

The longest lag phase (96 hours) was observed at 25% solids in APR-330 liquor, which contained the greatest initial amounts of the inhibitors acetic acid, furfural, and HMF (see Table C-2). As the concentration of these compounds decreased with dilution, the duration of the lag phase also decreased. It is interesting to note that the furfural and HMF present at the beginning of the experiment decreased to near zero before significant glucose consumption occurred (see Figure C-5). It has been reported in the literature that a lag phase is observed and cell death occurs in the presence of HMF and furfural, and cell growth initiates with the elimination of furfural and HMF from the culture (Chung and Lee, 1985).

Table C-2. Initial Inhibitor Concentrations

APR Sample	Solid	Acetic acid	Lactic acid	HMF	Furfural
330	25%	5.91	3.71	0.51	0.52
330	18%	3.88	2.78	0.35	0.37
330	12%	2.64	2.07	0.25	0.00
392	25%	4.59	1.42	0.43	0.52
392	18%	3.16	1.17	0.30	0.38
392	12%	2.15	0.98	0.21	0.28
392	25% whole slurry	4.86	1.60	0.39	0.49
392	12% whole slurry	2.11	1.02	0.18	0.00

C.4 Conclusions

The duration of the lag phase increases as the solids level increases. It appears that this lag phase is associated with the concentration of HMF and furfural, and as the concentration of these inhibitors decreases, the duration of the lag phase also decreases. The rate of glucose and xylose utilization are not affected by the solids concentration and the rates appear similar after the lag phase. The presence of solids do not affect fermentation performance, thus, performance on liquor (e.g., in chemostat studies) can be used to predict performance of whole slurries.

C.5 Reference

Chung, I. S., Lee, Y.Y. (1985) Ethanol Fermentation of Crude Acid Hydrolyzate of Cellulose Using High-Level Yeast Inocula. *Biotechnol. Bioeng.* 27 (3), 308-315.

Figure C-1. Glucose and Xylose Consumption by LNHST2 as a Function of Solids Concentration (APR Sample-330)

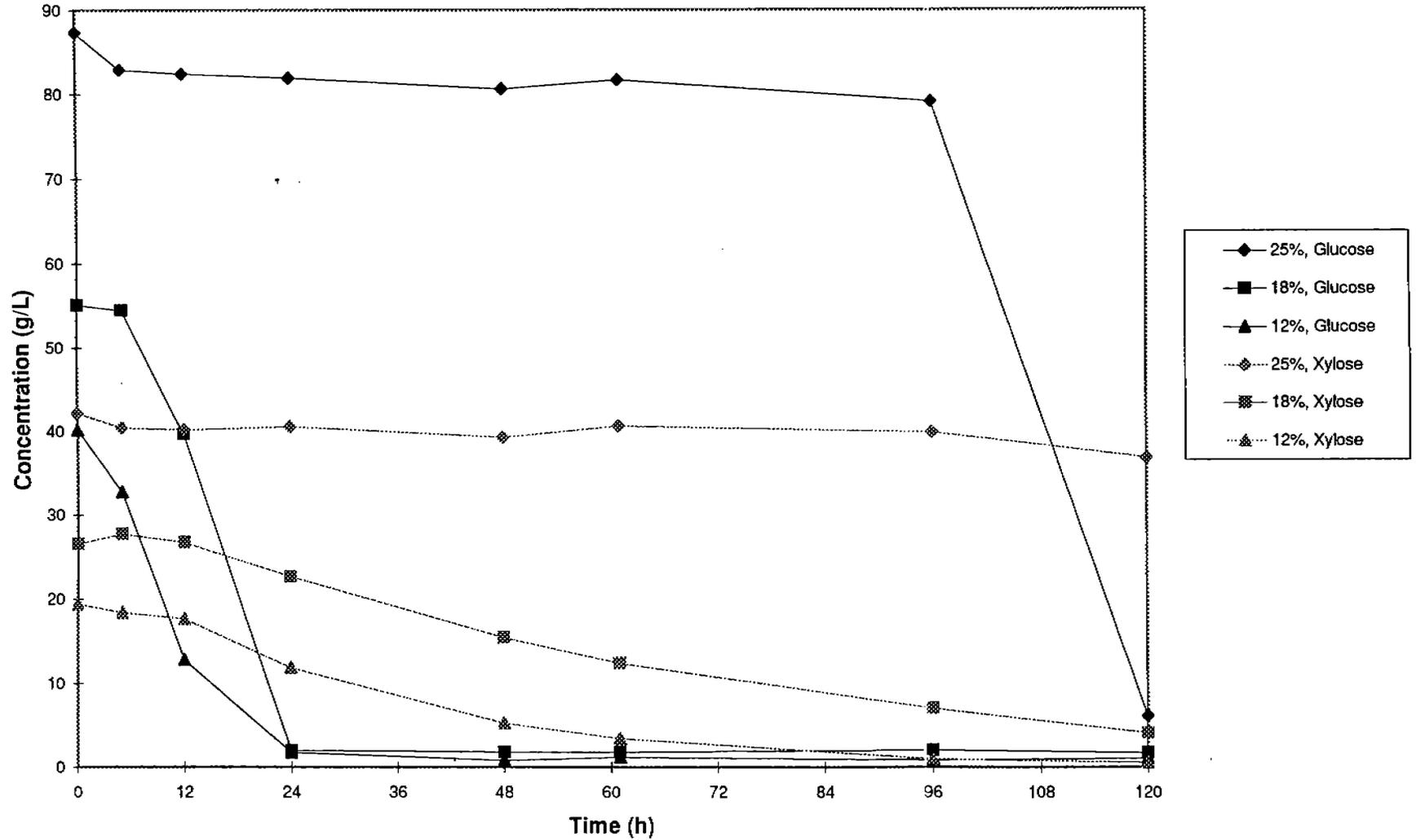


Figure C-2. Glucose and Xylose Consumption by LNHST2 as a Function of Solids Concentration (APR Sample-392)

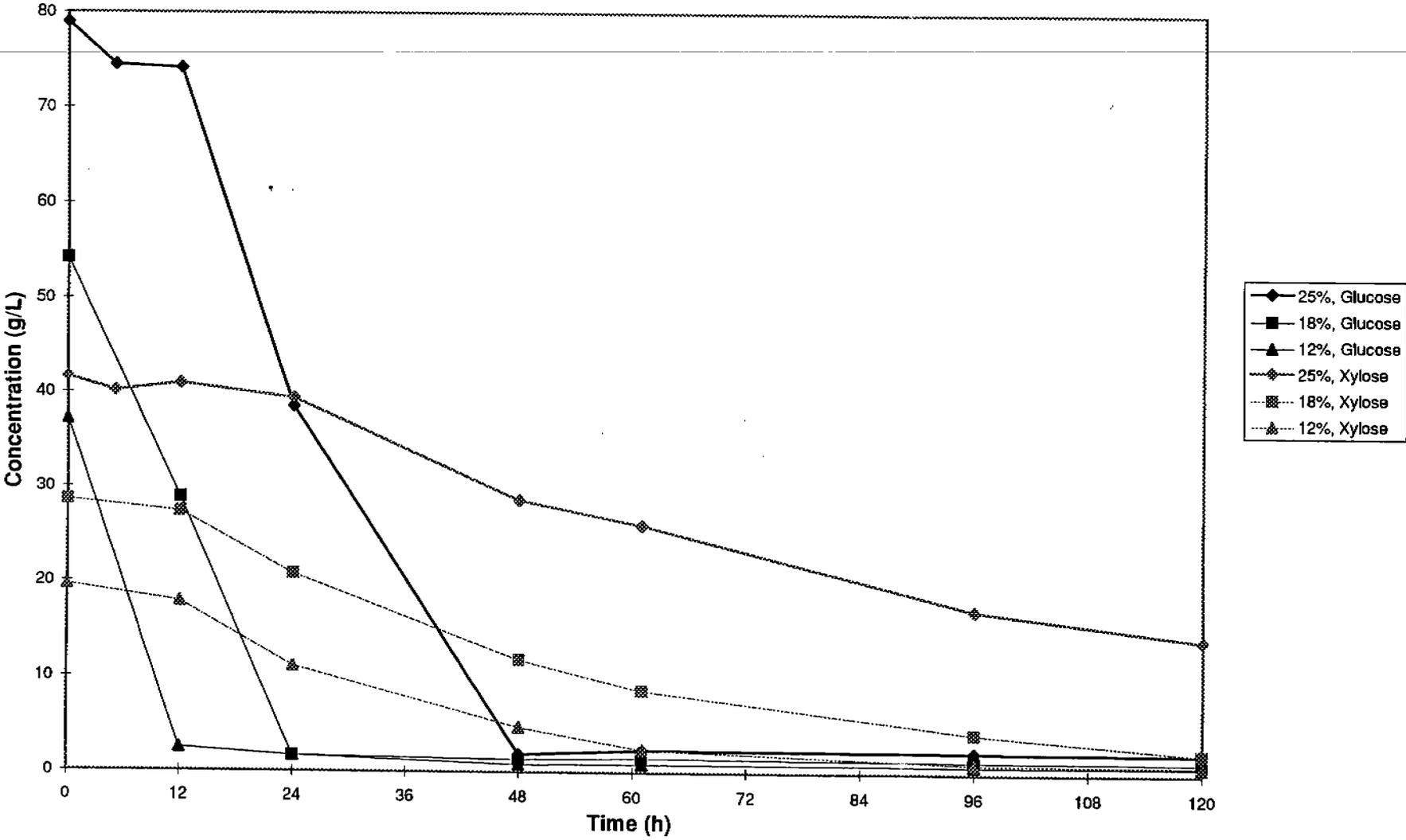


Figure C-4. Xylose and Glucose Consumption by LNHST2 at 12% Solids Concentration in Liquor and Whole Slurry

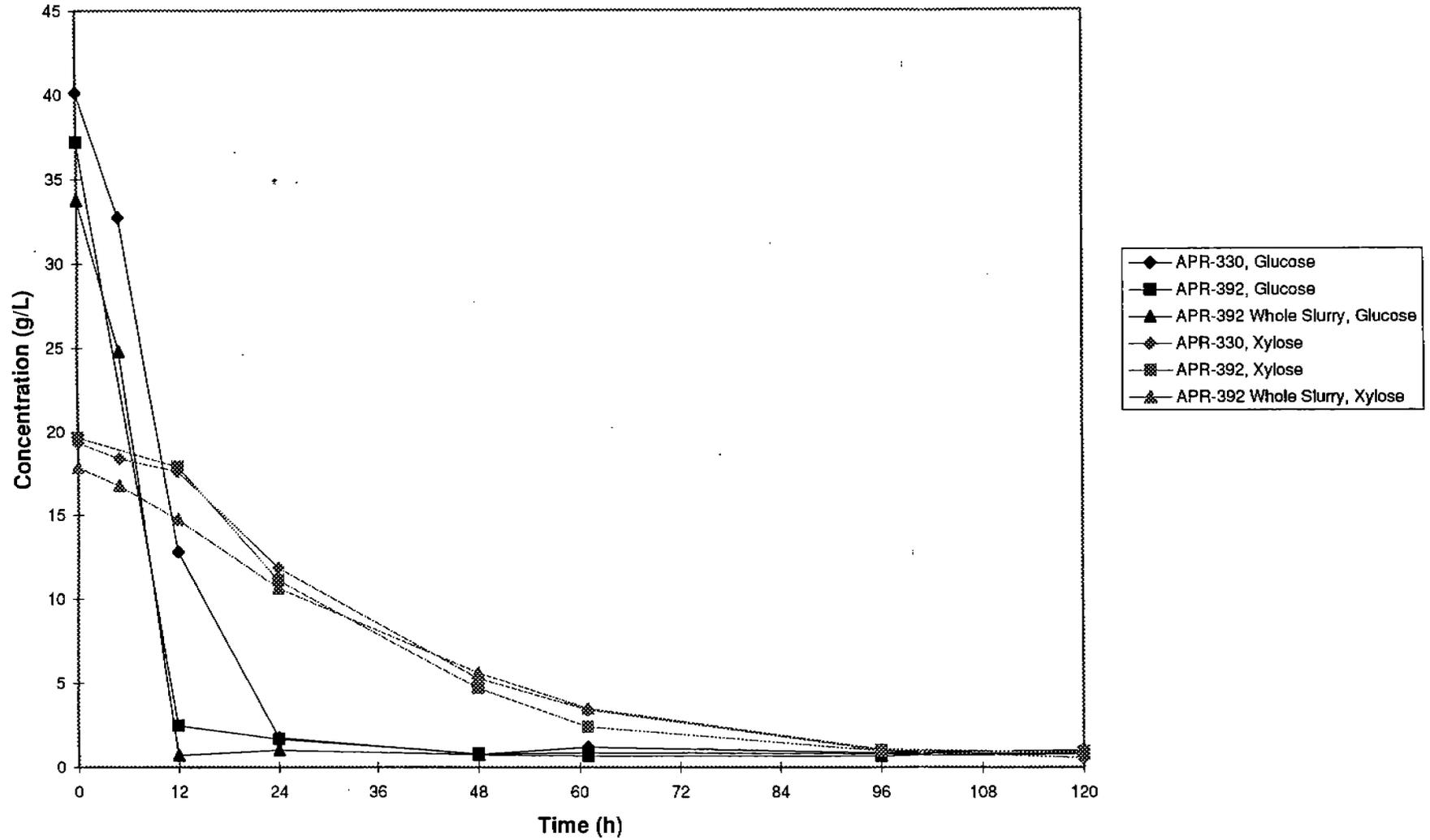
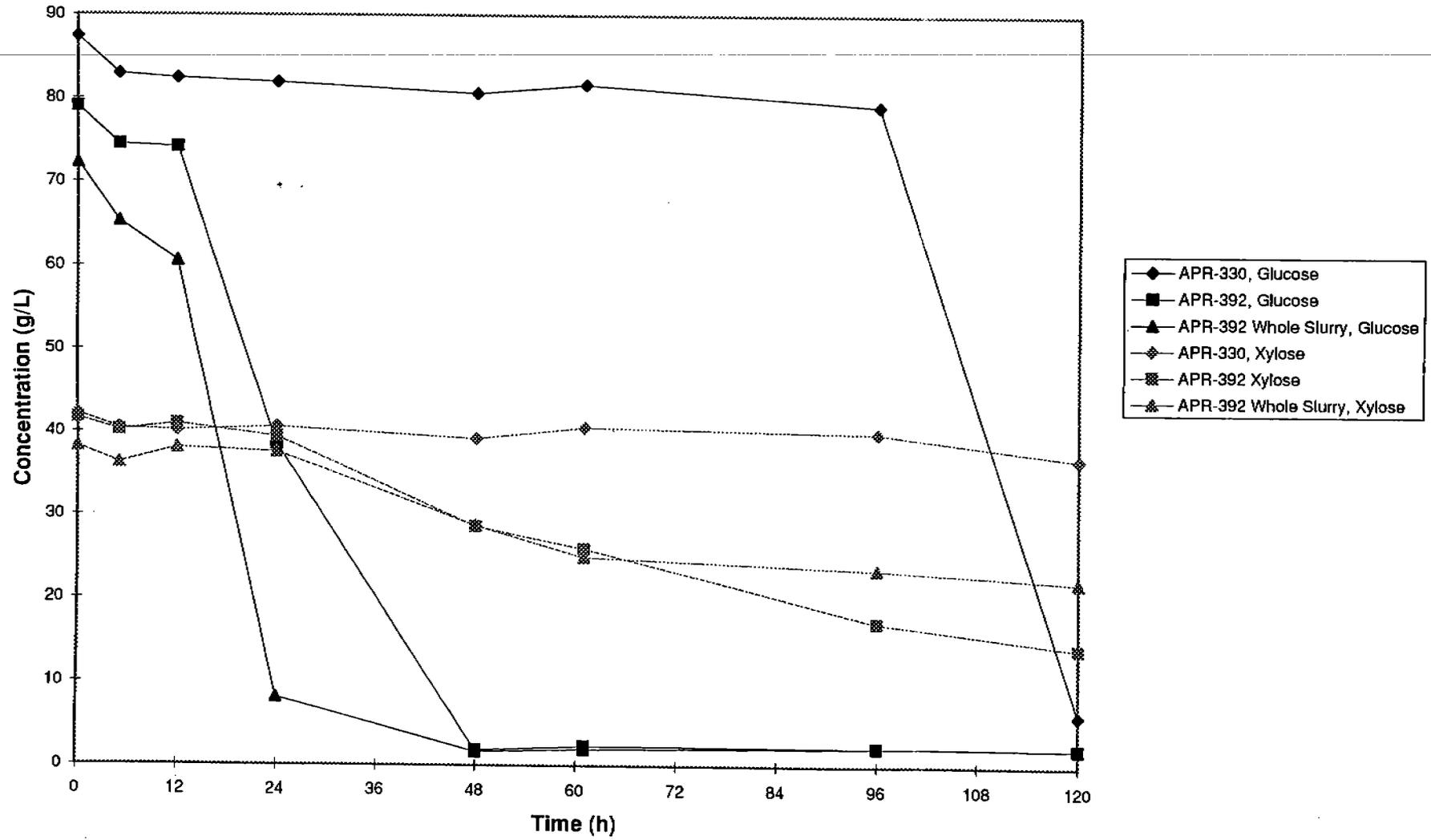
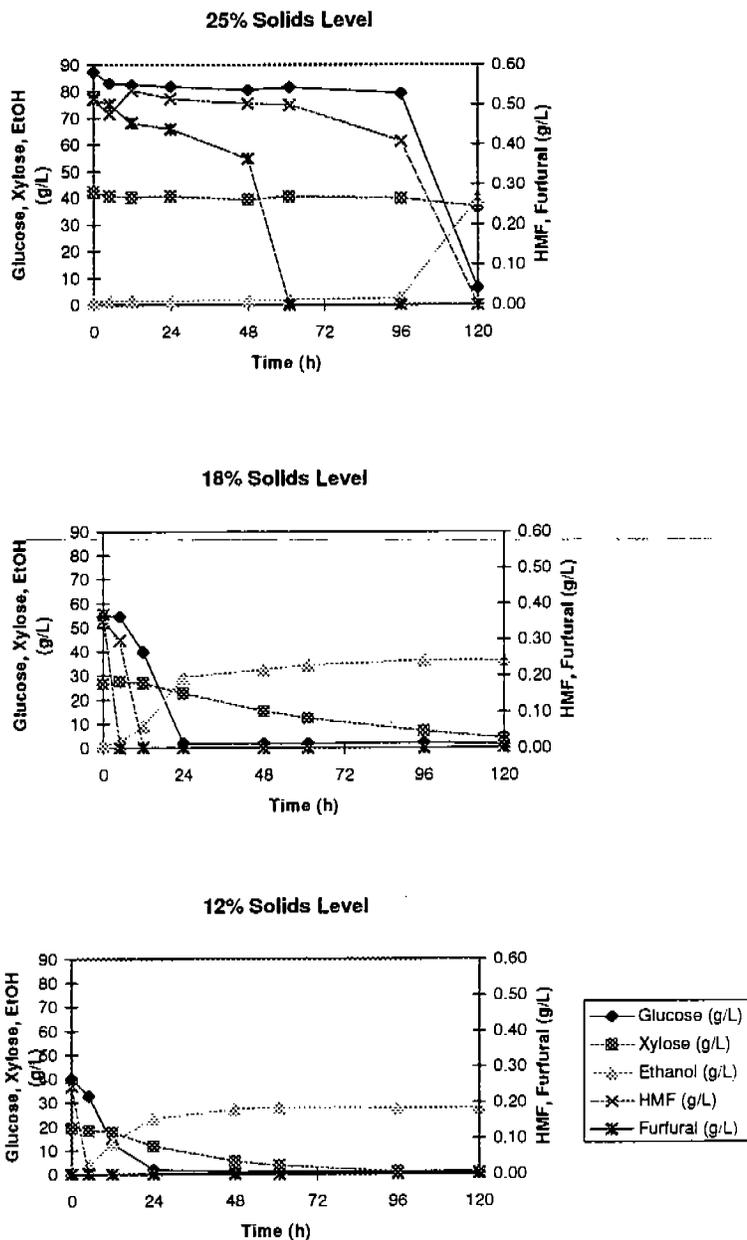


Figure C-3. Xylose and Glucose Consumption by LNHST2 at 25% Solids Concentration in Liquor and Whole Slurry



**Figure C-5. Fermentation Performance of LNHST2 on APR-330
Liquor at Various Solids Levels**



Raw Data

Flask 1		liquor - 25% equivalent solids (APR #330, generated on 4/21/96)										
Time (h)	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Xylitol (g/L)	acid (g/L)	acid (g/L)	Glycerol (g/L)	acid (g/L)	Ethanol (g/L)	HMF (g/L)	Furfural (g/L)
0	87.35	42.18	8.20	24.38	1.95	0.73	3.71	0.66	5.91	0.93	0.51	0.52
5	82.92	40.42	8.18	24.40	2.02	0.73	3.75	0.75	6.03	1.20	0.48	0.50
12	82.42	40.19	7.70	24.13			3.66	0.81	5.93	1.46	0.54	0.45
24	81.91	40.57	7.72	24.22	1.85	0.75	3.73	0.94	6.04	1.52	0.51	0.44
48	80.63	39.24	6.92	23.51			3.55	0.80	5.85	1.58	0.50	0.37
61	81.67	40.62	8.39	24.30	1.92	0.76	3.79	1.02	6.27	1.59	0.50	0.00
96	79.12	39.90	7.65	24.10	1.85	0.70	3.72	1.10	5.82	2.64	0.41	0.00
120	6.12	36.79	8.07	24.35	1.85	0.82	3.94	4.90	5.24	39.58	0.00	0.00

Flask 2		liquor - 18% equivalent solids (APR #339, generated on 4/21/96)										
Time (h)	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Xylitol (g/L)	Succinic acid (g/L)	Lactic acid (g/L)	Glycerol (g/L)	Acetic acid (g/L)	Ethanol (g/L)	HMF (g/L)	Furfural (g/L)
0	55.03	26.55	5.17	15.35	1.23	0.50	2.78	0.49	3.88	1.00	0.35	0.37
5	54.43	27.71	5.65	16.71	1.22	0.49	2.80	0.65	3.93	2.22	0.30	0.00
12	39.66	26.77	4.73	16.32			2.84	1.55	3.79	8.97	0.00	0.00
24	1.97	22.62	5.18	16.56	1.27	0.57	2.89	4.05	3.48	29.42	0.00	0.00
48	1.76	15.42	5.02	17.23			2.84	4.11	3.57	32.46	0.00	0.00
61	1.76	12.36	4.65	16.29	1.61	0.59	2.87	4.27	3.78	34.47	0.00	0.00
96	2.04	7.04	3.50	16.13	1.87	0.59	2.80	4.28	3.95	36.43	0.00	0.00
120	1.74	4.07	3.14	15.70	2.09	0.63	2.81	4.21	4.24	36.32	0.00	0.00

Flask 3		liquor - 12% equivalent solids (APR #330, generated on 4/21/96)										
Time (h)	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Xylitol (g/L)	Succinic acid (g/L)	Lactic acid (g/L)	Glycerol (g/L)	Acetic acid (g/L)	Ethanol (g/L)	HMF (g/L)	Furfural (g/L)
0	40.11	19.34	3.74	11.12	0.85	0.34	2.07	0.31	2.64	1.00	0.25	0.00
5	32.72	18.37	3.40	11.11	0.84	0.35	2.12	0.63	2.64	3.64	0.00	0.00
12	12.80	17.62	3.19	11.13			2.12	2.01	2.36	13.18	0.00	0.00
24	1.75	11.83	3.33	10.92	1.05	0.43	2.13	2.90	2.20	23.32	0.00	0.00
48	0.77	5.26	2.43	10.83			2.13	3.04	2.29	27.01	0.00	0.00
61	1.19	3.41	2.20	10.52	2.16	0.48	2.10	3.07	2.36	28.03	0.00	0.00
96	0.85	1.02	1.35	9.74	2.84	0.50	2.04	3.11	2.25	27.73	0.00	0.00
120	1.03	0.56	1.41	9.22	3.32	0.53	2.01	3.21	2.14	27.58	0.00	0.00

Flask 4		liquor - 25% equivalent solids (APR #392, generated on 6/5/96)										
Time (h)	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Xylitol (g/L)	Succinic acid (g/L)	Lactic acid (g/L)	Glycerol (g/L)	Acetic acid (g/L)	Ethanol (g/L)	HMF (g/L)	Furfural (g/L)
0	79.01	41.67	8.03	23.45	1.88	0.72	1.42	0.59	4.59	0.95	0.43	0.52
5	74.47	40.17	7.67	23.41	1.92	0.76	1.47	0.80	4.74	1.46	0.40	0.45
12	74.15	40.98	7.41	23.80			1.57	1.10	4.79	2.62	0.33	0.00
24	38.54	39.43	7.70	23.14	1.98	0.78	1.65	2.99	4.68	19.61	0.00	0.00
48	1.85	28.66	7.47	24.60			1.59	4.91	4.33	40.70	0.00	0.00
61	2.35	26.05	8.23	24.51	2.50	0.89	1.73	5.54	4.93	46.06	0.00	0.00
96	2.23	17.19	7.48	23.83	2.41	0.78	1.52	5.03	4.72	44.07	0.00	0.00
120	2.15	14.11	7.38	23.29	2.60	0.92	1.80	5.75	5.67	44.49	0.00	0.00

Flask 5		liquor - 18% equivalent solids (APR #392, generated on 6/5/96)										
Time (h)	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Xylitol (g/L)	Succinic acid (g/L)	Lactic acid (g/L)	Glycerol (g/L)	Acetic acid (g/L)	Ethanol (g/L)	HMF (g/L)	Furfural (g/L)
0	54.16	28.61	5.57	19.67	1.33	0.50	1.17	0.40	3.16	1.00	0.30	0.38
12	28.90	27.37	5.00	16.34			1.32	1.93	3.11	12.59	0.00	0.00
24	1.68	20.81	5.31	16.31	1.56	0.59	1.39	3.80	2.96	29.33	0.00	0.00
48	1.33	11.81	4.62	16.69	1.89	0.66	1.52	4.25	3.19	34.33	0.00	0.00
61	1.52	8.63	4.23	15.90	2.17	0.56	1.24	3.77	2.94	35.68	0.00	0.00
96	1.26	4.18	2.35	15.27	2.97	0.66	1.37	4.12	3.18	37.83	0.00	0.00
120	1.23	2.12	2.22	14.71	3.35	0.66	1.27	4.05	3.20	36.88	0.00	0.00

Flask 6 liquor - 12% equivalent solids (APR #392, generated on 6/5/96)												
	Glucose	Xylose	Galactose	Arabinose	Xylitol	Succinic acid	Lactic acid	Glycerol	Acetic acid	Ethanol	HMF	Furfural
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	37.18	19.62	3.81	11.01	0.93	0.35	0.98	0.30	2.15	0.99	0.21	0.28
12	2.45	17.87	3.69	11.49			1.12	2.06	1.90	17.68	0.00	0.00
24	1.66	11.11	3.42	10.83	1.25	0.42	1.09	2.32	1.78	22.70	0.00	0.00
48	0.78	4.69	2.71	10.87	1.99	0.45	1.09	2.38	1.83	25.59	0.00	0.00
61	0.85	2.39	1.97	10.26	2.63	0.51	1.12	2.54	2.00	26.47	0.00	0.00
96	0.80	0.99	1.39	9.39	3.45	0.59	1.14	2.70	1.84	25.27	0.00	0.00
120	0.81	0.93	1.44	8.78	4.15	0.57	0.84	2.56	1.60	25.31	0.00	0.00

Flask 7 Whole slurry - 25% equivalent solids (APR #392, generated on 6/5/96)												
	Glucose	Xylose	Galactose	Arabinose	Xylitol	Succinic acid	Lactic acid	Glycerol	Acetic acid	Ethanol	HMF	Furfural
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	72.26	38.22	7.33	21.63	1.80	0.73	1.60	0.68	4.86	1.51	0.39	0.49
5	65.29	36.23	6.97	21.29	1.72	0.67	1.41	0.88	4.61	3.93	0.29	0.00
12	60.60	38.10	6.87	22.28			1.69	1.82	5.02	8.06	0.00	0.00
24	8.15	37.59	7.59	22.80	2.40	0.80	1.78	6.24	6.10	42.95	0.00	0.00
48	1.67	28.81	7.34	24.67	2.24	0.81	1.77	5.98	5.07	42.61	0.00	0.00
61	1.96	24.99	7.19	22.42	2.27	0.81	1.72	5.96	5.17	41.69	0.00	0.00
96	2.23	23.52	7.40	24.18						41.80		
120	2.11	22.00	7.17	23.74	2.48	0.86	1.76	6.26	6.68	41.91	0.00	0.00

Flask 9 Whole slurry - 12% equivalent solids (APR #392, generated on 6/5/96)												
	Glucose	Xylose	Galactose	Arabinose	Xylitol	Succinic acid	Lactic acid	Glycerol	Acetic acid	Ethanol	HMF	Furfural
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	33.73	17.85	3.44	10.06	0.84	0.34	1.02	0.31	2.11	1.08	0.18	0.00
5	24.78	16.76	3.20	9.91	0.80	0.31	1.02	0.63	2.10	5.26	0.00	0.00
12	0.71	14.71	2.95	10.48			1.27	2.34	2.13	17.33	0.00	0.00
24	1.00	10.64	3.10	10.09	1.09	0.41	1.14	2.31	2.08	21.71	0.00	0.00
48	0.73	5.62	1.55	10.02	1.96	0.48	1.14	2.40	2.41	23.83	0.00	0.00
61	0.67	3.49	1.29	8.94	2.37	0.54	1.10	2.40	2.51	23.17	0.00	0.00
96	0.67	1.09	1.23	8.48		0.77	1.03	2.54	2.36	22.78	0.00	0.00
120	0.78	0.86	1.35	7.80	4.06	0.92	0.63	2.49	2.01	21.82	0.00	0.00

Appendix D

Effect of HMF and Furfural on Fermentation Performance of LNHST2

D.1 Background/Objective

In a previous shake flask study, it was discovered that the lag phase appeared to be associated with the depletion of HMF and furfural from the culture medium. Therefore, a shake flask experiment using clarified hydrolyzate (Task 5, 6/14/96, between APR samples 417 and 418) at 25%, 18%, and 12% equivalent solids levels, was performed to examine the effect of HMF and furfural on the fermentation performance of LNHST2 and to provide data for kinetic modeling.

D.2 Materials and Methods

D.2.1 Inoculum Preparation

Inoculum was generated in two stages in a shaking incubator at 30°C and 150 rpm. The first stage, consisting of 50 mL of YEPD (1% w/v yeast extract, 2% w/v peptone and 2% w/v glucose, pH 5) in a 250 mL baffled Erlenmeyer flask, was inoculated with 1 mL of LNHST2 from a frozen seed stock. After a 12 hour incubation, a 10% v/v inoculum was transferred to a second stage consisting of CSL medium (1% w/v CSL, 2% w/v glucose, pH 5) with a working volume of 120 mL in a 500 mL baffled Erlenmeyer flask. The second stage was incubated for only 5 hours by which time a majority of the glucose was consumed (exponential growth stage). A 10% v/v inoculum from the second stage was used to inoculate the liquor hydrolyzate shake flasks.

D.2.2 Preparation of Liquor Hydrolyzate

The liquor used in this study was clarified from hydrolyzate for a bench scale, two-stage continuous fermentation, using the PDU basket centrifuge and stored refrigerated. The pH of the liquor was adjusted to 5.0 with sodium hydroxide (NaOH) pellets, centrifuged for 30 minutes at 8000 rpm, and sterilized by filtering through a 0.2 µm filter the day the experiment began.

Based on an HPLC analysis, some concentration of the liquor occurred during processing, because APR samples generated before and after the material collected were significantly lower in sugars and inhibitors. Based on the average sugar and inhibitor levels during the PDU Task 5 run, and the sugar and inhibitor levels measured in the liquor, the effective solids concentration of the undiluted liquor was 44%. Therefore, the dilutions in this study were based on 44% solids, and not the measured 31.7% solids of the whole slurry.

D.2.3 Fermentation Conditions

The three flasks in this study, one each at 25%, 18%, and 12% effective solids, contained an initial level of 300 mL in a 500 mL baffled Erlenmeyer flask to minimize aeration. One percent corn steep liquor (CSL) was used as a nutrient source. The temperature was maintained at 30°C and mixing was controlled at 150 rpm. The pH of each flask was monitored and adjusted to pH 5 with 12.5 M NaOH as needed.

D.2.4 Sampling and Analysis

Samples were withdrawn every two hours for the first 14 hours, and every 12-24 hours thereafter, up to 78

hours. Samples were analyzed for ethanol and glucose with the Yellow Springs Instrument (YSI) analyzer. In addition, the hexose and pentose sugars were analyzed by liquid chromatography (HPLC) with an HP 1047 IR detector and a Biorad HPX87P column. Ethanol, xylitol, succinic acid, lactic acid, glycerol, acetic acid, HMF, and furfural were measured with an HPLC equipped with a Biorad HPX87H column. HMF and furfural were also analyzed by gas chromatography (GC). Dry cell weights (DCW), viable plate counts, and total cell counts were performed on every sample.

D.3 Results and Discussion

At the 25% effective solids level, 1.77 g/L HMF and 0.39 g/L furfural were measured at time zero (see Table D-1). (Note: The HMF values obtained by GC in this study were significantly higher (~4 times) than values obtained by LC. Amoco's analytical laboratory repeated the analysis of HMF using a LC with ultraviolet detector and also obtained the lower values. The Amoco values are reported in this paper.) At the 25% effective solids level, an initial lag phase of 6 hours was observed before the onset of exponential growth (see Figure D-1). During the lag phase, a majority of the furfural (69%) and some of the HMF (13.6%) was utilized. After the lag phase HMF utilization continued. This study showed that glucose utilization and cell growth occurs while a significant amount of HMF is still present.

Table D-1. Initial Inhibitor Concentrations

Solids Concentration (%)	Acetic Acid (g/L)	Lactic Acid (g/L)	HMF (g/L)	Furfural (g/L)
25	4.08	1.89	0.43	0.39
18	2.77	1.48	0.31	0.31
12	1.84	1.29	0.21	0.20

The duration of the lag phase decreased at the lower effective solids levels of 18% and 12% (see Figures D-2 and D-3). At the 18% effective solids level, a lag phase of approximately 4 hours was observed before exponential growth started. During this period, furfural concentration decreased from 0.31 g/L to 0.10 g/L (68%), and HMF concentration decreased from 1.32 g/L to 0.88 g/L (33%). Similarly, at the 12% effective solids level, the furfural decreased from 0.20 g/L to 0.14 g/L during a 2 hour lag phase. Again, significant amounts of HMF, 0.88 g/L and 0.54 g/L at the 18% and 12% effective solids levels, respectively, was present during exponential growth and decreased to zero by the end of exponential growth.

The ethanol metabolic yields were similar at all three solids loadings (see Table D-2). The ethanol process yield (75.7% theoretical) was lower at 25% effective solids due to incomplete xylose conversion (65.9%) by 78 hours. The xylose conversion was better at the lower solids levels (91%) giving higher ethanol process yields of 85.7% and 84.1% at the 18% and 12% solids levels, respectively. After the lag phase, the rate of glucose utilization was similar in all three flasks; 4.5 g/L-h, 5.1 g/L-h, and 4.4 g/L-h at 25%, 18%, and 12% effective solids, respectively. The rate of xylose utilization increased as the solids loading decreased (see Table D-2) probably due to the effect of acetic acid on xylose utilization, an observation from past

experiments.

D.4 Conclusions

It appears that the end of the lag phase and the initiation of exponential growth occurs when furfural drops to low levels (<0.1 g/L). HMF utilization occurs during the lag phase and exponential growth and reaches near zero as glucose reaches near zero. To better determine the effect of these two compounds on the fermentation performance of LNHST2, spiking studies should be performed. The data from this experiment will be used to create HMF and furfural inhibition terms in the kinetic model.

Table D-2. Sugar Conversions and Ethanol Yields (based on 78 hour time point)

Solids Concentration (%)	Glucose Conversion (%)	Xylose Conversion (%)	Glucose Utilization (g/L-h)	Xylose Utilization (g/L-h)	Ethanol Process Yield (%)	Ethanol Metabolic Yield (%)
25	96.3	65.9	5.52	0.33	75.7	87.4
18	96.8	91.2	5.14	0.47	85.7	90.2
12	97.7	90.9	4.38	0.52	84.1	87.9

Figure D-1. Fermentation Performance of LNHST2 at 25% Effective Solids Level

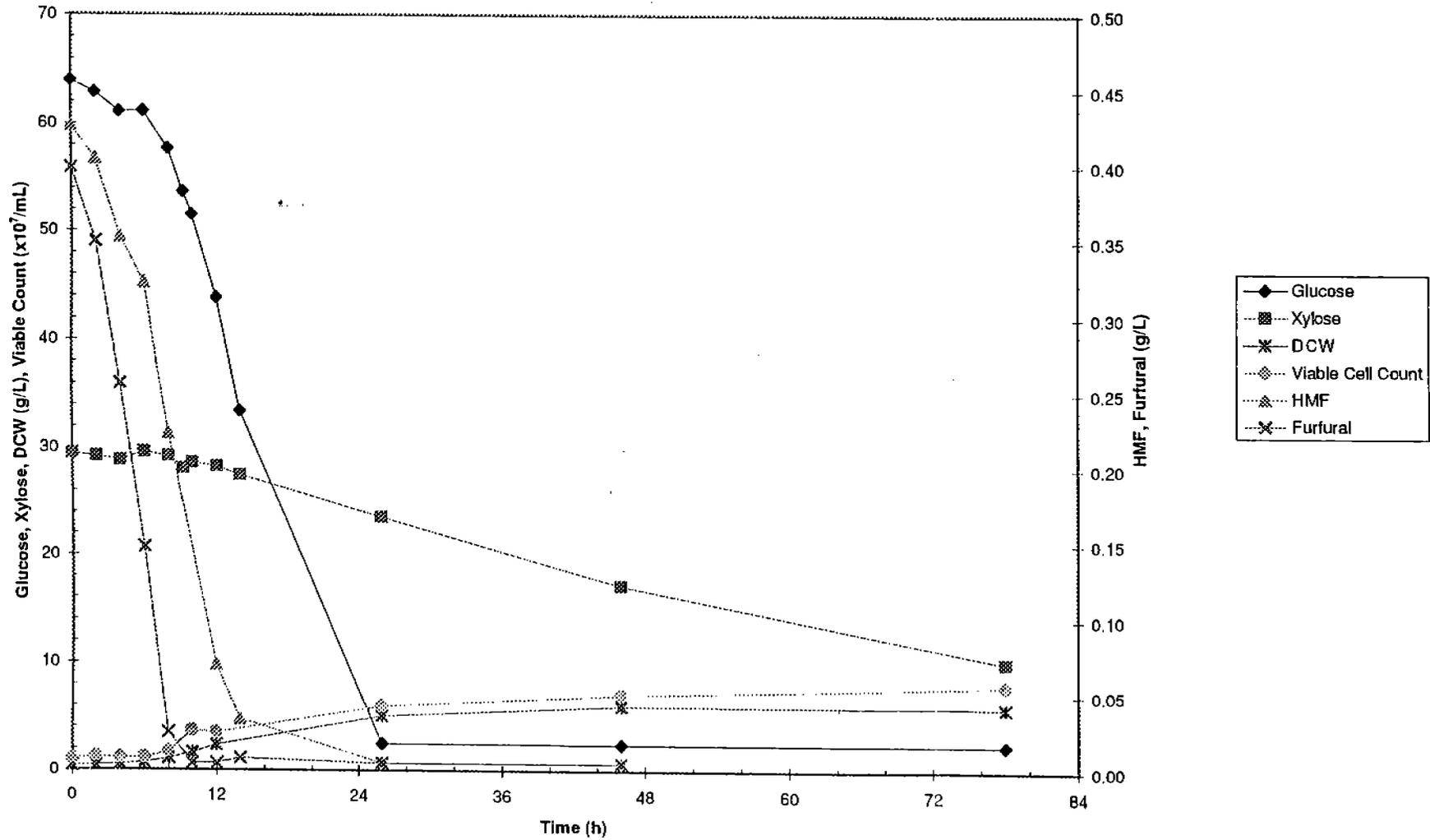


Figure D-2. Fermentation Performance of LNHST2 at 18% Effective Solids Level

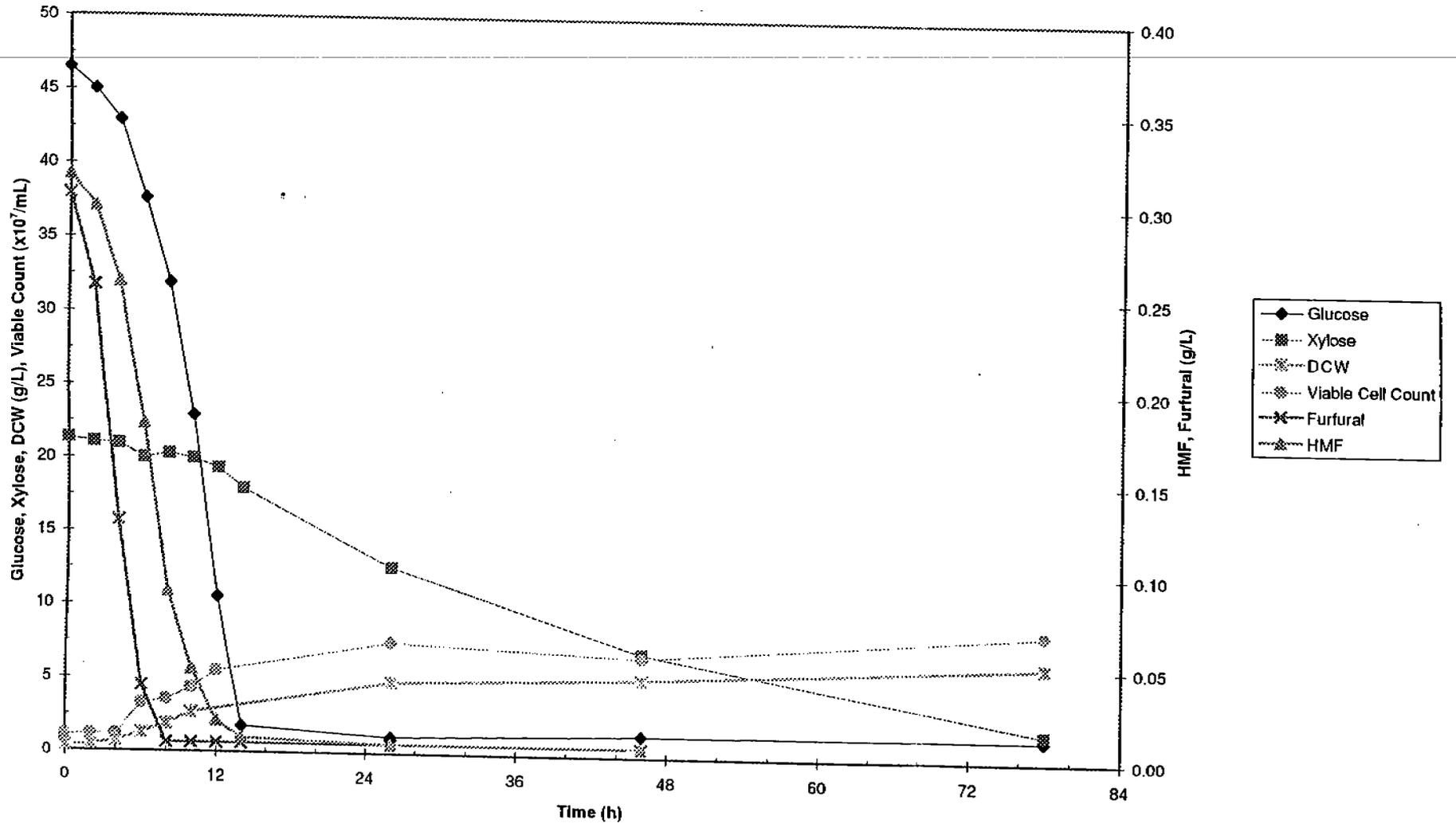
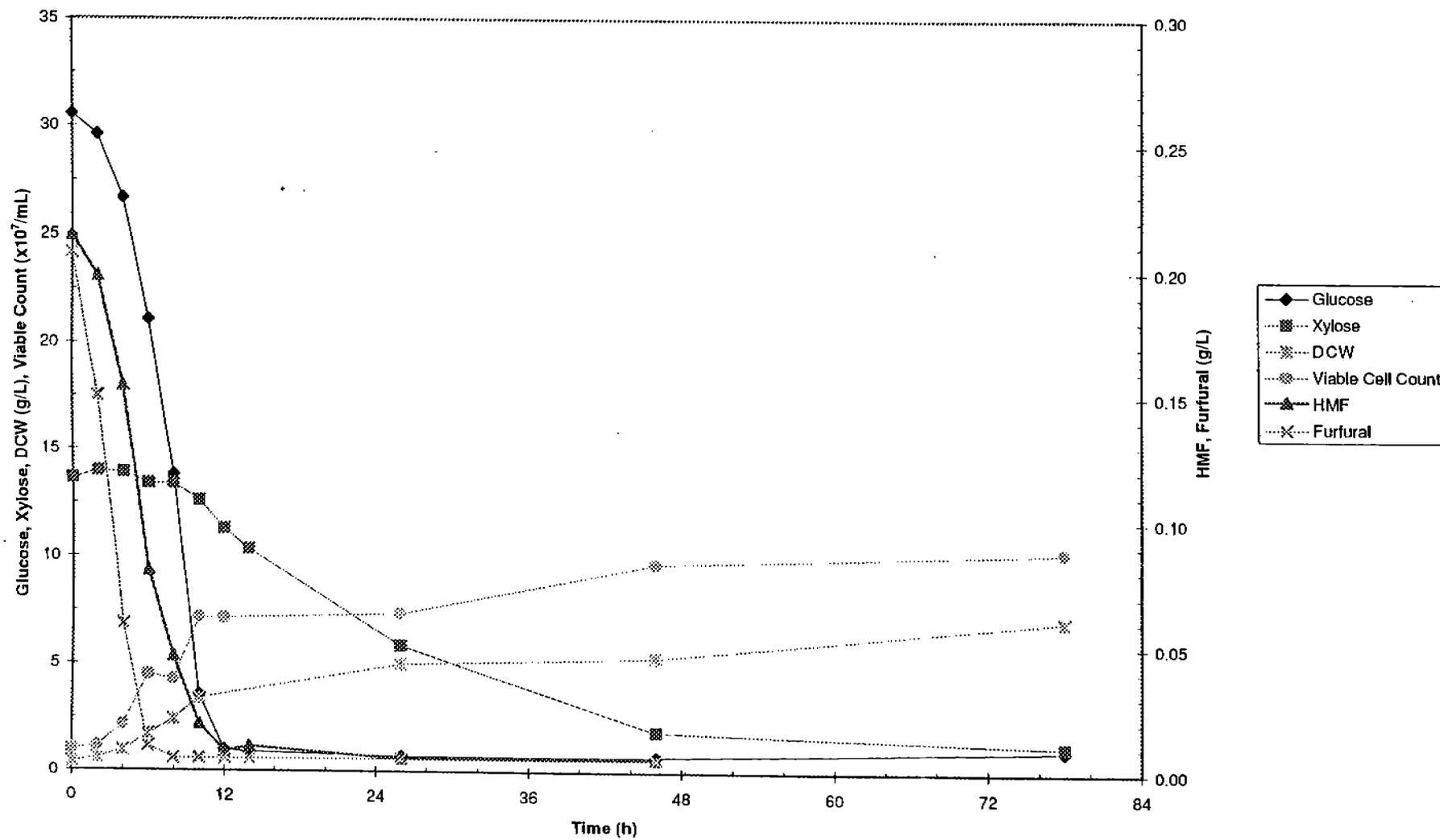


Figure D-3. Fermentation Performance of LNHST2 at 12% Effective Solids Level



Raw Data

Flask A 25% Equivalent Solids Concentration

Time (h)	Glucose	Xylose	Galactose	Arabinose	Xylitol	Succinic acid	Lactic acid	Glycerol	Acetic acid	Ethanol	GC	LC - Amoco	GC - Amoco	GC	DCW (g/L)	Hemacytometer count (cells/mL)	Viable count (cells/mL)	Viable count (x10 ⁷ /mL)	% viable	ln(OD)
	(g/L), 25%	(g/L), 25%									HMF (g/L), 25%	HMF (g/L), 25%	Furfural (g/L), 25%	Furfural (g/L), 25%						
0	63.99	29.44	6.23	17.09	0.63	0.00	1.89	0.63	4.08	0.76	1.77	0.43	0.40	0.39	0.37	2.23E+07	1.00E+07	1.00	44.84	-0.99
2	62.91	29.20	6.20	17.02	1.17	0.75	1.89	0.67	4.09	1.01	1.63	0.41	0.35	0.38	0.44	1.98E+07	1.22E+07	1.22	61.77	-0.81
4	61.08	28.82	6.14	16.88	1.16	0.00	1.93	0.80	4.15	1.52	1.48	0.35	0.26	0.25	0.51	2.16E+07	1.11E+07	1.11	51.51	-0.66
6	61.13	29.58	6.32	17.41	0.54	0.00	1.86	0.79	4.04	2.31	1.53	0.32	0.15	0.12	0.69	2.52E+07	1.12E+07	1.12	44.53	-0.38
8	57.64	29.23	6.28	17.28	1.14	0.00	1.92	1.00	4.11	3.67	1.15	0.22	0.03	0.04	1.04	3.80E+07	1.67E+07	1.67	44.01	0.04
9:25	53.68	28.05	5.53	16.24	1.11	0.00	1.89	1.05	4.03	4.78	0.86	0.14	0.01	0.03	1.59	5.59E+07	3.63E+07	3.63	65.00	0.46
10	51.53	28.60	6.16	17.05	1.17	0.00	2.07	1.41	4.22	6.18	0.86	0.07	0.01	0.03	2.33	9.45E+07	3.47E+07	3.47	36.72	0.85
12	43.85	28.27	5.65	16.61	1.11	0.00	1.98	1.61	4.02	9.93	0.29	0.03	0.01	0.02	5.01	1.69E+08	5.97E+07	5.97	35.33	1.61
14	33.44	27.42	5.51	16.30	1.12	0.00	1.97	2.14	3.90	14.61	0.01	0.01	0.01	0.01	6.01	1.84E+08	7.03E+07	7.03	38.21	1.79
26	2.49	23.49	6.10	17.44	0.67	0.00	2.08	3.94	3.92	32.18	0.01	0.01	0.01	0.01	5.86	1.54E+08	7.87E+07	7.87	51.10	1.77
46	2.40	17.14	5.79	17.10	0.60	0.00	2.01	3.87	4.08	34.78	0.01	0.01	0.01	0.01						
78	2.39	10.04	5.07	16.67	1.63	0.73	2.03	3.78	4.61	36.93	0.01	0.01	0.01	0.01						

Flask B 18% Equivalent Solids Concentration

Time (h)	Glucose	Xylose	Galactose	Arabinose	Xylitol	Succinic acid	Lactic acid	Glycerol	Acetic acid	Ethanol	GC	LC - Amoco	GC - Amoco	GC	DCW (g/L)	Hemacytometer count (cells/mL)	Viable count (cells/mL)	Viable count (x10 ⁷ /mL)	% viable	ln(OD)
	(g/L), 18%	(g/L), 18%									HMF (g/L), 25%	HMF (g/L), 25%	Furfural (g/L), 25%	Furfural (g/L), 25%						
0	46.50	21.33	4.52	12.39	0.79	0.47	1.48	0.30	2.77	0.76	1.32	0.31	0.30	0.31	0.39	2.39E+07	1.05E+07	1.05	44.03	
2	45.03	21.06	4.48	12.29	0.80	0.47	1.51	0.37	2.79	1.14	0.95	0.30	0.25	0.24	0.46	2.14E+07	1.16E+07	1.16	54.21	
4	42.92	20.97	4.49	12.32	0.79	0.00	1.56	0.48	2.82	2.11	0.88	0.26	0.13	0.10	0.70	2.80E+07	1.12E+07	1.12	40.00	
6	37.67	20.03	4.00	11.61	0.79	0.00	1.60	0.66	2.84	4.12	0.88	0.18	0.04	0.01	1.26	5.25E+07	3.26E+07	3.26	62.10	
8	31.91	20.31	4.41	12.19	0.80	0.00	1.63	0.97	2.80	7.31	0.36	0.18	0.04	0.01	1.90	7.80E+07	3.53E+07	3.53	45.26	
10	22.95	19.99	4.40	12.25	0.79	0.00	1.66	1.44	2.67	11.84	0.22	0.09	0.01	0.01	2.67	1.09E+08	4.37E+07	4.37	39.98	
12	10.52	19.34	4.39	12.33	0.79	0.00	1.67	1.89	2.55	18.11	0.10	0.05	0.01	0.01	4.84	1.65E+08	7.53E+07	7.53	45.55	
14	1.79	17.97	4.34	12.21	0.80	0.00	1.68	2.36	2.49	22.56	0.06	0.02	0.01	0.01	5.30	1.73E+08	6.71E+07	6.71	38.90	
26	1.12	12.64	3.77	11.87	1.00	0.00	1.66	2.55	2.51	26.55	0.01	0.01	0.01	0.01	6.43	1.69E+08	8.57E+07	8.57	50.62	
46	1.47	6.99	3.64	11.66	0.40	0.00	1.66	2.61	2.56	29.59	0.01	0.01	0.01	0.01						
78	1.50	1.88	2.48	10.88	0.48	0.00	1.61	2.71	2.04	30.46	0.01	0.01	0.01	0.01						

Flask C 12% Equivalent Solids Concentration

Time (h)	Glucose	Xylose	Galactose	Arabinose	Xylitol	Succinic acid	Lactic acid	Glycerol	Acetic acid	Ethanol	GC	LC - Amoco	GC - Amoco	GC	DCW (g/L)	Hemacytometer count (cells/mL)	Viable count (cells/mL)	Viable count (x10 ⁷ /mL)	% viable	ln(OD)
	(g/L), 12%	(g/L), 12%									HMF (g/L), 25%	HMF (g/L), 25%	Furfural (g/L), 25%	Furfural (g/L), 25%						
0	30.54	13.64	2.55	7.63	0.52	0.31	1.29	0.00	1.84	0.75	0.74	0.21	0.21	0.20	0.41	2.33E+07	1.01E+07	1.01	43.35	
2	29.60	13.97	2.99	8.16	0.54	0.31	1.32	0.27	1.85	1.24	0.54	0.20	0.15	0.14	0.60	2.41E+07	1.14E+07	1.14	47.40	
4	26.67	13.89	3.00	8.19	0.53	0.00	1.37	0.37	1.88	2.66	0.52	0.15	0.06	0.05	0.96	5.60E+07	2.14E+07	2.14	38.21	
6	21.08	13.39	2.67	7.81	0.53	0.00	1.39	0.57	1.84	5.24	0.28	0.08	0.01	0.01	1.70	9.20E+07	4.50E+07	4.5	48.91	
8	13.83	13.41	2.96	8.22	0.53	0.00	1.42	0.91	1.72	9.00	0.14	0.05	0.01	0.01	2.39	1.13E+08	4.27E+07	4.27	37.85	
10	3.56	12.61	2.91	8.22	0.53	0.00	1.44	1.36	1.62	14.28	0.06	0.02	0.01	0.01	3.40	1.13E+08	4.27E+07	4.27	37.85	
12	0.99	11.31	2.63	7.07	0.55	0.00	1.44	1.52	1.54	15.79	0.04	0.01	0.01	0.01	5.00	1.13E+08	4.27E+07	4.27	37.85	
14	0.90	10.37	2.77	8.02	0.59	0.00	1.43	1.52	1.52	16.49	0.03	0.01	0.01	0.01	5.30	1.54E+08	7.17E+07	7.17	46.71	
26	0.72	5.86	2.33	7.64	0.33	0.00	1.43	1.63	1.46	19.58	0.01	0.01	0.01	0.01	6.43	1.49E+08	7.13E+07	7.13	47.85	
46	0.71	1.86	1.20	6.65	1.80	0.35	1.40	1.65	1.07	20.82	0.01	0.01	0.01	0.01						
78	1.04	1.24	1.23	6.40	2.69	0.00	0.79	1.62	0.76	19.73	0.01	0.01	0.01	0.01						
102	0.67	0.80	0.88	5.72	2.97	0.00	0.00	1.78	0.88	18.53	0.01	0.01	0.01	0.01						
126	0.97	1.14	1.11	6.05	3.19	0.00	0.00	1.79	0.76	17.02	0.01	0.01	0.01	0.01						
174	0.96	1.13	1.01	6.02	3.39	0.00	0.00	1.37	0.00	13.56	0.01	0.01	0.01	0.01						

Appendix E

Two Stage Continuous Fermentation of Hydrolyzate by LNHST2

E-1 Objective

Examine the performance of LNHST2 under laboratory conditions, in a two-stage continuous fermentation scheme, using hydrolyzate collected from the PDU. Adjust pH, residence time, and other parameters to improve fermentation performance and transfer these conditions to the PDU for testing.

E-2 Material and Methods

E-2.1 Inoculum Preparation

Inoculum was generated in two stages in a shaking incubator at 30°C and 150 rpm. The first stage consisted of 50 mL of YEPD (1% w/v yeast extract, 2% w/v peptone, and 2% w/v glucose, pH 5) in a 250 mL baffled Erlenmeyer flask and was inoculated with 1 mL of LNHST2 from frozen feed stock. After a 12 hour incubation, a 10% v/v inoculum was transferred to a second stage consisting of 135 mL CSL medium (1% w/v CSL, 2% w/v glucose, pH 5) in a 500 mL baffled Erlenmeyer flask. The second stage was incubated for 5 hours, by which time a majority of the glucose was consumed (exponential growth stage). A 10% v/v inoculum from the second stage was used to inoculate the first chemostat.

E-2.2 Preparation of Hydrolyzate Liquor

The experiments consisted of four runs. Runs 1, 2, and 3 utilized hydrolyzate liquor obtained after APR sample 330. Run 4 was run with hydrolyzate liquor collected between APR samples 417 and 418. The hydrolyzate collected after APR sample 330 was collected in a 55 gallon drum and allowed to cool overnight. It was then transferred to the Bock Extractor (basket centrifuge) in the PDU where the clarified liquor was collected and the solids discarded. The hydrolyzate collected between APR samples 417 and 418 was collected in two 5 gallon buckets, allowed to cool overnight, and once again clarified using the Bock extractor.

Based on several analyses of both liquor aliquots collected, some sort of concentration effect was noted. The concentration of all sugars, acids, and reversion products monitored was consistently 40% higher than that recorded for the actual APR samples. While all dilutions of the liquor were based and recorded on a total solids basis, the actual concentration based on normalizing sugar concentrations are 40% higher. Therefore, the effective solids concentration was higher in all runs than targeted.

E-2.3 Fermentation Conditions and Configuration

For the fermentations, two 1.7-L New Brunswick BioFlo III fermenters were employed. To minimize ethanol evaporation, the condensers on each unit were packed with 1 mm glass beads and equipped with 4°C water circulation. The working volume of each vessel was 1.3 liters, temperature was maintained at 30°C, and the pH was controlled at 5.0 with the addition of 50% caustic (NaOH). Air was not supplied to the fermenters. The fermenters were agitated at 150 rpm.

The first stage fermenter was prepared and autoclaved with CSL (diluted with tap water to yield a concentration of 1% w/v). Once cooled, an appropriate amount of hydrolyzate liquor was added and pH was

adjusted to 5.0 with 50% caustic (NaOH). A 10% v/v inoculum was transferred to the fermenter and was allowed to grow for 24 hours (or until the glucose level was below 5 g/l) before being switched to continuous operation. The effluent from the first vessel was directed to the second stage. The feed for the continuous mode consisted of hydrolyzate liquor and CSL (with adjustments in concentration and flow rates performed to maintain the proper concentrations in the fermenters).

The second stage was sterilized with enough water to cover the pH probe membrane. After autoclaving, and before the effluent from the first stage was started, the water was pumped out of the fermenter. The residence time of the first fermenter (36 hours in Runs 1,2 and 3; 24 hours in Run 4) determined the time required to fill the second vessel to the 1.3 liter working volume. The effluent from the second vessel was collected in a sterile carboy.

The hydrolyzate liquor, CSL, and caustic addition vessels were placed on scales and the weights were recorded daily in order to monitor the dilution rate for the fermenters. Runs 1 through 3 were performed without interruption (although fresh inoculum was added at the beginning of each run due to problems with the pH control system). The fermenters were taken down, cleaned, and re-started for Run 4.

E-2.4 Sampling and Analysis

Samples were withdrawn every 24 hours and analyzed on the Yellow Springs Instrument (YSI) Analyzer for ethanol, glucose, and lactate. In addition, samples were analyzed by the CAT Team for glucose, xylose, acetic acid, lactic acid, glycerol, ethanol, furfural and HMF by HPLC. Cell counts via hemacytometer and dry cell weight were obtained for every sample to monitor cell growth and population maintenance. The dry cell weight was determined by centrifuging 5 ml of fermentation broth for 5—10 minutes at 5000 rpm. The supernatant was collected and frozen, and the pellet was washed with 5 mL of deionized water twice to remove dissolved solids. The pellets were transferred to weighed pans and allowed to dry overnight in a 105°C oven. Optical density for determining cell number was not used due to material that precipitated out of solution upon neutralization to pH 5.0.

E-3 Results and Discussion

E-3.1 Overview

A total of four runs were made in the chemostats. The conditions employed for each run are shown in Table E-1.

Table E-1. Chemostat Run Conditions

	APR Sample	Target Solids Concentration (%)	Actual Solids Concentration (%)	pH	Residence Time (h)
Run 1	330	25	35.0	5.0	36
Run 2	330	25	35.0	6.5	36
Run 3	330	27	23.8	5.0	36
Run 4	417/418	15	21.0	5.0	24

E-3.2 Run 1

The purpose of Run 1 was to establish a base case by duplicating conditions utilized in the PDU. After 24 hours of batch growth, the first stage fermenter contained 33 g/L ethanol and essentially no glucose. After being switched to continuous operation, the ethanol concentration peaked at 40 g/L (56 hours) and then proceeded to decrease throughout the remainder of the run (see Figures E-1 and E-2). During this time, the glucose concentration in vessel 1 increased to over 40 g/L. The yeast population appears to have stabilized at a low level. In the second vessel, as illustrated in Figures E-3 and E-44, the ethanol concentration peaked at 43 g/L (102 hours) and decreased throughout the remainder of the run. The glucose concentration increased to 8 g/L in vessel 2 by the end of the run. In general, the media appeared to be toxic to the yeast and a stable steady state was not achieved within the 340 hours of this experiment run.

The data from the end of the run is summarized in Table E-2. HPLC results showing sugar and product concentrations in the two vessels during the run are presented in Figure E-1.

Table E-2. Steady State Performance of Run 1

	Run 1, Vessel 1	Run 2, Vessel 2	Run 2, Overall
Overall Glucose Conversion	54.8%	80.8%	91.3%
Overall Xylose Conversion	10.0%	6.1%	15.4%
Ethanol Process Yield (% Theoretical)	32.8%	43.4%	58.6%
Ethanol Metabolic Yield (% Theoretical)	80.9%	96.6%	87.1%

E-3.3 Run 2

For Run 2, all conditions, except for pH, were maintained at the same settings as Run 1. The pH was increased to 6.5 to determine if higher pH would reduce the toxicity of the acetic and lactic acids and improve yield. This run was started with fresh inoculant after the pH control system on the chemostat over adjusted the pH to 10 (this is why the glucose concentration starts out at over 50 g/L - after readjusting the pH, the continuous feeds were kept running to determine if the cells had survived). In the first stage fermenter, as in Run 1, the glucose concentration started increasing and the ethanol concentration decreased after approximately 50 hours. As illustrated in Figure E-2 and Table E-3, these conditions enabled the yeast to produce glycerol at the expense of ethanol (12.5 g/L glycerol in the second stage at the end of the run). Cell counts and mass and xylose conversion were higher in this run than Run 1. Run 2 was terminated after 170 hours.

E-3.4 Run 3

For Run 3, the hydrolyzate liquor was diluted to the equivalent of 17% total solids (23.8% solids on sugar basis) to reduce the toxicity of the fermentation media. The temperature was maintained at 30°C, the pH at 5.0, and all other conditions as in previous runs. Stage 1 was operated in batch mode for approximately 24 hours after being inoculated. After being switched to continuous operation, the glucose concentration increased and ethanol decreased, as seen in the previous runs. During this run, the inner wall of the first vessel

was coated with material (which had been accumulating since the chemostat had been in operation non-stop for about 700 hours) and there appeared to be zones of poor mixing. Thus, the experiment was terminated after approximately 200 hours (even though a true steady-state had not yet been reached). By decreasing the solids level, the conversion of xylose improved, increasing the ethanol process yield to 65% of theoretical. The performance summary data for Run 3 follows in Table E-4, and HPLC data can be found in Figure E-3.

Table E-3. Performance of Run 2 at End of Run

	Run 2, Vessel 1	Run 2, Vessel 2	Run 2, Overall
Overall Glucose Conversion	64.2%	83.4%	94.1%
Overall Xylose Conversion	17.9%	13.6%	29.0%
Ethanol Process Yield (% Theoretical)	31.4%	40.1%	51.6%
Ethanol Metabolic Yield (% Theoretical)	63.4%	84.7%	70.4%

Table E-4. Performance of Run 3 at End of Run

	Run 3, Vessel 1	Run 3, Vessel 2	Run 3, Overall
Overall Glucose Conversion	77.0%	78.9%	95.1%
Overall Xylose Conversion	22.6%	10.4%	30.6%
Ethanol Process Yield (% Theoretical)	49.3%	38.4%	64.9%
Ethanol Metabolic Yield (% Theoretical)	82.8%	104%	87.1%

E-3.5 Run 4

This run was performed to determine if less severely pretreated material would be less toxic to the yeast and to evaluate the fermentation performance by diluting the liquor hydrolyzate to the equivalent of 15% total solids and reducing the residence time in each fermenter to 24 hours (also, this was compared against PDU performance in which the solids level had been dropped to 15% [residence time maintained at 36 hours vessel] to increase xylose utilization).

The hydrolyzate obtained between APR samples 417 and 418 was analyzed, before and after sampling, for glucose (YSI) and total solids and was similar to the analyses performed on APR samples 417 and 418. It was after the liquor was analyzed and was found to contain concentrations of all components 40% higher than that reported for the PDU, that we realized that the material collected for the chemostat runs was somehow being concentrated. As a result, the effective solids concentration was 21% instead of the targeted 15%.

As in the previous runs, once the continuous feeds were started, glucose concentration increased and ethanol decreased in the first vessel. The system appeared to stabilize and reach steady state after 215 hours. Due to the concentration effect on the hydrolyzate liquor, the feed material was somewhat toxic to the fermentation,

and conversions and yields of ethanol from xylose were lower than expected, but higher than those obtained from the previous 3 runs. A final ethanol concentration of 28 g/L was obtained. Xylose conversion was 36 % and process yield was 67% of theoretical. A sample of the material in the product collection vessel was analyzed and it was found to contain 33 g/L ethanol and that 62% of the xylose was consumed. This vessel contained material collected during the entire 309 hours the fermentation ran without any type of agitation or control.

Samples of material collected during the batch phase, middle of run, and the end of Run 4 were sent to Lee Polite for HMF and furfural analysis. His results indicate that furfural concentration dropped to <5 ug/mL in the both vessels during continuous operation and that HMF concentration was maintained at approximately 40 ug/mL in the first stage fermenter and 10 ug/mL in the second stage fermenter. Based on a preliminary analysis of shake flask results (Appendix D), neither species concentration is large enough to be causing any measurable effect on the fermentation. However, more studies into the effect of HMF and furfural on LNHST2 are required before a definitive statement can be made. Acetic acid concentrations were also reported at 2.8 g/L which was lower than HPLC results from NREL (3.3 to 4.3 g/L). The HMF and furural data is summarized in Table E-5.

Table E-5. HMF and Furfural Concentrations During Run 4

	HMF (ug/mL)	Furfural (ug/mL)
Hydrolyzate	805	850
Vessel 1, Batch	301	251
Vessel 1, Batch, t=19.5	13	12
Vessel 1, Continuous, t=0	21	5
Vessel 1, t=116.5	41	5
Vessel 2, t=116.5	9	<5
Vessel 1, t=309	38	<5
Vessel 2, t=309	8	<5

A summary of the results from Run 4 is contained in Table E-6 below. Process data, based on HPLC results is presented in Figure E-4.

Table E-6. Performance of Run 4 (steady-state data)

	Run 4 Vessel 1	Run 4 Vessel 2	Run 4, Overall
Overall Glucose Conversion	80.1%	79.6%	95.9%
Overall Xylose Conversion	24.1%	16.1%	36.3%
Ethanol Process Yield (% Theoretical)	52.2%	39.1%	67.4%
Ethanol Metabolic Yield (% Theoretical)	84.7%	101%	87.8%

E-4 Conclusions

The following conclusions can be drawn from the data obtained during the four chemostat runs:

1. The concentration effect seen with the hydrolyzate liquor, though unexplained, was useful in observing the effect of toxic material on a continuous fermentation. While exact duplication of PDU fermentation performance was not achieved, the effect of reducing the concentration of solids (and toxins) was an increase in xylose conversion. This data was useful in predicting the PDU performance at a 15% total solids concentration.
 - a) Clarifying hydrolyzate in the Bock Extractor (basket centrifuge) may be responsible for the concentration effect seen with all clarified material obtained.
2. Run 4 indicates that the fermentation can be run using a residence time of 24 hours per vessel without having washout.
3. Increasing the pH to 6.5 reduced the toxicity of the fermentation media but stimulated the yeast to produce approximately 3 times the glycerol normally seen during fermentation.
4. Glucose was not completely utilized in the first stage fermenter during any of the runs.
5. At steady state, HMF and furfural concentrations do not appear to be high enough to adversely affect the fermentation.

E-5 Recommendations

1. Future attempts to clarify hydrolyzate using the Bock Extractor should include sending samples of the slurry for standard analysis for comparison with analysis of clarified material.
2. Experiments need to be run using material diluted to yield sugar/toxin concentrations similar to the PDU at 15, 17, and 25% total solids.
3. Shake flask studies to determine nutrient requirements, ethanol tolerance, and the effect of process conditions (pH, temperature) on the fermentation. Also additional data on inhibitors is required.
4. Mixing studies to determine whether any mass transfer issues arise for scale-up.
5. Chemostat studies with ethanol recycle to measure the effect of ethanol and acetic/lactic acid on the fermentation. May also want to try different process configurations at this scale to determine if more optimum conditions can be obtained.

Figure E-1. Run 1 Component Concentrations

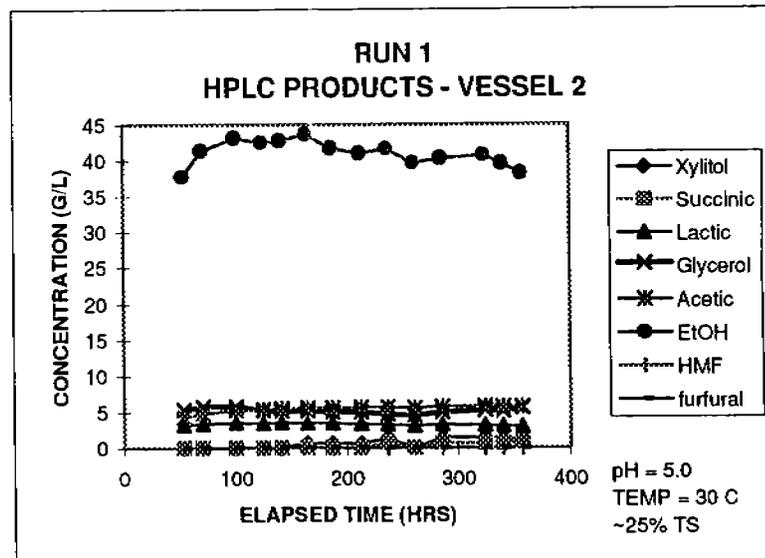
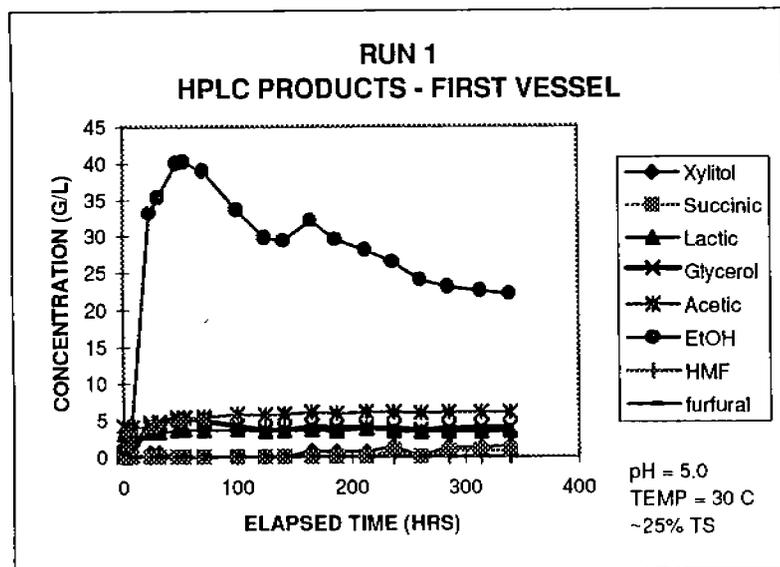
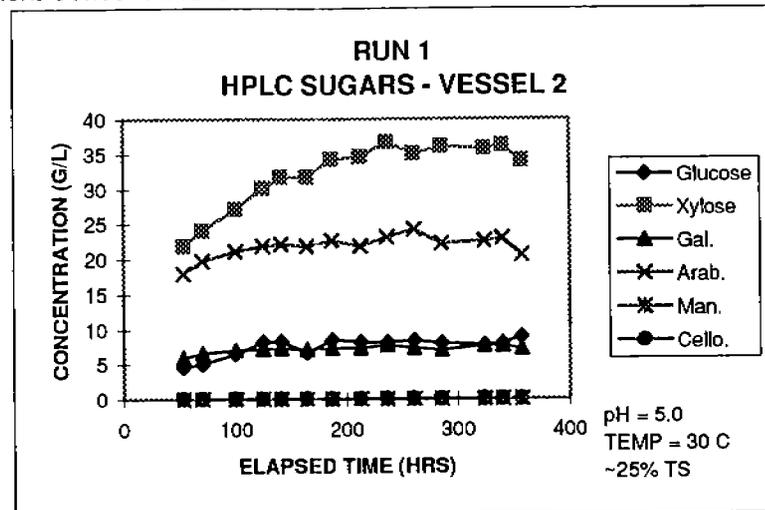
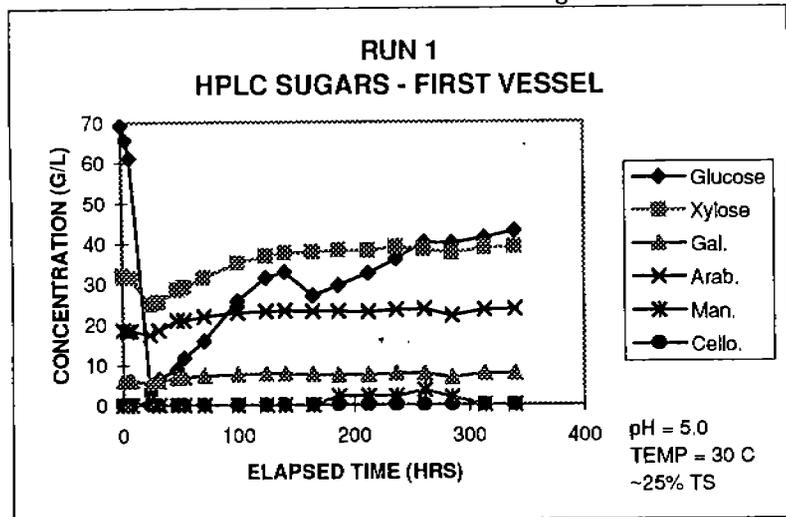


Figure E-2. Run 2 Component Concentrations

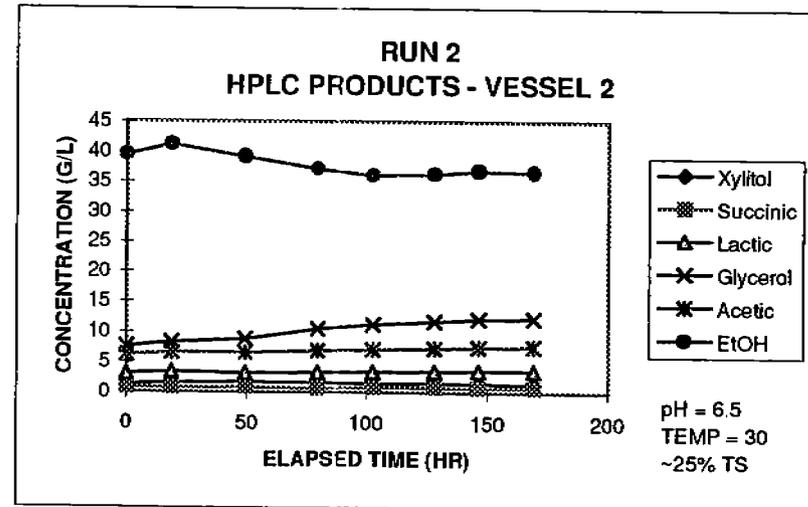
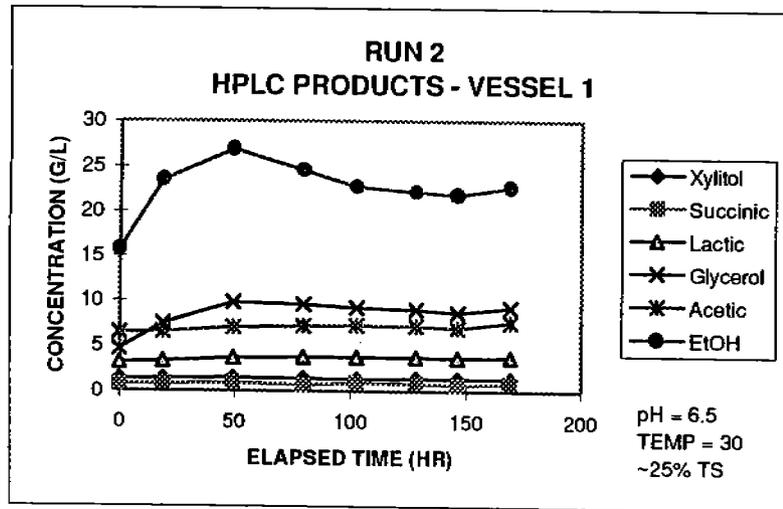
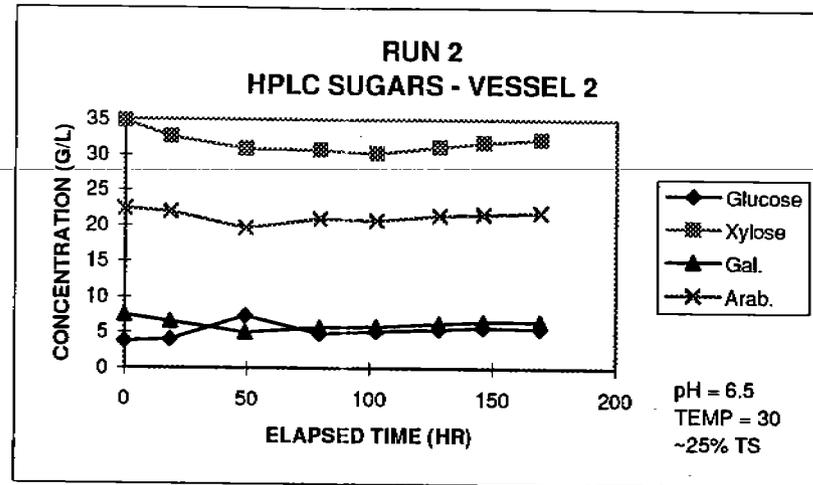
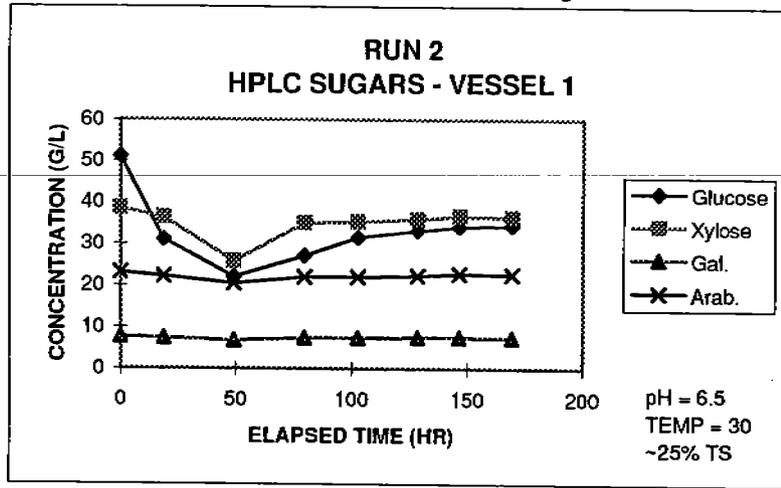


Figure E-3. Run 3 Component Concentrations

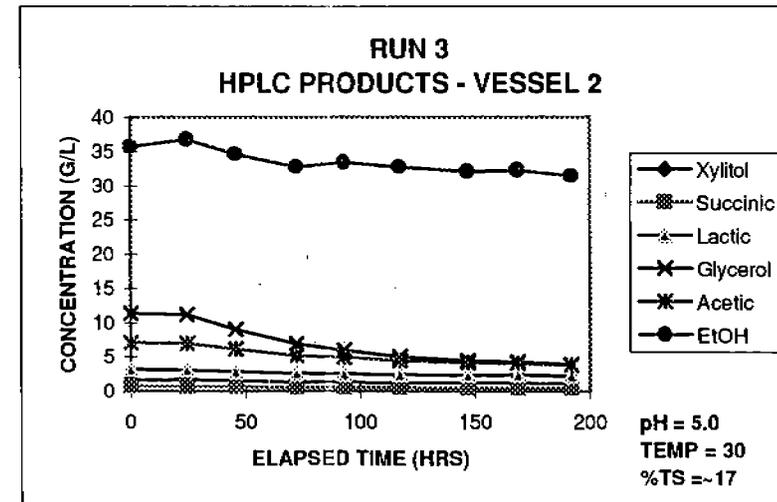
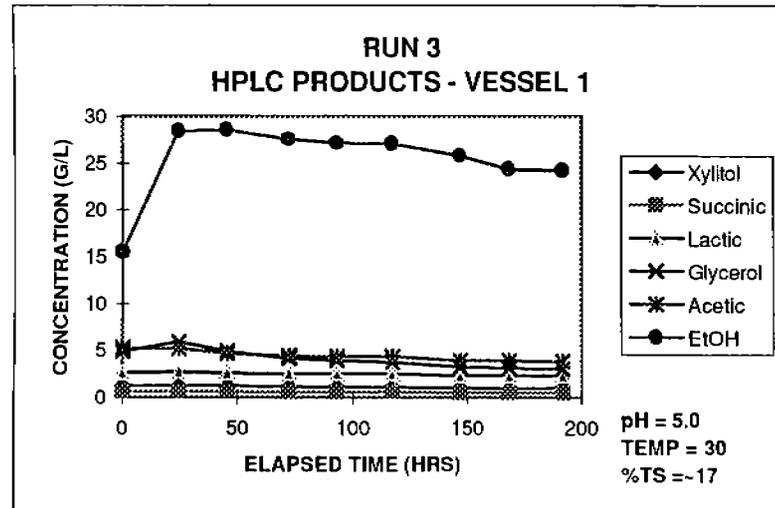
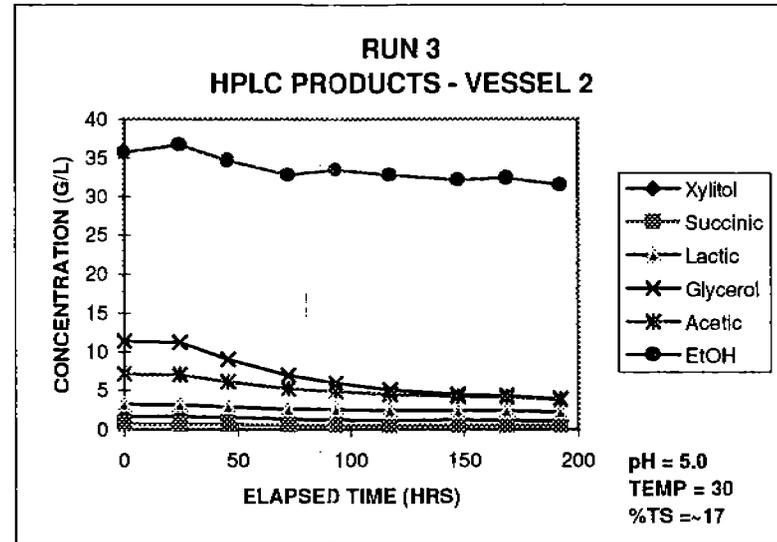
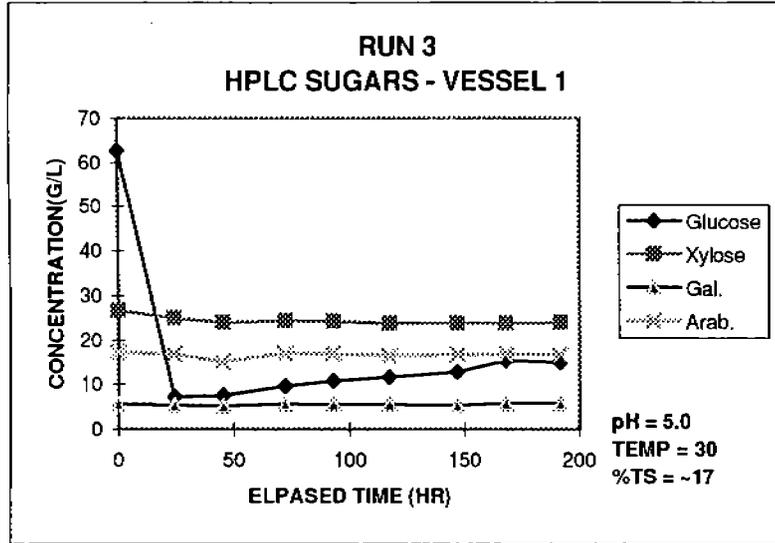
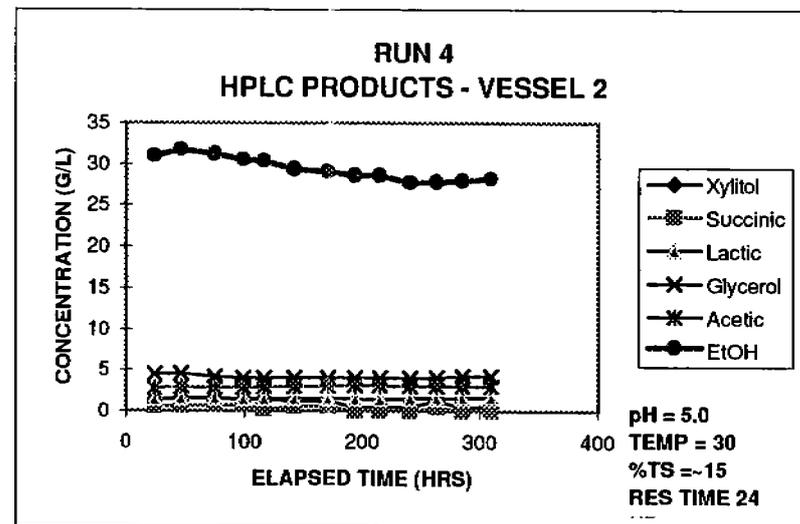
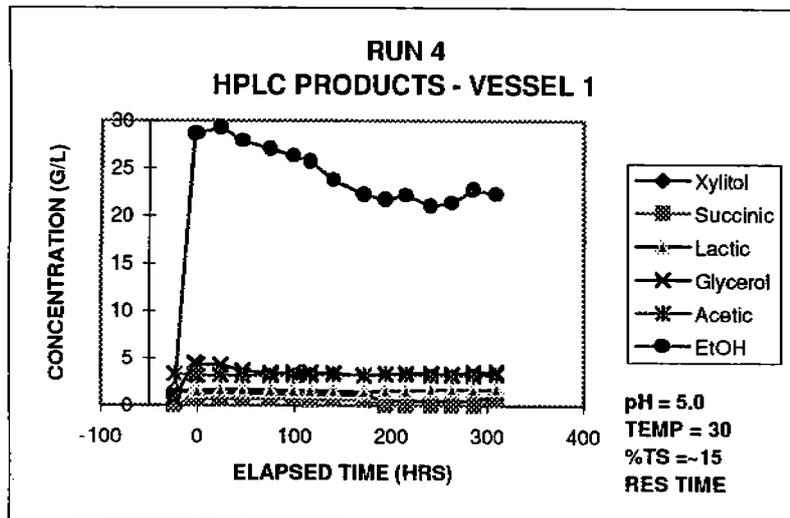
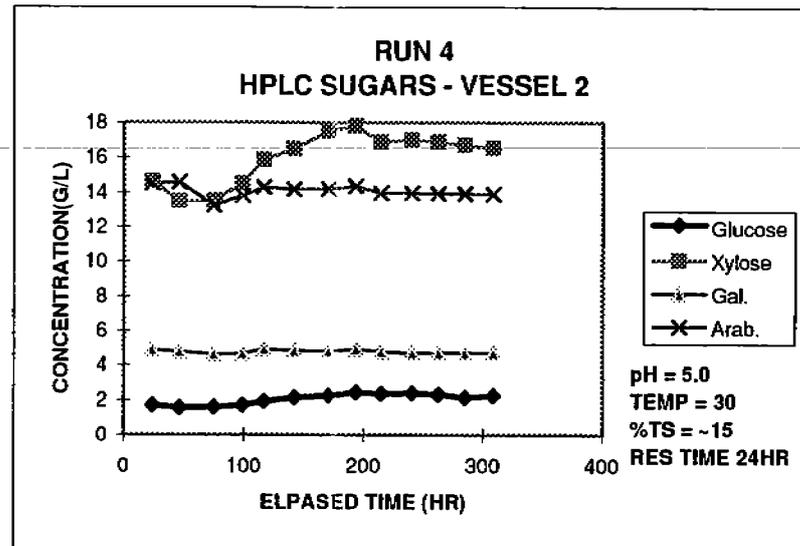
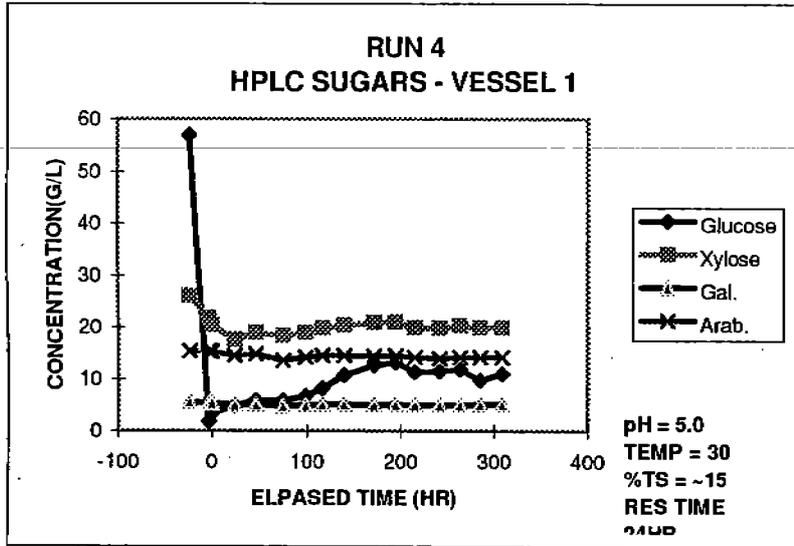


Figure E-4. Run 4 Component Concentrations



Run 1

SAMPLE ID	TOTAL Run Time (HR)	HPLC (g/L)													
		Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xyllitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
INOCULUM	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.25	0.25	1.12	0.00	9.11	0.00	0.00
T=0, V1	0.00	69.19	32.07	6.04	18.71	0.00	0.00	0.00	0.00	3.09	0.65	4.25	0.89	0.33	0.35
T=4, V1	4.08	65.55	31.51	5.93	18.38	0.00	0.00	0.00	0.00	2.94	0.61	4.02	2.04	0.27	0.00
CSL		0.40	0.00	0.09	0.02	0.00	0.00	0.00	0.10	3.26	0.23	0.65	0.00	0.00	0.00
HYDROLYSATE	7.33	129.42	60.17	11.28	35.01	0.00	0.00	0.00	0.00	3.66	0.00	7.75	0.00	0.80	0.00
T=7, V1	7.33	61.16	31.52	5.94	18.46	0.00	0.00	0.00	0.00	2.97	0.77	4.03	4.27	0.00	0.00
T=24, V1	23.58	2.82	25.02	5.52	17.48	1.17	0.00	0.61	0.00	3.40	4.69	4.10	33.17	0.00	0.00
T=32, V1	31.08	6.42	25.51	6.02	18.71	0.00	0.00	0.58	0.00	3.34	4.75	4.27	35.34	0.00	0.00
T=47, V1	47.08	8.85	28.52	6.81	21.06	0.00	0.00	0.00	0.00	3.60	5.38	5.04	39.98	0.00	0.00
HYDR2		128.68	62.40	13.35	37.29	0.00	0.00								
T=56, V1	53.25	11.67	29.02	6.91	20.98	0.00	0.00	0.00	0.00	3.69	5.39	5.32	40.13	0.00	0.00
T=56, V2	53.25	4.58	21.85	5.91	17.90	0.00	0.00	0.00	0.00	3.17	5.32	4.29	37.72	0.00	0.00
T=74, V1	70.58	15.97	31.39	7.22	21.94	0.00	0.00	0.00	0.00	3.60	4.95	5.45	38.95	0.00	0.00
T=74, V2	70.58	4.98	24.06	6.54	19.69	0.00	0.00	0.00	0.00	3.38	5.76	4.76	41.37	0.00	0.00
T=102, V1	100.08	25.79	35.08	7.56	22.93	0.00	0.00	0.00	0.00	3.71	4.22	5.77	33.60	0.00	0.00
T=102, V2	100.08	6.51	27.14	7.05	21.12	0.00	0.00	0.00	0.00	3.52	5.74	5.20	43.12	0.00	0.00
T=126.5, V1	124.58	31.47	36.78	7.67	23.29	0.00	0.00	0.00	0.00	3.48	3.64	5.67	29.76	0.00	0.00
T=126.5, V1	124.58	30.44	36.00	7.44	22.75	2.27	0.00								
T=126.5, V2	124.58	8.22	30.13	7.25	21.87	0.00	0.00	0.00	0.00	3.47	5.28	5.35	42.54	0.00	0.00
T=126.5, V2	124.58	7.78	29.81	7.00	21.45	0.00	0.00								
T=143, V1	141.17	32.79	37.63	7.70	23.40	0.00	0.00	0.00	0.00	3.53	3.61	5.78	29.34	0.00	0.00
T=143, V2	141.17	8.32	31.65	7.34	22.13	0.00	0.00	0.00	0.00	3.52	5.15	5.52	42.79	0.00	0.00
T=165, V1	165.08	27.04	37.84	7.51	23.27	0.00	0.00	0.81	0.00	3.64	4.18	6.03	32.08	0.00	0.00
T=165, V2	165.08	6.66	31.73	7.10	21.85	0.00	0.00	0.69	0.00	3.49	5.12	5.61	43.64	0.00	0.00
T=187, V1	187.08	29.62	38.19	7.48	23.27	2.21	0.00	0.73	0.00	3.50	3.98	5.92	29.54	0.00	0.00
T=187, V2	187.08	8.46	34.29	7.33	22.69	0.00	0.00	0.62	0.00	3.45	4.93	5.63	41.76	0.00	0.00
T=213, V1	213.08	32.54	38.07	7.45	23.03	2.27	0.00	0.70	0.00	3.78	4.00	6.06	28.08	0.00	0.00
T=213, V2	213.08	8.24	34.57	7.29	21.86	0.00	0.00	0.58	0.00	3.38	4.95	5.72	41.04	0.00	0.00
T=237, V1	237.08	35.96	39.05	7.80	23.53	2.25	0.00	1.38	0.86	3.44	3.93	6.04	26.39	0.00	0.00
T=237, V2	237.08	8.09	36.74	7.78	23.13	0.00	0.00	1.35	0.74	3.27	4.74	5.70	41.62	0.00	0.00
T=261, V1	261.08	40.14	38.46	7.71	23.66	3.65	0.00	0.00	0.00	3.40	3.64	5.94	24.01	0.00	0.00
T=261, V2	261.08	8.43	35.06	7.30	24.21	0.00	0.00	0.00	0.00	3.13	4.57	5.57	39.67	0.00	0.00
T=285, V1	285.33	39.94	37.43	6.87	22.12	1.92	0.00	1.40	0.75	3.40	3.93	6.02	23.02	0.00	0.00
T=285, V2	285.33	8.00	36.03	6.99	22.17	0.00	0.00	1.40	0.72	3.29	4.98	5.80	40.34	0.00	0.00
T=324, V1	314.08	41.27	38.64	7.78	23.53	0.00	0.00	1.28	0.91	3.41	3.94	6.04	22.52	0.00	0.00
T=324, V2	314.08	9.69	43.96	9.45	29.70	0.00	0.00	1.68	0.91	3.89	6.25	6.90	47.51	0.00	0.00
t=324, v2 - 2nd	324.00	7.79	35.83	7.66	22.53	0.00	0.00	1.41	0.65	3.20	5.23	5.88	40.74	0.00	0.00
T=340, V1	340.00	42.99	39.03	7.74	23.75	0.00	0.00	1.51	0.78	3.44	3.95	6.09	22.07	0.00	0.00
T=340, V2	340.00	8.00	36.24	7.64	22.94	0.00	0.00	1.45	0.66	3.15	5.18	5.76	39.57	0.00	0.00
T=357, V1	357.00	42.88	36.35	7.22	20.49	0.00	0.00	0.93	0.70	2.70	3.06	5.35	19.54	0.00	0.00
T=357, V2	357.00	8.93	34.09	7.19	20.58	0.00	0.00	1.08	0.90	3.04	5.55	5.79	38.18	0.00	0.00

Run 2		HPLC (g/L)														
SAMPLE ID	TOTAL RUN TIME (HR)	Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural	
T=0, V1	0.00	51.09	38.44	7.65	22.98	0.00	0.00	1.34	0.80	3.19	4.64	6.46	15.76	0.00	0.00	
T=0, V2	0.00	3.77	34.86	7.44	22.45	0.00	0.00	1.30	0.70	3.07	7.47	6.17	39.53	0.00	0.00	
T=18.75, V1	18.75	30.96	36.41	7.42	22.03	2.59?	0.00	1.42	0.76	3.29	7.48	6.59	23.51	0.00	0.00	
T=18.75, V2	18.75	4.01	32.62	6.55	21.96	0.00	0.00	1.58	0.77	3.35	8.24	6.54	41.14	0.00	0.00	
T=37.25, V1	49.25	21.87	25.67	6.78	20.47	0.00	0.00	1.47	0.92	3.65	9.81	7.08	26.87	0.00	0.00	
T=37.25, V2	49.25	7.37	30.86	4.99	19.69	0.00	0.00	1.72	0.83	3.21	8.83	6.56	39.13	0.00	0.00	
T=67.75, V1	79.75	27.04	35.03	7.39	21.96	0.00	0.00	1.42	0.73	3.71	9.60	7.18	24.56	0.00	0.00	
T=67.75, V2	79.75	4.82	30.72	5.76	21.03	0.00	0.00	1.57	0.66	3.24	10.51	6.84	37.15	0.00	0.00	
T=90.75, V1	102.75	31.52	35.37	7.36	21.99	0.00	0.00	1.35	0.90	3.77	9.25	7.25	22.75	0.00	0.00	
T=90.75, V2	102.75	5.17	30.23	5.85	20.73	0.00	0.00	1.48	0.81	3.38	11.26	7.11	36.08	0.00	0.00	
T=116.25, V1	128.25	33.17	35.85	7.45	22.20	0.00	0.00	1.40	0.82	3.77	9.01	7.20	22.20	0.00	0.00	
T=116.25, V2	128.25	5.48	31.18	6.29	21.46	0.00	0.00	1.54	0.80	3.51	11.79	7.34	36.32	0.00	0.00	
T=134.25, V1	146.25	34.09	36.61	7.57	22.65	0.00	0.00	1.32	0.73	3.64	8.66	7.02	21.82	0.00	0.00	
T=134.25, V2	146.25	5.76	31.74	6.51	21.67	0.00	0.00	1.52	0.78	3.60	12.13	7.53	36.82	0.00	0.00	
HYDR3		133.42	62.39	12.56	36.62	0.00	0.00	1.10	0.30	0.83	0.00	6.18	0.00	0.00	0.00	
T=169.25, V1	169.25	34.32	36.36	7.42	22.52	0.00	0.00	1.32	0.86	3.73	9.25	7.61	22.67	0.00	0.00	
T=169.25, V2	169.25	5.58	32.26	6.55	21.94	0.00	0.00	1.36	0.77	3.57	12.30	7.65	36.63	0.00	0.00	
T=0, V1	0.00	51.09	38.44	7.65	22.98	0.00	0.00	1.34	0.80	3.19	4.64	6.46	15.76	0.00	0.00	
T=18.75, V1	18.75	30.96	36.41	7.42	22.03	2.59?	0.00	1.42	0.76	3.29	7.48	6.59	23.51	0.00	0.00	
T=37.25, V1	49.25	21.87	25.67	6.78	20.47	0.00	0.00	1.47	0.92	3.65	9.81	7.08	26.87	0.00	0.00	
T=67.75, V1	79.75	27.04	35.03	7.39	21.96	0.00	0.00	1.42	0.73	3.71	9.60	7.18	24.56	0.00	0.00	
T=90.75, V1	102.75	31.52	35.37	7.36	21.99	0.00	0.00	1.35	0.90	3.77	9.25	7.25	22.75	0.00	0.00	
T=116.25, V1	128.25	33.17	35.85	7.45	22.20	0.00	0.00	1.40	0.82	3.77	9.01	7.20	22.20	0.00	0.00	
T=134.25, V1	146.25	34.09	36.61	7.57	22.65	0.00	0.00	1.32	0.73	3.64	8.66	7.02	21.82	0.00	0.00	
T=169.25, V1	169.25	34.32	36.36	7.42	22.52	0.00	0.00	1.32	0.86	3.73	9.25	7.61	22.67	0.00	0.00	
T=0, V2	0.00	3.77	34.86	7.44	22.45	0.00	0.00	1.30	0.70	3.07	7.47	6.17	39.53	0.00	0.00	
T=18.75, V2	18.75	4.01	32.62	6.55	21.96	0.00	0.00	1.58	0.77	3.35	8.24	6.54	41.14	0.00	0.00	
T=37.25, V2	49.25	7.37	30.86	4.99	19.69	0.00	0.00	1.72	0.83	3.21	8.83	6.56	39.13	0.00	0.00	
T=67.75, V2	79.75	4.82	30.72	5.76	21.03	0.00	0.00	1.57	0.66	3.24	10.51	6.84	37.15	0.00	0.00	
T=90.75, V2	102.75	5.17	30.23	5.85	20.73	0.00	0.00	1.48	0.81	3.38	11.26	7.11	36.08	0.00	0.00	
T=116.25, V2	128.25	5.48	31.18	6.29	21.46	0.00	0.00	1.54	0.80	3.51	11.79	7.34	36.32	0.00	0.00	
T=134.25, V2	146.25	5.76	31.74	6.51	21.67	0.00	0.00	1.52	0.78	3.60	12.13	7.53	36.82	0.00	0.00	
T=169.25, V2	169.25	5.58	32.26	6.55	21.94	0.00	0.00	1.36	0.77	3.57	12.30	7.65	36.63	0.00	0.00	

Run 2

SAMPLE ID	TOTAL RUN TIME (MIN)	TOTAL RUN TIME (HR)	TARE	TARE+DCW	DCW (G/L)	VOLUME (ML)	CELL COUNT (HEMACYTOMETER)		PLATE COUNT
T=0, V1	0	0.00	1.1148	1.1512	7.28	5/ML	2.28E+07	/ML	8.25E+06
T=0, V2	0	0.00	1.1107	1.1348	4.82	5/ML	2.80E+07	/ML	1.86E+07
T=18.75, V1		18.75	1.1035	1.1352	6.34	5/ML	3.45E+07	/ML	1.36E+07
T=18.75, V2		18.75	1.1104	1.1496	7.84	5/ML	3.50E+07	/ML	1.33E+07
T=37.25, V1		49.25	1.1126	1.1478	7.04	5/ML	4.89E+07	/ML	
T=37.25, V2		49.25	1.1002	1.1420	8.36	5/ML	4.53E+07	/ML	
T=67.75, V1		79.75	1.1028	1.1328	6.00	5/ML	1.23E+07	/ML	
T=67.75, V2		79.75	1.1018	1.1451	8.66	5/ML	3.96E+07	/ML	
T=90.75, V1		102.75	1.0782	1.1053	5.42	5/ML	1.72E+07	/ML	3.95E+06
T=90.75, V2		102.75	1.0841	1.1208	7.34	5/ML	4.39E+07	/ML	1.31E+07
T=116.25, V1		128.25	1.0811	1.1058	4.94	5/ML	1.40E+07	/ML	
T=116.25, V2		128.25	1.0962	1.1355	7.86	5/ML	2.65E+07	/ML	
T=134.25, V1		146.25	1.1040	1.1294	5.08	5/ML	2.26E+07	/ML	
T=134.25, V2		146.25	1.1071	1.1445	7.48	5/ML	5.03E+07	/ML	
T=169.25, V1		169.50	1.1135		-222.70	5/ML	1.68E+07	/ML	
T=169.25, V2		169.50	1.0873		-217.46	5/ML	4.83E+07	/ML	

Run 3		HPLC (g/L)													
SAMPLE ID	TOTAL RUN TIME (HR)	Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
		HYDR3		92.65	43.59	7.58	27.24	0.00	0.00	1.29	0.54	2.22	0.00	5.49	0.00
T=0, V1	0.00	62.62	26.69	5.65	17.28	0.00	0.00	1.20	0.59	2.64	4.89	5.28	15.53	0.00	0.00
T=0, V2	0.00	2.25	27.79	5.40	19.54	0.00	0.00	1.61	0.71	3.17	11.32	7.08	35.73	0.00	0.00
t=24.5, V1	24.50	7.24	24.99	5.29	16.73	0.00	0.00	1.23	0.64	2.69	5.92	5.16	28.45	0.00	0.00
t=24.5, V2	24.50	2.23	26.57	5.09	19.29	0.00	0.00	1.66	0.68	3.11	11.19	6.96	36.74	0.00	0.00
t=45.5 V1	45.50	7.44	23.96	5.20	15.02	0.00	0.00	1.19	0.59	2.57	4.91	4.69	28.60	0.00	0.00
t=45.5, V2	45.50	2.37	24.58	5.00	16.70	0.00	0.00	1.54	0.64	2.87	9.00	6.11	34.64	0.00	0.00
t=72.25, V1	72.25	9.54	24.40	5.55	17.08	0.00	0.00	1.12	0.57	2.49	4.14	4.39	27.54	0.00	0.00
t=72.25, V2	72.25	3.58	23.85	5.32	17.99	0.00	0.00	1.33	0.56	2.56	6.88	5.21	32.73	0.00	0.00
T=93, V1	93.25	10.78	24.18	5.47	16.89	0.00	0.00	1.11	0.56	2.54	3.93	4.34	27.12	0.00	0.00
T=93, V2	93.25	3.57	23.38	5.37	17.64	0.00	0.00	1.27	0.53	2.52	5.94	4.89	33.43	0.00	0.00
T=117, V1	117.25	11.64	23.76	5.49	16.54	0.00	0.00	1.10	0.61	2.54	3.76	4.31	27.09	0.00	0.00
T=117, V2	117.25	4.53	24.51	6.00	18.54	0.00	0.00	1.18	0.53	2.40	5.03	4.47	32.79	0.00	0.00
T=147, V1	147.00	12.81	23.78	5.35	16.70	1.37	0.00	0.99	0.47	2.31	3.26	3.93	25.77	0.00	0.00
T=168.5, V1	168.50	15.33	23.78	5.80	16.78	2.04	0.00	0.98	0.46	2.32	3.13	3.94	24.37	0.00	0.00
T=168.5, V2	168.50	3.80	21.13	5.34	16.40	0.99	0.00	1.18	0.49	2.40	4.25	4.11	32.32	0.00	0.00
T=192, V1	192.00	14.89	23.99	5.69	16.67	0.00	0.00	0.99	0.49	2.29	3.08	3.87	24.23	0.00	0.00
T=192, V2	192.00	2.04	20.85	5.35	16.24	0.00	0.00	1.09	0.49	2.24	3.88	3.79	31.46	0.00	0.00
T=0, V1	0.00	62.62	26.69	5.65	17.28	0.00	0.00	1.20	0.59	2.64	4.89	5.28	15.53	0.00	0.00
t=24.5, V1	24.50	7.24	24.99	5.29	16.73	0.00	0.00	1.23	0.64	2.69	5.92	5.16	28.45	0.00	0.00
t=45.5 V1	45.50	7.44	23.96	5.20	15.02	0.00	0.00	1.19	0.59	2.57	4.91	4.69	28.60	0.00	0.00
t=72.25, V1	72.25	9.54	24.40	5.55	17.08	0.00	0.00	1.12	0.57	2.49	4.14	4.39	27.54	0.00	0.00
T=93, V1	93.25	10.78	24.18	5.47	16.89	0.00	0.00	1.11	0.56	2.54	3.93	4.34	27.12	0.00	0.00
T=117, V1	117.25	11.64	23.76	5.49	16.54	0.00	0.00	1.10	0.61	2.54	3.76	4.31	27.09	0.00	0.00
T=147, V1	147.00	12.81	23.78	5.35	16.70	1.37	0.00	0.99	0.47	2.31	3.26	3.93	25.77	0.00	0.00
T=168.5, V1	168.50	15.33	23.78	5.80	16.78	2.04	0.00	0.98	0.46	2.32	3.13	3.94	24.37	0.00	0.00
T=192, V1	192.00	14.89	23.99	5.69	16.67	0.00	0.00	0.99	0.49	2.29	3.08	3.87	24.23	0.00	0.00

Run 3

SAMPLE ID	TOTAL RUN TIME (MIN)	TOTAL RUN TIME (HR)	TARE	TARE+DCW	DCW (G/L)	VOLUME (ML)	CELL COUNT (HEMACYTOMETER)		PLATE COUNT
T=0, V1		0	0.00	1.1156	1.1342	3.72	5/ML	2.35E+07	/ML
T=0, V2		0	0.00	1.1074	1.1464	7.80	5/ML	3.95E+07	/ML
t=24.5, V1		24.50	1.1122	1.1359	4.74	5/ML	4.40E+07	/ML	3.60E+07
t=24.5, V2		24.50	1.1043	1.1424	7.62	5/ML	3.50E+07	/ML	1.15E+07
t=45.5 V1		45.50	1.0996	1.1184	3.76	5/ML	2.00E+07	/ML	
t=45.5, V2		45.50	1.0956	1.1266	6.20	5/ML	1.74E+07	/ML	
t=72.25, V1		72.25	1.0824	1.0994	3.40	5/ML	2.80E+07	/ML	
t=72.25, V2		72.25	1.0982	1.1264	5.64	5/ML	3.00E+07	/ML	
T=93, V1		93.25	1.2521	1.2667	2.92	5/ML	1.58E+07	/ML	
T=93, V2		93.25	1.2667	1.2884	4.34	5/ML	1.39E+07	/ML	
T=117, V1		117.25	1.2620	1.2772	3.04	5/ML	1.26E+07	/ML	
T=117, V2		117.25	1.2509	1.2709	4.00	5/ML	1.63E+07	/ML	
T=147, V1		147.00	1.2751	1.2893	2.84	5/ML	2.21E+07	/ML	
T=147, V2		147.00	1.2548	1.2741	3.86	5/ML	1.95E+07	/ML	
T=168.5, V1		168.50	1.2653	1.2787	2.68	5/ML	1.69E+07	/ML	
T=168.5, V2		168.50	1.2595	1.2776	3.62	5/ML	2.02E+07	/ML	
T=192, V1		192.00	1.2713	1.2851	2.76	5/ML	1.59E+07	/ML	
T=192, V2		192.00	1.2637	1.2833	3.92	5/ML	2.00E+07	/ML	

Run 4		HPLC (g/L)														
SAMPLE ID	TOTAL RUN TIME (HR)	Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural	
V1, T0, BATCH	-24.00	56.99	26.04	5.54	15.35	0.00	0.00	1.60	0.00	1.41	0.62	3.35	1.01	0.37	0.39	
HYDR1		136.32	62.56	13.09	36.74	3.24	0.00	0.00	0.00	1.39	0.00	6.82	0.00	0.84	0.85	
FEED1		83.43	38.19	7.99	22.40	2.01	0.00	1.77	0.00	0.80	0.00	3.84	0.00	0.48	0.49	
V1, T3, BATCH		55.00	26.07	5.47	15.31	1.37	0.00	1.41	0.00	1.24	0.46	3.13	1.00	0.32	0.00	
V1, T19.5, BATCH	-3.50	1.90	21.72	5.28	15.33	0.00	0.00	1.26	0.71	1.69	4.48	3.29	28.60	0.00	0.00	
V1, T0, CONT	0.00	3.28	20.41	5.23	15.18	0.00	0.00	1.24	0.64	1.56	4.26	3.15	28.66	0.00	0.00	
t=24, cont, v1	24.00	4.82	17.60	4.91	14.47	0.00	0.00	1.25	0.66	1.65	4.27	3.19	29.25	0.00	0.00	
t=24, cont, v2	24.00	1.69	14.61	4.89	14.51	0.00	0.00	1.33	0.57	1.42	4.45	2.82	30.95	0.00	0.00	
t=46.5, cont, v1	46.50	5.71	18.86	4.97	14.70	0.00	0.00	1.25	0.64	1.64	3.66	3.15	27.87	0.00	0.00	
t=46.5, cont, v2	46.50	1.56	13.47	4.79	14.57	0.00	0.00	1.58	0.65	1.53	4.57	2.92	31.76	0.00	0.00	
t=75, v1	75.00	5.75	18.27	4.78	13.48	0.00	0.00	1.20	0.65	1.61	3.38	3.11	26.96	0.00	0.00	
t=75, v2	75.00	1.58	13.51	4.62	13.21	0.00	0.00	1.51	0.66	1.52	4.17	2.88	31.19	0.00	0.00	
t=99, v1	99.00	6.66	18.94	4.89	14.15	0.00	0.00	1.13	0.73	1.56	3.45	3.12	26.29	0.00	0.00	
t=99, v2	99.00	1.69	14.52	4.69	13.79	0.00	0.00	1.38	0.73	1.50	4.03	2.87	30.55	0.00	0.00	
t=116.5, v1	116.50	8.12	19.81	5.05	14.52	1.21	0.00	1.18	0.56	1.57	3.48	3.23	25.67	0.00	0.00	
t=116.5, v2	116.50	1.90	15.85	4.94	14.30	0.00	0.00	1.40	0.20	1.48	3.98	2.90	30.38	0.00	0.00	
feed2		69.48	30.90	5.02	16.73	0.00	0.00	1.30	0.62	0.80	0.00	3.93	0.00	0.48	0.52	
t=140, v1	140.00	10.62	20.34	5.02	14.47	0.00	0.00	1.09	0.58	1.56	3.42	3.26	23.75	0.00	0.00	
t=140, v2	142.00	2.12	16.50	4.86	14.18	0.00	0.00	1.22	0.56	1.48	4.00	2.93	29.37	0.00	0.00	
t=171, v1	171.00	12.49	20.90	4.96	14.35	0.00	0.00	1.04	0.52	1.42	3.14	3.19	22.15	0.00	0.00	
t=171, v2	171.00	2.24	17.51	4.84	14.19	0.00	0.00	1.15	0.54	1.44	3.98	3.04	29.03	0.00	0.00	
t=194, v1	194.00	13.15	21.04	5.09	14.43	1.77	0.00	0.46	0.00	1.54	3.29	3.33	21.62	0.00	0.00	
t=194, v2	194.00	2.42	17.83	4.93	14.35	0.00	0.00	0.45	0.00	1.45	4.03	3.09	28.56	0.00	0.00	
FEED3		72.53	34.91	6.58	19.94	1.78	0.00	0.53	0.00	0.78	0.00	3.96	0.00	0.46	0.48	
t=215, v1	215.00	11.31	19.85	4.94	14.07	1.76	0.00	0.50	0.00	1.53	3.39	3.25	22.09	0.00	0.00	
t=215, v2	215.00	2.34	16.91	4.79	13.95	0.00	0.00	0.44	0.00	1.42	4.00	3.06	28.62	0.00	0.00	
t=241, v1	241.00	11.43	19.72	4.85	13.84	1.82	0.00	0.47	0.00	1.67	3.38	3.15	21.01	0.00	0.00	
t=241, v2	241.00	2.40	17.00	4.73	13.95	0.00	0.00	0.43	0.00	1.51	4.04	3.02	27.75	0.00	0.00	
t=263, v1	263.00	11.82	20.21	4.95	14.15	1.77	0.00	0.44	0.00	1.64	3.36	3.13	21.34	0.00	0.00	
t=263, v2	263.00	2.33	16.90	4.73	13.93	0.00	0.00	1.21	0.49	1.57	4.11	3.01	27.85	0.00	0.00	
t=285, v1	285.00	9.67	19.93	4.97	14.15	1.57	0.00	0.45	0.00	1.67	3.56	3.17	22.72	0.00	0.00	
t=285, v2	285.00	2.12	16.70	4.72	13.91	0.00	0.00	0.45	0.00	1.62	4.24	3.04	27.99	0.00	0.00	
FEED4		70.75	33.32	6.28	18.85	1.60	0.00	1.25	0.57	0.77	0.00	4.05	0.00	0.46	0.49	
FEED5 (hydr		120.16	57.21	12.07	33.19	3.12	0.00	0.91	0.00	1.37	0.00	7.04	0.00	0.78	0.85	
t=309, v1	309.00	10.95	19.85	4.98	14.17	1.73	0.00	1.04	0.57	1.77	3.64	3.28	22.26	0.00	0.00	
t=309, v2	309.00	2.25	16.54	4.72	13.88	0.00	0.00	0.46	0.00	1.64	4.27	3.02	28.19	0.00	0.00	
product	309.00	1.55	9.87	4.42	13.57	0.00	0.00	1.84	0.51	1.51	4.65	2.95	33.09	0.00	0.00	

Run 4

SAMPLE ID	TOTAL RUN TIME (MIN)	TOTAL RUN TIME (HR)	TARE	TARE+DCW	DCW (G/L)	VOLUME (ML)	CELL COUNT (HEMACYTOMETER)		PLATE COUNT
T=0, V1		-24.00	1.0979	1.1074	1.90	5/ML	1.82E+07	/ML	
T=0, V2						5/ML		/ML	
T=19.5, V1 BATCH		-3.50	1.2583	1.2857	5.48	5/ML	1.33E+08	/ML	
T=0, CONTINUOUS, V1		0.00	1.2554	1.2824	5.40	5/ML	9.25E+07	/ML	
t=24, cont, v1		24.00	1.2682	1.2900	4.36	5/ML	5.00E+07	/ML	
t=24, cont, v2		24.00	1.2683	1.2936	5.06	5/ML	7.30E+07	/ML	
t=46.5, cont, v1		46.50	1.2640	1.2827	3.74	5/ML	3.75E+07	/ML	
t=46.5, cont, v2		46.50	1.2700	1.2937	4.74	5/ML	6.07E+07	/ML	
t=75, v1		75.00	1.2420	1.2594	3.48	5/ML	2.45E+07	/ML	
t=75, v2		75.00	1.2495	1.2714	4.38	5/ML	4.30E+07	/ML	
t=99, v1		99.00	1.2734	1.2895	3.22	5/ML	3.05E+07	/ML	
t=99, v2		99.00	1.2664	1.2792	2.56	5/ML	2.40E+07	/ML	
t=116.5, v1		116.50	1.2736	1.2882	2.92	5/ML	2.39E+07	/ML	
t=116.5, v2		116.50	1.2735	1.2922	3.74	5/ML	2.87E+07	/ML	
t=140, v1		140.00	1.2644	1.2787	2.86	5/ML	2.19E+07	/ML	
t=140, v2		140.00	1.2607	1.2786	3.58	5/ML	2.76E+07	/ML	
t=171, v1		171.00	1.2741	1.2881	2.80	5/ML	9.00E+06	/ML	
t=171, v2		171.00	1.2655	1.2831	3.52	5/ML	1.50E+07	/ML	
t=194, v1		194.00	1.2673	1.2806	2.66	5/ML	2.42E+07	/ML	
t=194, v2		194.00	1.2531	1.2698	3.34	5/ML	2.54E+07	/ML	
t=215, v1		215.00	1.2391	1.2532	2.82	5/ML	2.63E+07	/ML	
t=215, v2		215.00	1.2445	1.2609	3.28	5/ML	3.23E+07	/ML	
t=241, v1		241.00	1.2732	1.2869	2.74	5/ML	2.81E+07	/ML	
t=241, v2		241.00	1.2736	1.2904	3.36	5/ML	3.30E+07	/ML	
t=263, v1		263.00	1.2610	1.2753	2.86	5/ML	2.98E+07	/ML	
t=263, v2		263.00	1.2618	1.2773	3.10	5/ML	2.93E+07	/ML	
t=285, v1		285.00	1.2724	1.2865	2.82	5/ML	2.84E+07	/ML	
t=285, v2		285.00	1.2468	1.2629	3.22	5/ML	3.34E+07	/ML	
t=309, v1		309.00	1.2509	1.2642	2.66	5/ML	3.05E+07	/ML	
t=309, v2		309.00	1.2708	1.2862	3.08	5/ML	3.46E+07	/ML	

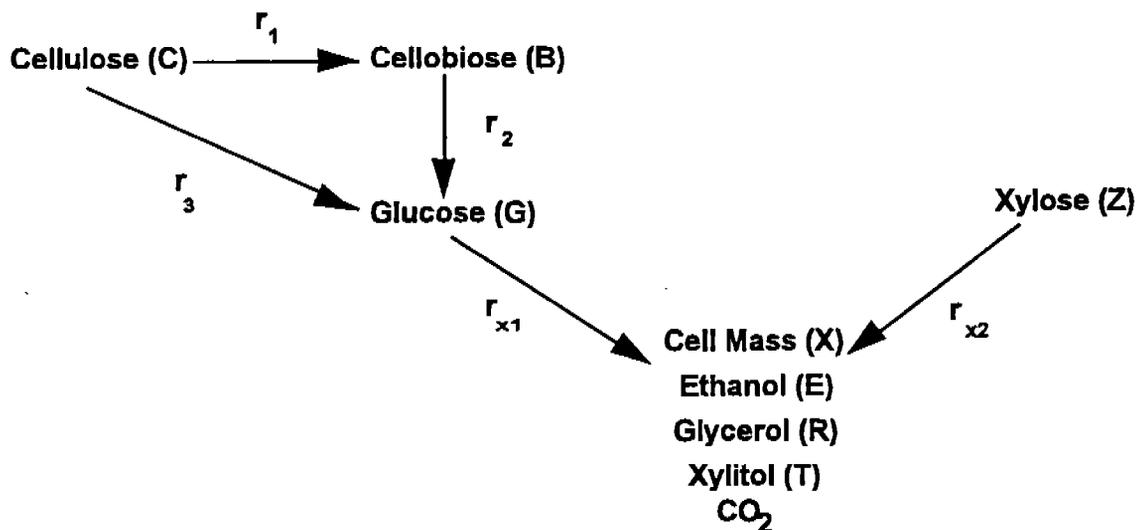
Appendix F

Update of the Kinetic Model

F.1 Introduction

The kinetic model has been updated to include terms describing the effect of acids on xylose utilization, the effects of HMF on glucose utilization, and the effect of furfural on cell mass production in a continuous train. These effects were modeled to fit experimental data from shake flasks and the PDU.

The SSCF model consists of two interdependent parts. The first describes the enzymatic hydrolysis kinetics for cellulose and depends on the characteristics of the particular enzyme-substrate system. The second describes the fermentation kinetics for glucose and xylose and depends on the characteristics of the fermentative organism. In the present version of the model, the fermentation kinetics are formulated specifically for the Purdue recombinant yeast strains, which ferment glucose and xylose to ethanol with cell mass, CO₂, glycerol, and xylitol being the main by-products, according to the following scheme:



Based on previous experimental data, the rates for cellulose and cellobiose hydrolysis are given by the following expressions for cellulose hydrolysis to cellobiose;

$$r_1 = \frac{k_1' C e^{-\lambda(1-C/c_0)}}{1 + \frac{B}{K_{1B}} + \frac{G}{K_{1G}}} \frac{K_{1E}}{K_{1E} + E} \quad (1)$$

cellulose hydrolysis to glucose;

$$r_3 = \frac{k_3' C e^{-\lambda(t-t_0)}}{1 + \frac{B}{K_{1B}} + \frac{G}{K_{1G}}} \frac{K_{1E}}{K_{1E} + E} \quad (2)$$

and cellobiose hydrolysis to glucose;

$$r_2 = \frac{k_2' B}{K_m \left(1 + \frac{G}{K_{2G}}\right) + B} \quad (3)$$

where C , B , G , and E are the concentrations (g/L) of cellulose, cellobiose, glucose, and ethanol, respectively, C_0 is the initial cellulose concentration (g/L), K_{1B} , K_{1G} , K_{1E} , and K_{2G} are inhibition constants (g/L), as detailed in the "Nomenclature" section, k_1' , k_2' , and k_3' are the lumped specific rate constants for cellulose (h^{-1}) and cellobiose (g/L-h) hydrolysis, and λ is the rate of decrease in the specific surface area of cellulose (h^{-1}) during the course of the enzymatic hydrolysis.

At the enzyme loadings being used in SSCF (5 to 20 IFPU/g cellulose), the specific rate constants for cellulose hydrolysis to cellobiose were experimentally determined to be proportional to the enzyme loading according to the following expression;

$$k_1' = k_1^* (e)_T e_c^* \quad (4)$$

where $(e)_T$ is the total (free and bound) concentration of the cellulase and β -glucosidase enzyme complex (g/L), e_c^* is the specific cellulase activity of the enzyme preparation (IFPU/g protein), and k_1^* is the maximum specific cellulose hydrolysis rate (h^{-1}). Similarly, the specific rate constants for cellobiose hydrolysis to glucose was experimentally determined to be proportional to the enzyme loading according to the following expression;

$$k_2' = k_2^* (e)_T e_g^* \quad (5)$$

where k_2^* is the maximum specific cellobiose hydrolysis rate (h^{-1}), and e_g^* is the specific β -glucosidase activity of the enzyme preparation (IFPU/g protein). The specific rate constant for cellulose hydrolysis to glucose was experimentally determined to be constant.

The fermentation part of SSCF was modeled with the following expressions describing the rate of glucose utilization as;

$$r_{X1} = \mu_{ml} X \frac{G}{K_G + G} \frac{K_{RG}}{K_{RG} + E} \quad (6)$$

and xylose utilization as;

$$r_{xz} = \mu_{m2} X \frac{Z}{K_Z + Z} \frac{K_{EZ}}{K_{EZ} + E} \frac{1}{1 + G/n} \quad (7)$$

where X and Z are cell mass and xylose concentrations (g/L), respectively, μ_{m1} and μ_{m2} are maximum specific growth rates (h^{-1}) of the yeast on glucose and xylose, respectively, K_G and K_Z are glucose and xylose saturation constants (g/L), respectively, and $K_{E,G}$ and $K_{E,Z}$ are product (ethanol) inhibition constants (g/L) for the glucose and xylose pathways, respectively. The parameter n is a factor that accounts for the experimentally documented preferential uptake of glucose over xylose (diauxic phenomenon). Better cofermentation performance is associated with a larger n .

Based on the outlined rate expressions, the following mass balance equations describe the batch SSCF process for cellulose concentration;

$$\frac{dC}{dt} = -r_1 - r_3 \quad (8)$$

cellobiose concentration;

$$\frac{dB}{dt} = 1.056 r_1 - r_2 \quad (9)$$

glucose concentration;

$$\frac{dG}{dt} = 1.111 r_3 - 1.053 r_2 - \frac{r_{X1}}{Y_{XG}} \quad (10)$$

xylose concentration;

$$\frac{dZ}{dt} = \frac{r_{X2}}{Y_{XZ}} \quad (11)$$

cell mass concentration;

$$\frac{dX}{dt} = r_{X1} + r_{X2} \quad (12)$$

glycerol concentration;

$$\frac{dR}{dt} = r_{X1} \frac{Y_{RG}}{Y_{XG}} + r_{X2} \frac{Y_{RZ}}{Y_{XZ}} \quad (13)$$

xylitol concentration;

$$\frac{dT}{dt} = r_{X2} \frac{Y_{TZ}}{Y_{XZ}} \quad (14)$$

and ethanol concentration;

$$E = E_0 - 1.278[0.444(C - C_0) + 0.4(G - G_0) + 0.4(Z - Z_0) + 0.421(B - B_0) + 0.391(R - R_0) + 0.394(T - T_0) + 0.479(X - X_0)] \quad (15)$$

where Y_{XG} and Y_{XZ} are cell mass yields from glucose and xylose (g/g), respectively, Y_{RG} and Y_{RZ} are glycerol yields (g/g) from glucose and xylose, respectively, and Y_{TZ} is xylitol yield (g/g) from xylose. Cell mass is formed from the consumption of both glucose and xylose. Glycerol is a by-product generated during the catabolism of both sugars. In contrast, xylitol formation takes place only as a result of inefficient xylose metabolism. In equations (9) and (10), the numeric constants account for the mass gain per mole of reactant caused by hydration during the hydrolysis reactions (if concentrations are expressed in moles, all constants should be set equal to one). It should be noted that the mass balance expression for ethanol, equation (15), ensures carbon balance closure for the fermentation and is based on carbon and degree of reduction balance considerations¹.

The fermentation constants for LNHST2 were initially developed using batch data from bench scale experiment 1.6 and are shown in Table F-1. The cell mass yields calculated from experiment 1.6 data on glucose and xylose were 0.15 g/g and 0.044 g/g, respectively. Previous experience found that the yields are closer to 0.05 g/g, so both the yields were set to 0.05 g/g for all following runs. After the Task 3 batch fermentation in a 9000-L fermenter, the constants μ_{m1} , μ_{m2} , K_z , and n were modified so that the model's predictions would better fit Task 3 data. Task 3 fermentation kinetic parameters, also shown in Table F-1, were used as starting points for all work done during Task 5.

Table F-1. Kinetic Parameters Determined from Shake Flask Fitting and Task 3 Data

Kinetic Parameters ^a	Shake Flask ^b	Task 3 ^c
μ_{m1} (h ⁻¹)	0.292	0.13
$K_{E,G}$ (g/L)	73.7	73.7
K_G (g/L)	0.385	0.385
μ_{m2} (h ⁻¹)	0.024	0.08
K_z (g/L)	7.25	250
$K_{E,Z}$ (g/L)	21	21
Y_{XG} (g/g)	0.150	0.05
Y_{XZ} (g/g)	0.044	0.05
Y_{RG} (g/g)	0.082	0.082
Y_{RZ} (g/g)	0.038	0.038
Y_{TZ} (g/g)	0.077	0.077
n (g/L)	8.75	50

^aThe parameters are listed in the nomenclature at the end of this report.

^b Cultivated in 1% yeast extract, 2% peptone, 22.86 g/L glucose, and 30.61 g/L xylose in batch mode for 24 hours before switching the operation to continuous (see Report 1.6).

^c Cultivated in APR pretreated corn fiber/screenings at 20.9% solids, batch mode with 1% CSL and 10 IFPU cellulase/g cellulose.

F.2 Organic Acid Inhibition of Xylose Utilization

A shake flask study was started on June 6, 1996 to study the effects of dilution on fermentation performance of two hydrolyzates (APR-330 and APR-392). Shake flask fermentations of the liquor from each hydrolyzate were performed at the equivalent of 25%, 18%, and 12% solids. Shake flask fermentations of the whole hydrolyzate from APR-392 were performed at 25% and 12% solids. The fermentations are discussed in Appendix C.

The maximum xylose utilization rate in each flask from the experiment is plotted against acetic acid concentration in Figure F-1 and against lactic acid concentration in Figure F-2. Each point is marked with the shake flask number. Numbers 1-3 are 25%, 18%, and 12% equivalent solids of APR-330 liquor, respectively. Numbers 4-6 are 25%, 18%, and 12% equivalent solids of APR-392 liquor, respectively. Numbers 7 and 8 are the 25% and 12% whole slurry dilutions of APR-392 material, respectively.

Figure F-3 shows the maximum xylose utilization rate in each flask plotted against the sum of the acetic and lactic acid concentrations. The points are numbered the same way as Figures 1 and 2. The xylose utilization rate in flasks 1-7 seems to be more dependent upon the sum of the acid concentrations than on either acetic or lactic acid alone.

The xylose rate expression needs a term that accounts for the effect of the sum of the acid concentrations. To develop the term, the xylose utilization rate from the data was divided by the maximum xylose utilization rate, the cell mass, the xylose saturation term, the ethanol inhibition term, and the cofermentation term and multiplied by the cell mass yield on xylose. The value is 1 if the acids do not inhibit xylose utilization. The values, termed "Corrected Xylose Utilization," are plotted against the total acid concentration (sum of the acetic and lactic acid concentrations) in Figure F-4.

In Figure F-4, the June 6, 1996 experimental points are numbered 1-8 as in Figures F-1—F-3. The points ND-3, ND-4, ND-6, ND-7, and ND-8, are data from an eight shake flask experiment with pure sugars and CSL. The experiment was started by Nancy Dowe on February 22, 1996. Different amounts of acetic acid were added to each shake flask to reach concentrations of 2—10 g/L. Shake flasks 1, 2, and 5 were not included in this analysis because of an unexplained significant loss of ethanol during xylose utilization. The point "P3" is data from the third flask in the experiment ran at LORRE (Purdue University) to determine the effect of acetic acid, lactic acid, and ethanol on fermentation of pure sugars. The third flask was the only flask of the 10 in which xylose was utilized. The data from the ten shake flasks run without acetic or lactic acid was not used, because acetic and lactic acid concentrations were not reported. Some lactic acid must have been present, because CSL was used as a nutrient source and CSL contains a high concentration of lactic acid. The point "T3" is data from the 9000 L batch fermentation performed during Task 3.

The line drawn through the data are values calculated after the acid inhibition term that was added to the xylose utilization equation. The new xylose utilization expression is;

$$r_{xz} = \mu_{wz} X \frac{Z}{K_Z Z} \frac{K_{RZ}}{K_{RZ} E} \frac{1}{1+G/n} e^{-K_{ZTA} A} \quad (16)$$

where K_{ZTA} is the acid inhibition constant and A is the sum of the acetic and lactic acid concentrations (g/L). The exponential expression was chosen because it fit the data better than a Monod term or a straight line with a Y intercept equal to 1. The new term must equal 1 when no organic acid are present.

The maximum utilization rate of xylose (μ_{m2}), the xylose saturation term (K_z), and the cofermentation constant (n) were modified to fit the new model to both the June 6 experimental data and Task 3 data. The kinetic parameter values before and after the modifications are listed in Table F-2. When Task 3 data was initially fit, the xylose saturation constant was set to 250 g/L to account for the increased xylose utilization rate soon after glucose disappears. However, the xylose saturation constant was determined to be 15 g/L from the organic acid inhibition experiment data, so increased xylose utilization soon after glucose disappears needs to be accounted for in another way. The cofermentation constant was increased to 1000 g/L because glucose concentration seems to have little impact on xylose utilization. The slow xylose utilization rate at the beginning of batch fermentations is probably caused by lower cell mass concentrations. When glucose has disappeared the resulting increase in cell mass concentration increases the xylose utilization rate. The measured and modeled glucose, xylose, and ethanol concentrations and the modeled cell mass concentration for the 8 shake flasks are shown in Figures F-5—F-12. The measured and modeled glucose, xylose, and ethanol concentrations and the modeled cell mass concentration for Task 3 data are shown in Figure F-13. The modeled fermentation start time was estimated to fit the glucose data in each flask, because there was not enough information to properly model inhibition.

Table F-2. Kinetic Parameters after Task 3 and after Hydrolyzate Dilution Experiment

Kinetic Parameters ^a	Task 3	From Experiment
μ_{m1} (h ⁻¹)	0.13	0.13
$K_{E,G}$ (g/L)	73.7	73.7
K_G (g/L)	0.385	0.385
μ_{m2} (h ⁻¹)	0.08	0.06
K_z (g/L)	250	15
$K_{E,Z}$ (g/L)	21	21
$K_{z,TA}$ (L/g)	N/A	0.25
Y_{XG} (g/g)	0.05	0.05
Y_{XZ} (g/g)	0.05	0.05
Y_{RG} (g/g)	0.082	0.082
Y_{RZ} (g/g)	0.038	0.038
Y_{TZ} (g/g)	0.077	0.077
n (g/L)	50	1000

^aThe parameters are listed in the nomenclature at the end of this report.

Xylose concentration at each data point in Figures F-5—F-13 is close to the model's prediction. The measured and predicted xylose concentrations are close when glucose is present, the measured value is lower for approximately 30 hours after glucose is gone, and near the end of the fermentation the values are close again. Apparently, another factor needs to be added to the model to account for the increased xylose utilization rate for a period of time after glucose disappearance.

F.3 HMF Inhibition of Glucose Utilization

The model used to predict concentrations shown on Figures F-5—F-13 could not predict the inhibition of

glucose fermentation, so the starting point of the glucose fermentation was estimated. In the literature, it has been noted that furfural can cause inhibition at the start of batch ethanol fermentations until it has been metabolized². An experiment was started July 11, 1996 to further investigate the possible inhibitory effects of both HMF and furfural. The experiment consisted of 3 shake flask fermentations of clarified hydrolyzate produced by the APR on June 14, 1996 (between APR-417 and APR-418). Flasks A, B, and C were diluted to 25%, 18%, and 12% equivalent solids, respectively.

The data showed disappearance of both HMF and furfural. Following are the kinetic expressions that were developed to model the disappearance of furfural;

$$r_U = k'_U X \frac{U}{K_{U,U} + U} \quad (17)$$

and the disappearance of HMF;

$$r_H = k'_H X \frac{H}{K_{H,H} + H} \quad (18)$$

where r_U is the rate of furfural conversion (g/L-h), k'_U is the maximum furfural conversion rate (h^{-1}), X is the cell mass concentration (g/L), U is furfural concentration (g/L), $K_{U,U}$ is the furfural saturation constant (g/L), r_H is the rate of HMF conversion (g/L-h), k'_H is the maximum HMF conversion rate (g/L-h), H is the HMF concentration (g/L), and $K_{H,H}$ is the HMF saturation constant (g/L). The measured and modeled furfural and HMF concentrations in flasks A, B, and C are shown in Figures F-14, F-15, and F-16, respectively.

To investigate the effects of HMF and furfural on glucose utilization, the glucose utilization rate was calculated from experimental data. The rate was then divided by cell mass concentration, the glucose saturation term, and the ethanol inhibition term and multiplied by cell mass yield on glucose to calculate a corrected glucose utilization rate (h^{-1}). After this calculation the corrected glucose utilization rate would be equal to the maximum specific growth rate on glucose (μ_m) if there was no inhibition. The corrected glucose utilization rates are plotted against furfural and HMF in Figures F-17 and F-18, respectively.

Visually, the trend seems to be more dependent upon HMF than on furfural. Therefore, a Monod kinetic term was added to the glucose utilization rate expression to account for HMF inhibition. The updated glucose utilization rate equation follows;

$$r_{X1} = \mu_m X \frac{G}{K_G + G} \frac{K_{E,G}}{K_{E,G} + E} \frac{K_{G,H}}{K_{G,H} + H} \quad (19)$$

where $K_{G,H}$ is the HMF inhibition constant (g/L). The corrected glucose utilization term was further corrected by dividing out the HMF inhibition term. Figure F-19 plots this term against furfural concentration. No correlation appears to be present between furfural concentration and further inhibition. Furfural may be a cause of inhibition, but the inhibition seen in this data was better expressed by the HMF inhibition term. More experimental work is necessary to separate the effects of HMF and furfural. More experimental work is also necessary to correctly model the effects of HMF or furfural concentrations greater than 0.4 g/L.

The fermentation performance of each of the three shake flasks was then modeled with the updated glucose utilization rate equation. The maximum glucose utilization rate (μ_m) was modified to fit the HMF inhibition

term. All of the fermentation terms used for the acid inhibition experiment and for the HMF inhibition experiment are shown in Table F-3. Figures F-20 and F-21 show the modeled and measured glucose, xylose, ethanol, and cell mass data for flask A. Figures F-22—F-25 show the same information for flasks B and C, respectively.

Table F-3. Kinetic Parameters Determined From Hydrolyzate Dilution and HMF Inhibition Experiment

Kinetic Parameters ^a	Hydrolyzate Dilution Experiment	HMF Inhibition Experiment
μ_{m1} (h ⁻¹)	0.13	0.22
$K_{E,G}$ (g/L)	73.7	73.7
K_G (g/L)	0.385	0.385
$K_{G,H}$ (g/L)	N/A	0.3
μ_{m2} (h ⁻¹)	0.06	0.06
K_Z (g/L)	15	15
$K_{E,Z}$ (g/L)	21	21
$K_{Z,TA}$ (L/g)	0.25	0.25
Y_{XG} (g/g)	0.05	0.05
Y_{XZ} (g/g)	0.05	0.05
Y_{RG} (g/g)	0.082	0.082
Y_{RZ} (g/g)	0.038	0.038
Y_{TZ} (g/g)	0.077	0.077
n (g/L)	1000	1000

^aThe parameters are listed in the nomenclature at the end of this report.

The modeled xylose concentrations at the 46 and 78 hour time points are higher than the measured concentrations in all three flasks. Most likely, this is the same effect as described earlier (Section F-2). The cell mass concentrations in all three flasks are higher than predicted. The predicted yield on glucose is 0.05 g/g for all three flasks. The measured cell mass yields were 0.06 g/g, 0.08 g/g, and 0.12 g/g in flasks A, B, and C, respectively. The measured yield in the PDU was between 0.03 g/g and 0.04 g/g during Task 5. The yields in the chemostat were close to those in the PDU. The yield was not measured during Task 3 or during the acid inhibition experiment. Two possible causes of the discrepancy between batch and continuous data are increased oxygen transfer in the shake flask and higher glucose concentrations at the beginning of a batch fermentation when compared to a continuous fermentation.

Most likely, HMF and/or furfural also inhibit xylose utilization. However, the predicted xylose consumption while HMF and furfural were present was minimal, so any change in xylose consumption was not detected.

F.4 Continuous Fermentation

Predictions from the continuous model were compared to steady state conditions in the PDU during Task 5 and from chemostat operation. The batch expressions (eq. 8-14) were integrated to develop the continuous model, under the assumption that all the fermenters are continuous stirred tank reactors. The parameters developed from the inhibition experiments were used.

At steady state conditions, the continuous kinetic model overpredicted xylose utilization. The overprediction seemed to be most prevalent in the first fermenter, so a term was added to reduce the predicted cell mass concentration in the first fermenter. Before this change, the cell mass concentration in all of the fermenters was modeled with the following expression;

$$\frac{F_{i-1}X_{i-1}}{V} - \frac{F_i X_i}{V} + (r_{x1} + r_{x2}) = 0 \quad (20)$$

where F_{i-1} is the volumetric flow rate (L/h) entering the fermenter, X_{i-1} is cell mass concentration entering the fermenter (g/L), V is the fermenter volume (L), F_i is the volumetric flow rate leaving the fermenter (L/h), X_i is the cell mass concentration in the fermenter (g/L), and r_{x1} and r_{x2} are cell mass production rates (g/L-h) as described in equations 19 and 16, respectively. The equation for the first fermenter was changed to the following;

$$\frac{F_{i-1}X_{i-1}}{V} - \frac{F_i X_i}{V} + (r_{x1} + r_{x2} - r_{x3}) = 0 \quad (21)$$

where r_{x3} is the cell mass reduction term. The cell mass reduction term is described by the following expression;

$$r_{x3} = \frac{F_i}{V} r'_{x3} \quad (22)$$

where r'_{x3} is the cell mass reduction term without a time unit (g/L). This term (r'_{x3}) accounts for a reduction in cell mass yield while metabolizing furfural and/or HMF. However, the reason for the cell mass yield reduction is unknown so the use of this term should be studied further. Since all of the furfural and HMF disappear from the first fermenter at residence times of 24—36 hours used in the chemostat and PDU, the reduction term was made independent of time.

The cell mass reduction term (r'_{x3}) was found for each of the four steady states achieved in the chemostat and PDU, by forcing the predicted xylose concentration in the last fermenter (the second in the chemostat and the third in the PDU) to be equal to the measured xylose concentration. Figure F-26 shows cell mass reduction versus the initial furfural level with a line fit to the data. Cell mass reduction also appears to be dependent upon the HMF level (Figure F-27). Further experiments are needed to separate the effects of HMF from those of furfural and to investigate if cell mass yields are reduced in continuous fermentation when little or no HMF or furfural is present.

The regressed equation cell mass reduction term is;

$$r'_{x3} = 5.27 U_0 - 1.07 \quad (23)$$

where U_0 is furfural concentration in the feed (g/L). This equation was used with the constants developed after the inhibition experiment (Table F-3) to model each of the four steady states.

Modeled data is compared to measured data for chemostat run 3 (Appendix E) in Figures F-28—F-30. Chemostat run 4 data is shown in Figures F-31—F-33. It is not known why there is a large concentration of

glucose left in the first fermenter. This unmetabolized glucose may be caused by metabolism of furfural and HMF that is not accounted for by the cell mass removal term. If unconverted glucose in the first fermenter were converted, the ethanol concentration would be close to the predicted value. The xylose concentration in the first fermenter is lower than predicted at both steady states. This lower concentration may be related to a rapid utilization of xylose after glucose disappears that is not accounted for by the kinetic model.

Modeled data is compared to measured data for first Task 5 mass balance point in Figures F-34—F-37 and second mass balance point in Figures F-38—F-41. Oligomeric glucose and xylose were converted to ethanol during the second point. These amounts were entered into the model as additional monomeric sugars, because conversion of oligomeric sugars has not been modeled.

The measured xylose concentrations in the first and second 9000-L fermenters is lower than predicted in both cases. The discrepancy may be caused by the extra utilization of xylose during and after glucose utilization, as was seen in the chemostat. The cellulose concentration in the third 9000-L fermenter is lower than predicted in both cases. The conversion constants were developed in shake flasks on pretreated corn fiber. Better pretreatment, different mixing properties, running in continuous mode, or the presence of the corn screenings could increase the conversion of cellulose to glucose. If the predicted cellulose conversion were closer to the measured conversion, the ethanol concentration would be closer to the measured value.

The predicted ethanol concentration in third fermenter is 5.5% lower than the measured concentration for both mass balance points. The 5.5% error is within the 20% error specification.

F.5 Nomenclature

A	Sum of the concentrations of acetic acid and lactic acid (g/L)
B	Concentration of cellobiose (g/L)
C	Concentration of cellulose (g/L)
$(e)_T$	Concentration of cellulase and β -glucosidase enzyme complex (g protein /L)
e_c^*	Specific cellulase activity of the enzyme preparation (IFPU/g protein)
e_g^*	Specific β -glucosidase activity of the enzyme preparation (IU/g protein)
E	Concentration of ethanol (g/L)
$F_{i,j}$	Volumetric flowrate entering the fermenter (L/h)
F_i	Volumetric flowrate leaving the fermenter (L/h)
G	Concentration of glucose (g/L)
H	Concentration of HMF (g/L)
k_1^*	Maximum specific rate of cellulose hydrolysis to cellobiose (h^{-1})
k_2^*	Maximum specific rate of cellobiose hydrolysis to glucose (g/IU-h)
k_1'	Lumped specific rate of cellulose hydrolysis to cellobiose, defined in Eq. (4) (h^{-1})
k_2'	Lumped specific rate of cellobiose hydrolysis to glucose, defined in Eq. (5) (g/L-h)
k_3'	Specific rate of cellulose hydrolysis to glucose (h^{-1})
$K_{E,G}$	Ethanol inhibition constant for glucose pathway in the microorganism (g/L)
$K_{E,Z}$	Ethanol inhibition constant for xylose pathway in the microorganism (g/L)
K_G	Glucose saturation constant for the microorganism (g/L)
k_H'	Maximum HMF conversion rate (g/L-h)
$K_{G,H}$	HMF inhibition of glucose utilization constant (g/L)
$K_{H,H}$	HMF saturation constant (g/L)
K_m	Cellobiose saturation constant for β -glucosidase (g/L)

K_{IB}	Inhibition constant of cellulase by cellobiose (g/L)
K_{IE}	Inhibition constants of cellulase by ethanol (g/L)
K_{IG}, K_{2G}	Inhibition constants of cellulase and β -glucosidase, respectively, by glucose (g/L)
k_U'	Maximum furfural conversion rate (g/L-h)
$K_{U,U}$	Furfural saturation constant (g/L)
K_Z	Xylose saturation constant for the microorganism (g/L)
$K_{Z,TA}$	Inhibition constants of xylose utilization by acetic and lactic acids (L/g)
L	Concentration of lignin (g/L)
n	Diauxic Phenomenon (Cofermentation) Term (g/L)
R	Concentration of glycerol (g/L)
r_1	Volumetric rate of cellulose hydrolysis to cellobiose (g/L-h)
r_2	Volumetric rate of cellobiose hydrolysis to glucose (g/L-h)
r_3	Volumetric rate of cellulose hydrolysis to glucose (g/L-h)
r_H	Volumetric rate of HMF conversion (g/L-h)
r_U	Volumetric rate of furfural conversion (g/L-h)
r_{x1}	Volumetric rate of cell mass production from glucose (g/L-h)
r_{x2}	Volumetric rate of cell mass production from xylose (g/L-h)
r_{x3}	Volumetric cell mass reduction in continuous train's first fermenter (g/L-h)
r_{x3}'	Volumetric cell reduction term (g/L) (not time dependent)
T	Concentration of xylitol (g/L)
t	Time (h)
U	Concentration of furfural (g/L)
V	Fermenter Volume (L)
X	Concentration of cell mass (g/L)
Y_{XG}	Yield coefficient of cell mass from glucose (g/g)
Y_{XZ}	Yield coefficient of cell mass from xylose (g/g)
Y_{RG}	Yield coefficient of glycerol from glucose (g/g)
Y_{RZ}	Yield coefficient of glycerol from xylose (g/g)
Y_{TZ}	Yield coefficient of xylitol from xylose (g/g)
Z	Concentration of xylose (g/L)

Greek symbols

λ	Rate of decrease in cellulose specific surface area (h^{-1})
μ_{m1}	Maximum specific growth rate of the yeast, when grown on glucose (h^{-1})
μ_{m2}	Maximum specific growth rate of the yeast, when grown on xylose (h^{-1})

Subscripts

T	Total value
0	Initial value

F.6 References

1. Hatzis, C. and Philippidis, G.P. Kinetics and Modeling of the Enzymatic Hydrolysis and Fermentation of Cellulose for Biochemical Production of Ethanol. *Biotechnol. Bioeng.* (submitted for publication).
2. Boyer, L.J., Vega, J.L., Klassen, E.C., Clausen, E.C., Gaddy, J.L. 1992. The Effects of Furfural on Ethanol Production by *Saccharomyces Cerevisiae* in Batch Culture. *Biomass and Bioenergy*. Vol. 3, No. 1, pp. 41-48.

Figure F-1. Maximum Xylose Utilization versus Acetic Acid Concentration

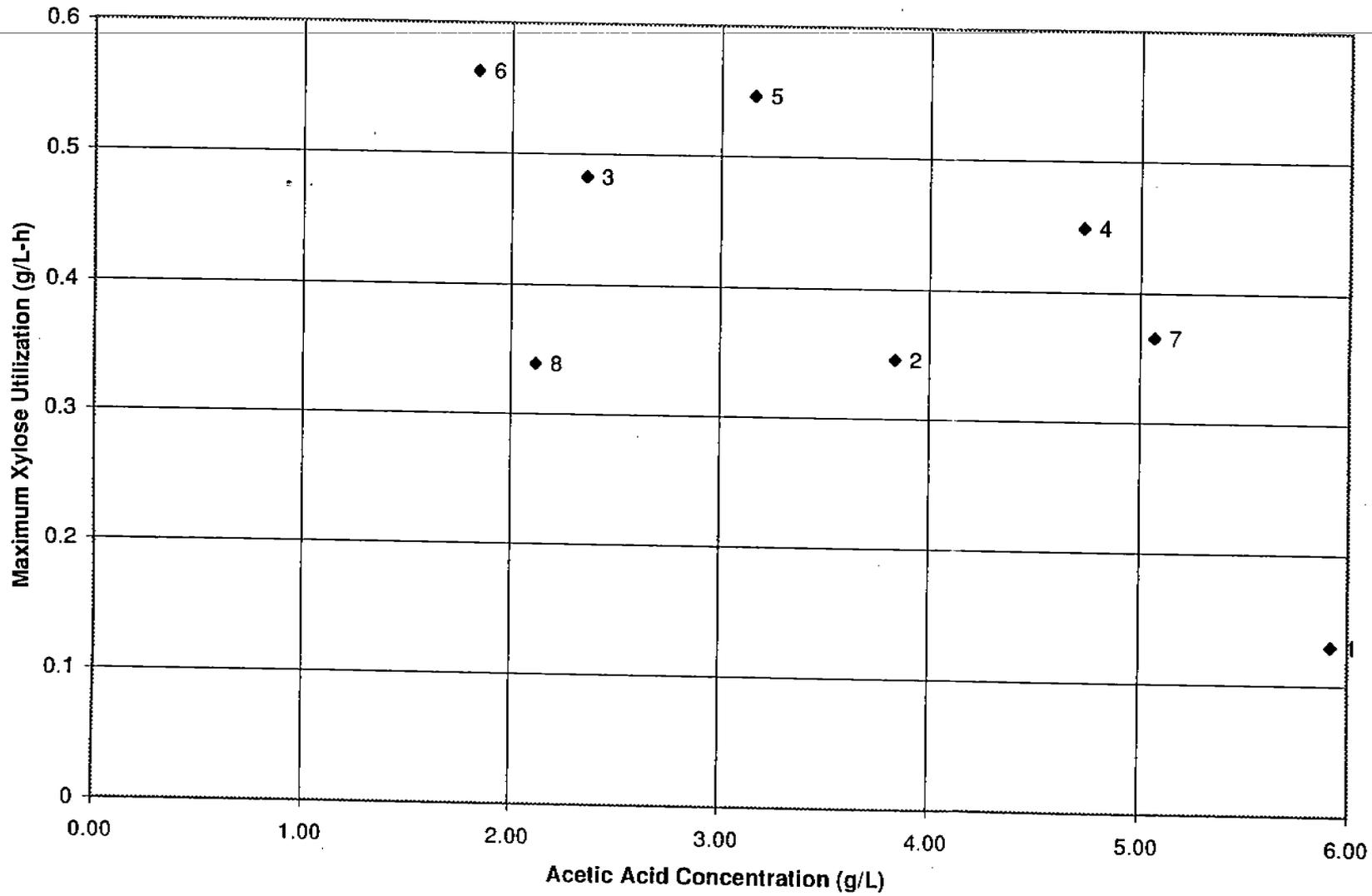


Figure F-2. Maximum Xylose Utilization versus Lactic Acid Concentration

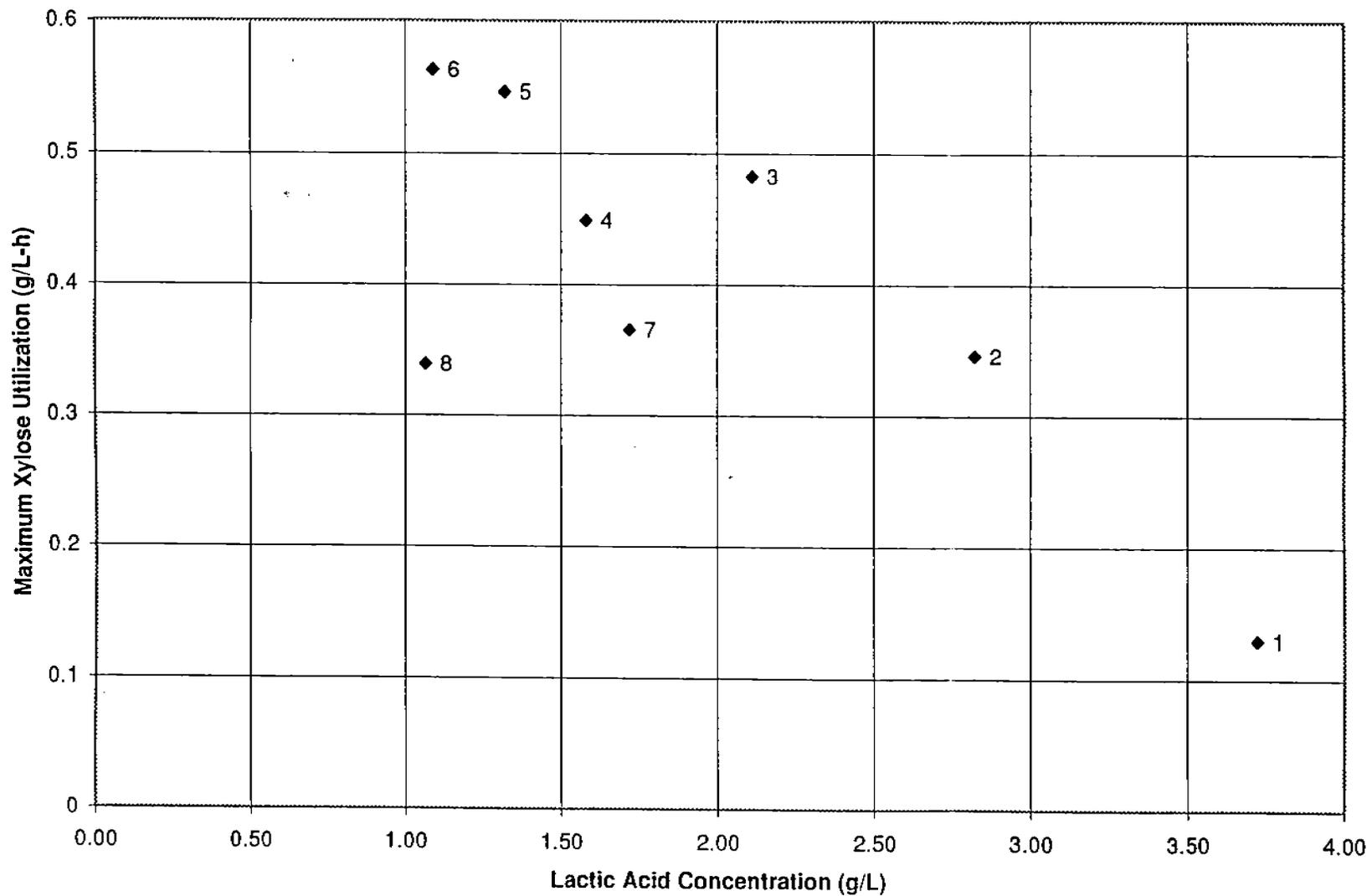


Figure F-3. Maximum Xylose Utilization versus Sum of Acetic Acid and Lactic Acid Concentrations

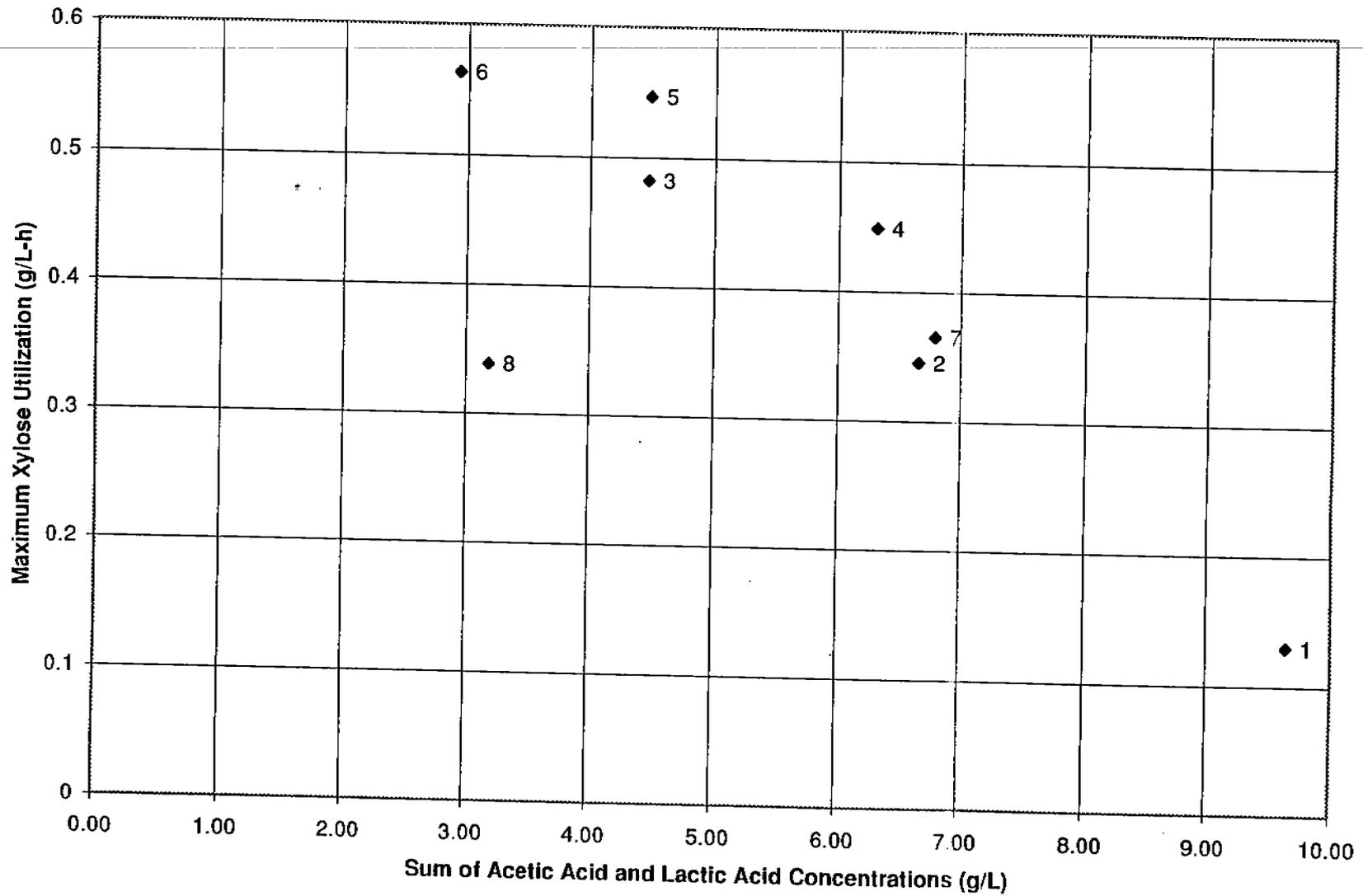


Figure F-4. Corrected Xylose Utilization versus Total Acid Concentration

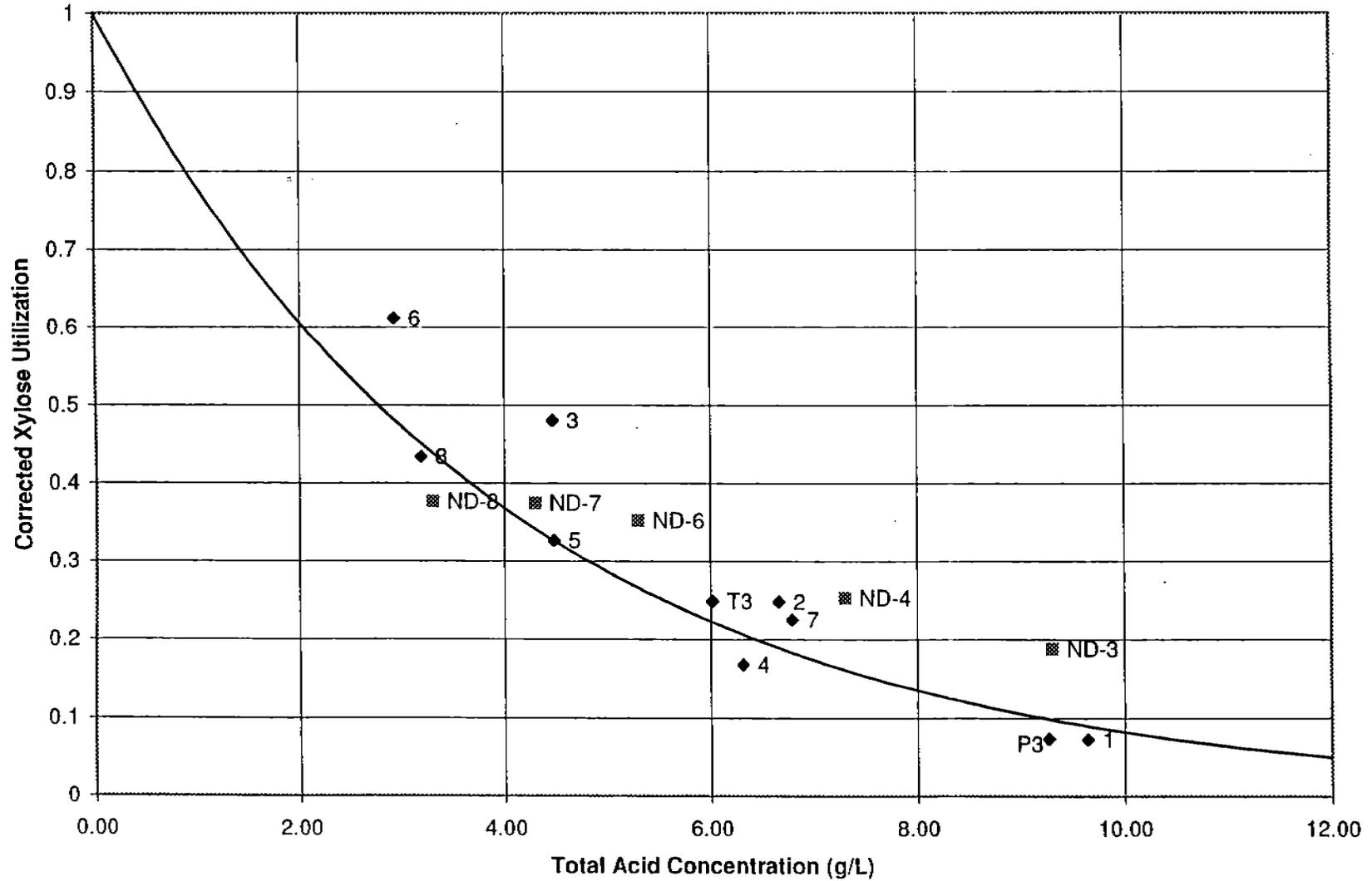


Figure F-5. Flask 1 (APR #330 -- 25% Equivalent Solids at Start)

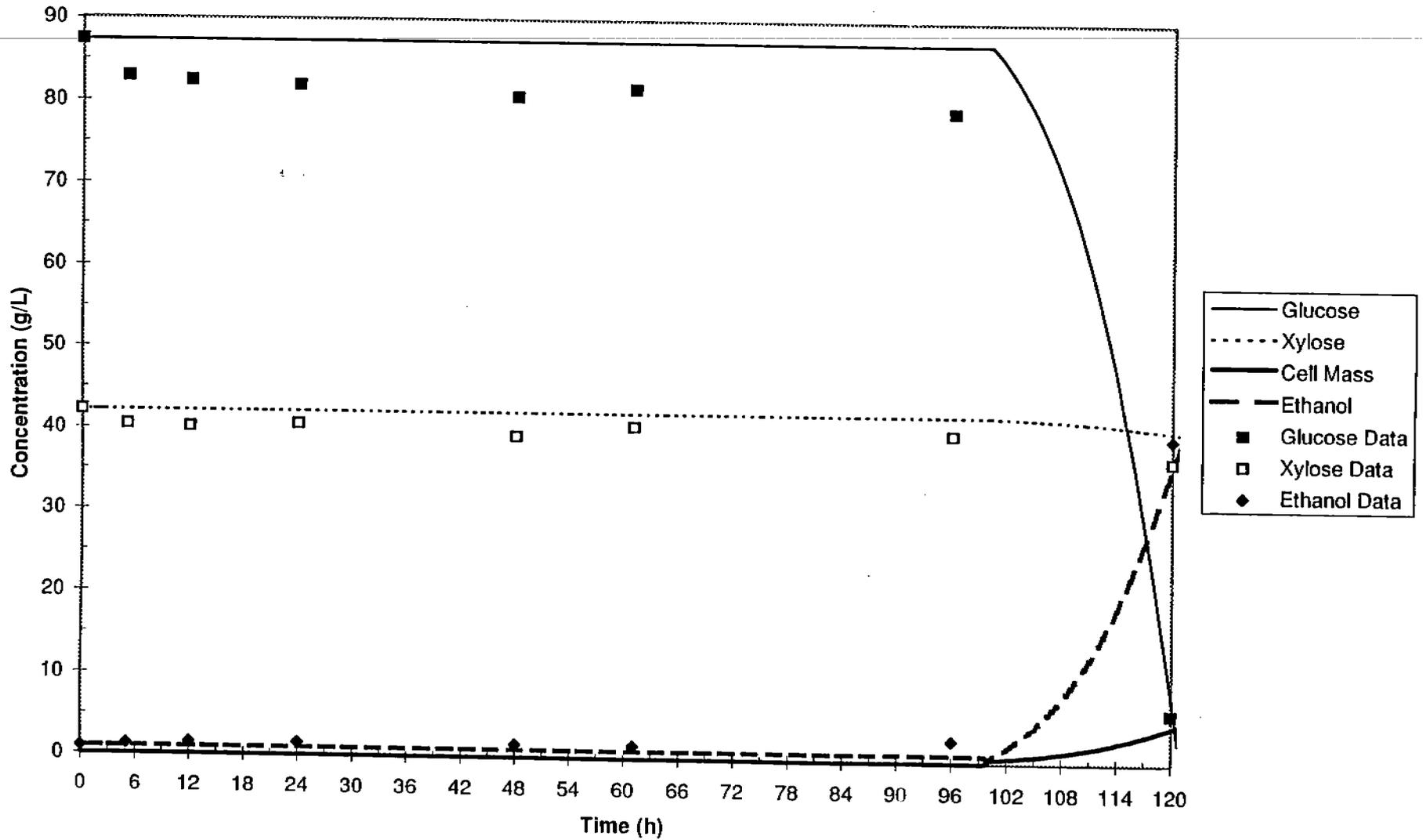


Figure F-6. Flask 2 (APR #330 -- 18% Equivalent Solids at Start)

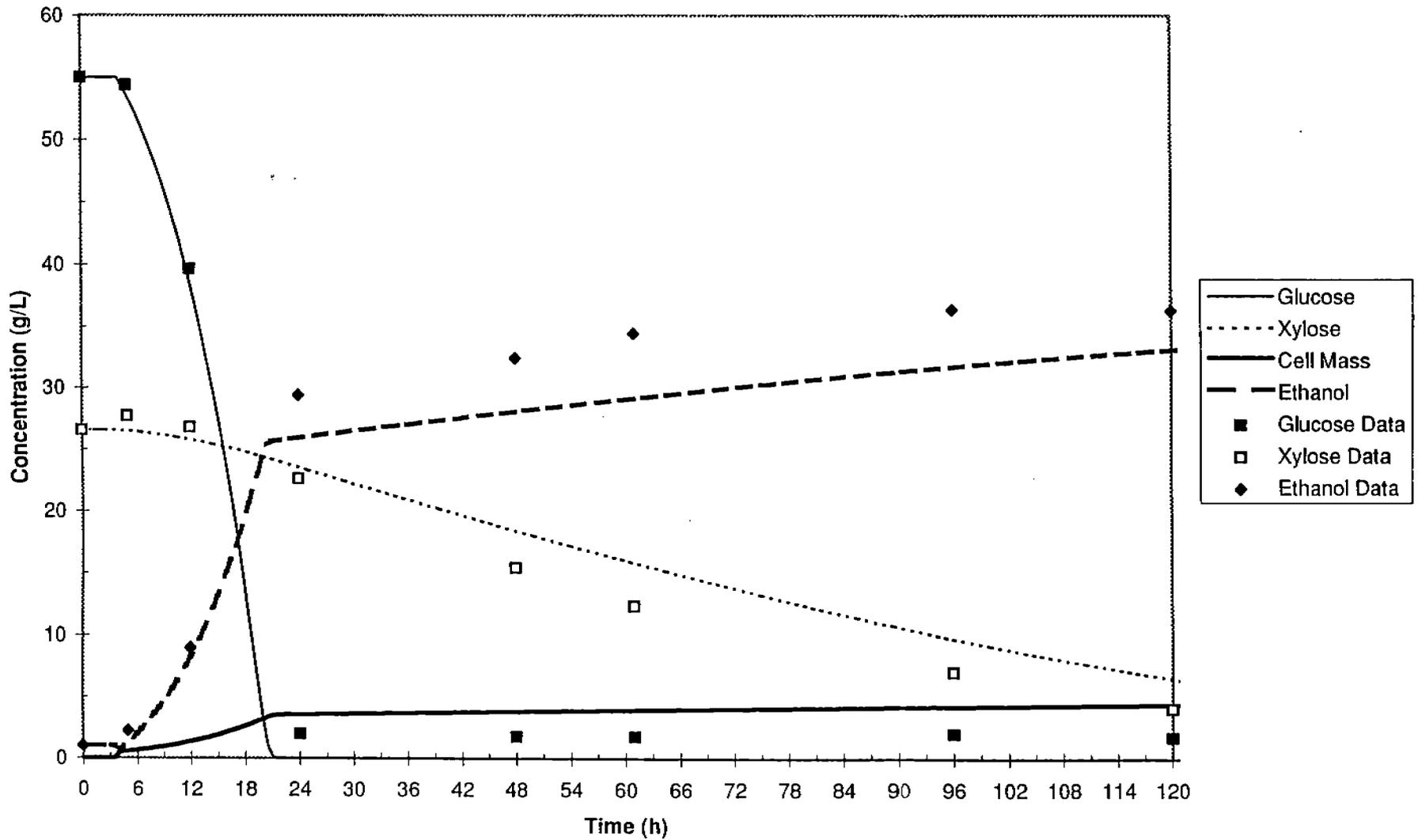


Figure F-7. Flask 3 (APR #330 -- 12% Equivalent Solids at Start)

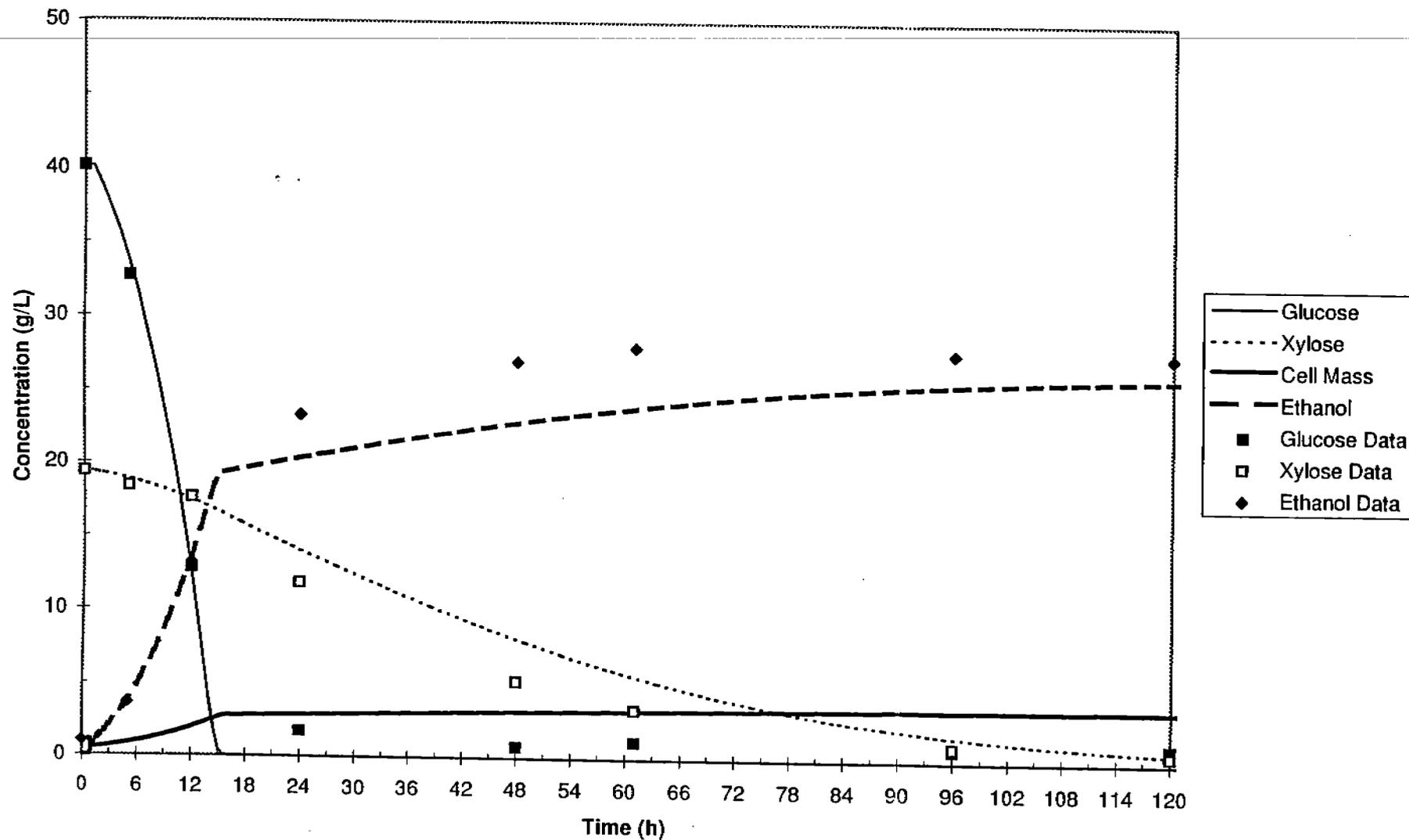


Figure F-8. Flask 4 (APR #392 -- 25% Equivalent Solids at Start)

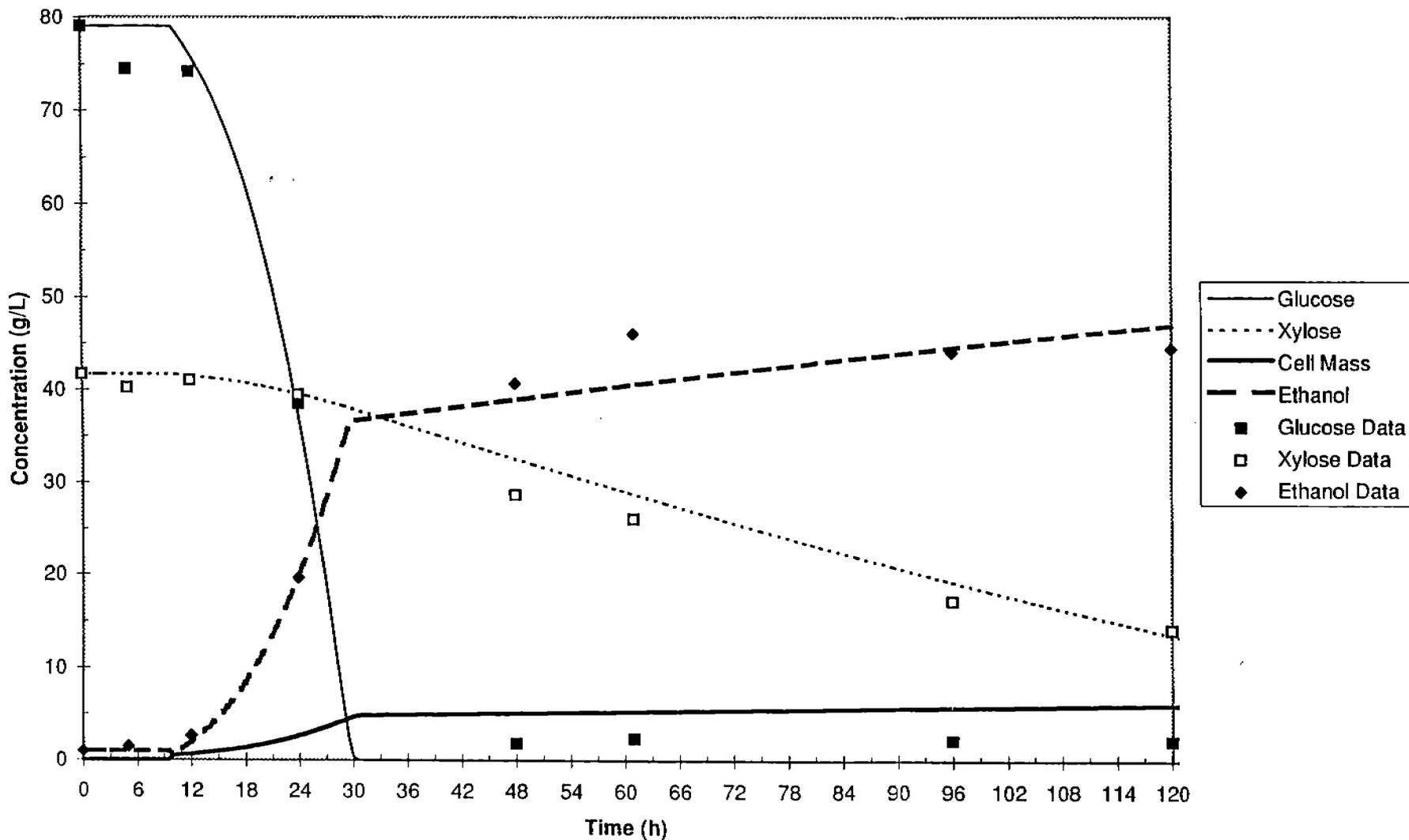


Figure F-9. Flask 5 (APR #392 -- 18% Equivalent Solids at Start)

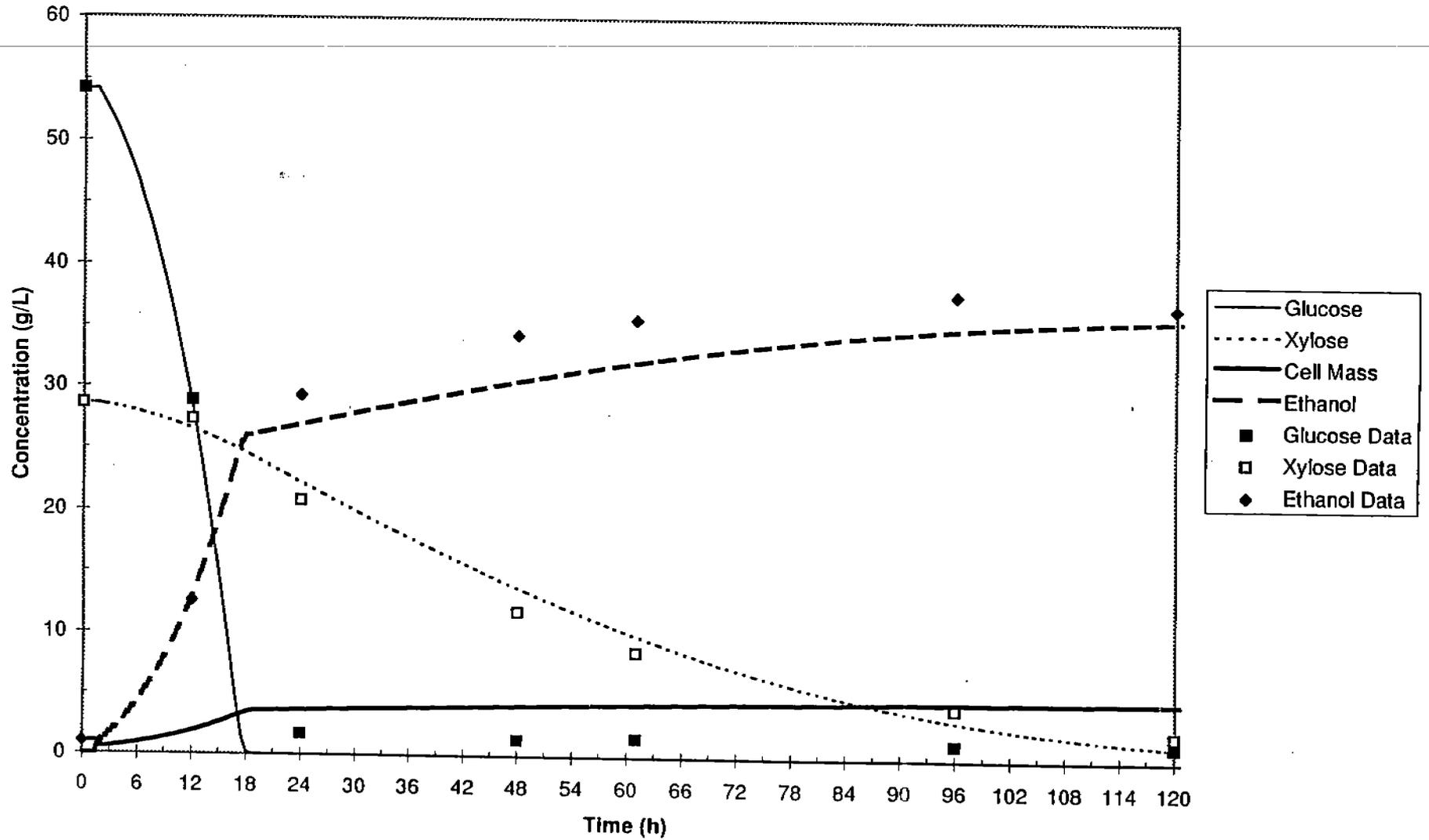


Figure F-10. Flask 6 (APR #392 -- 12% Equivalent Solids at Start)

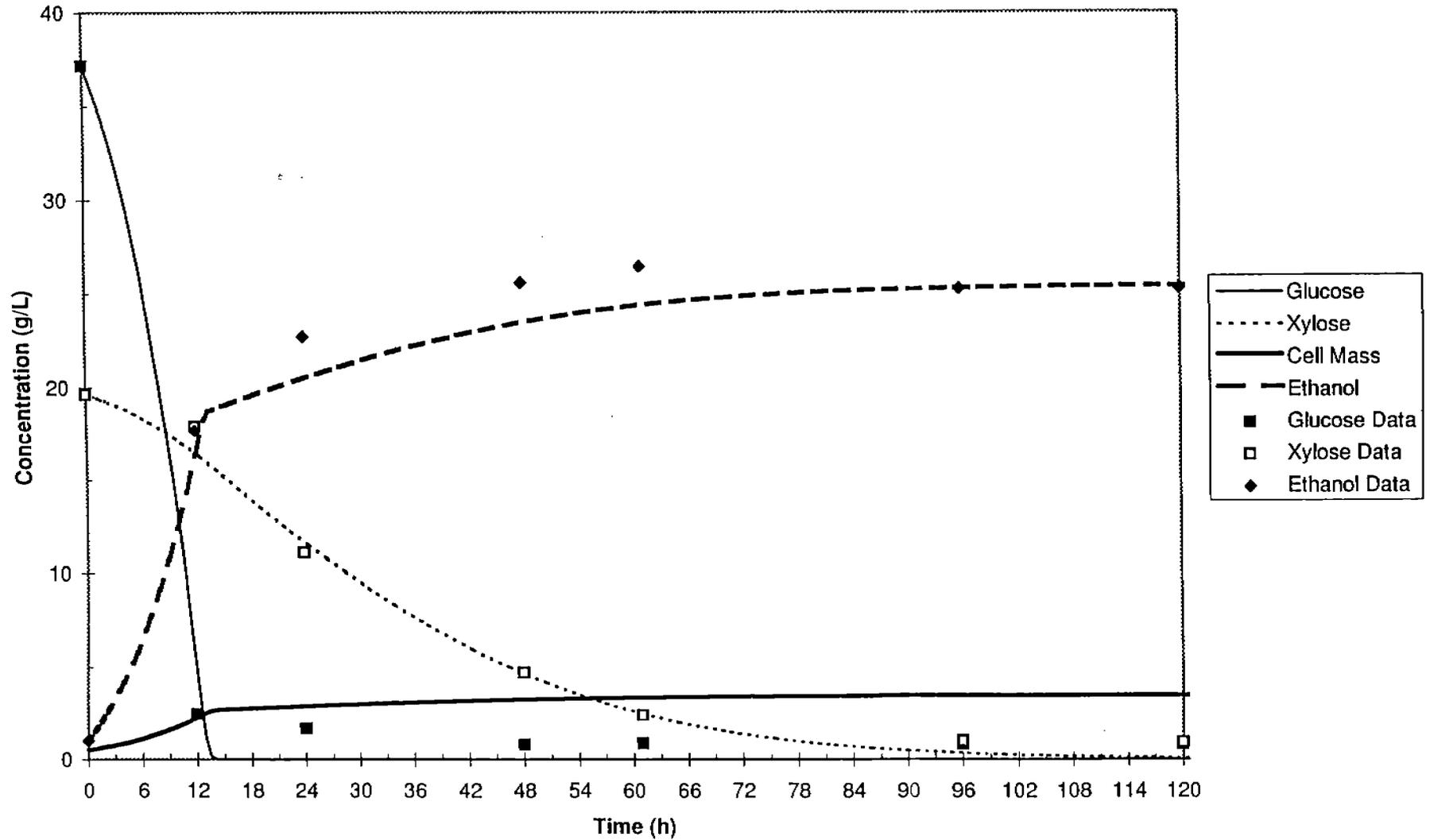


Figure F-11. Flask 7 (APR #392 -- 25% Solids at Start)

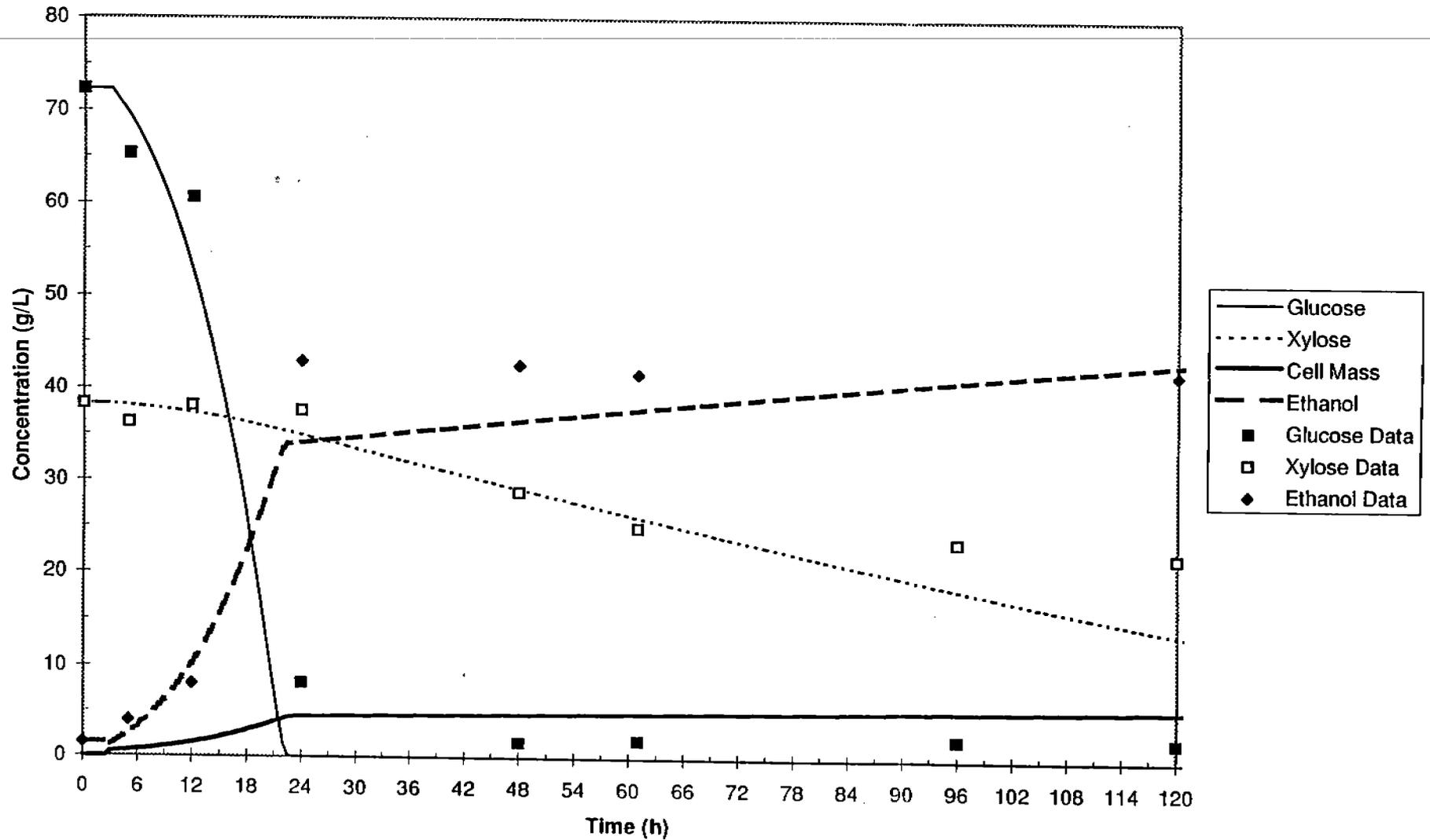


Figure F-12. Flask 8 (APR #392 -- 12% Solids at Start)

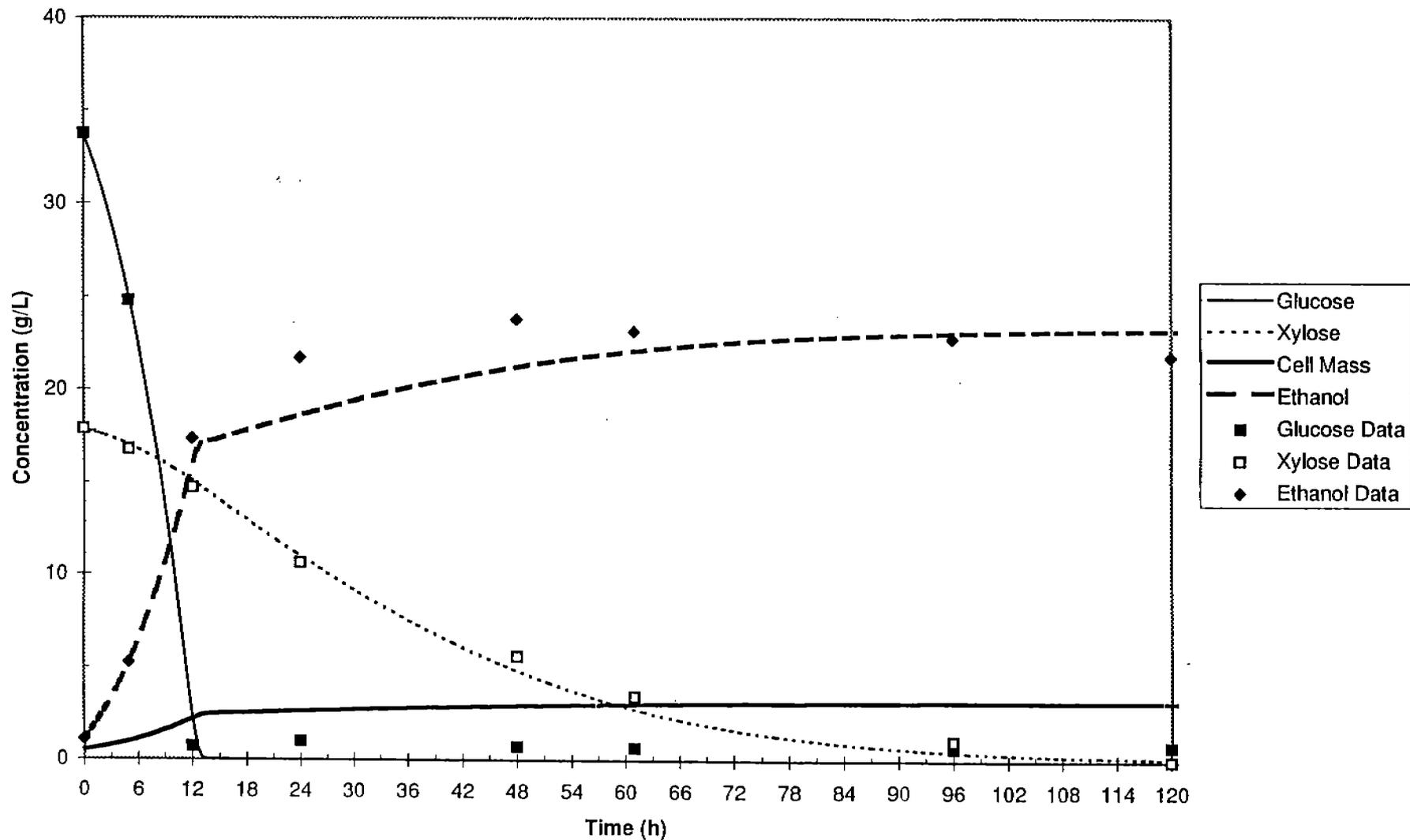


Figure F-13. Task #3 Batch (20% Solids at Start)

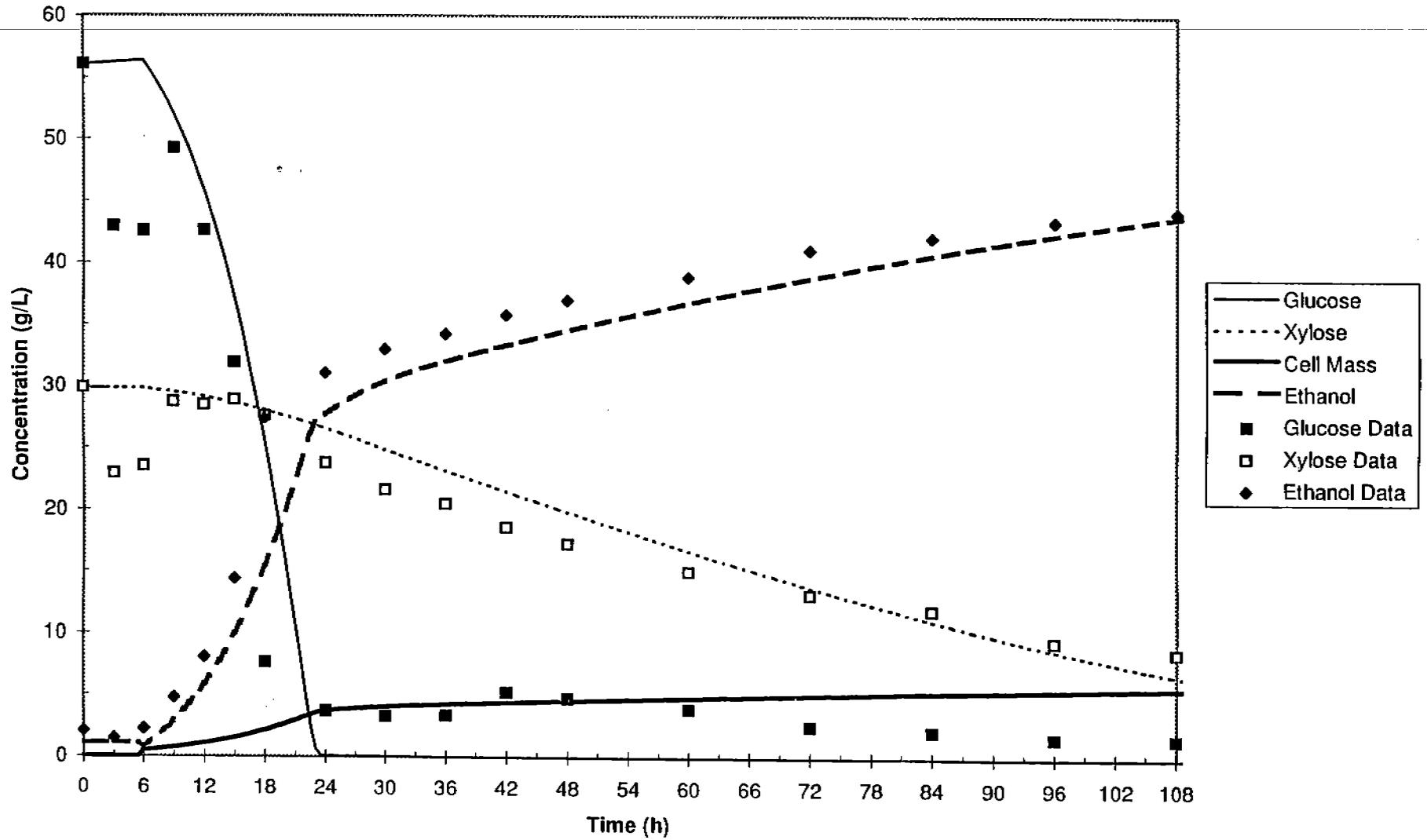


Figure F-14. Flask A (25% Equivalent Solids at Start)

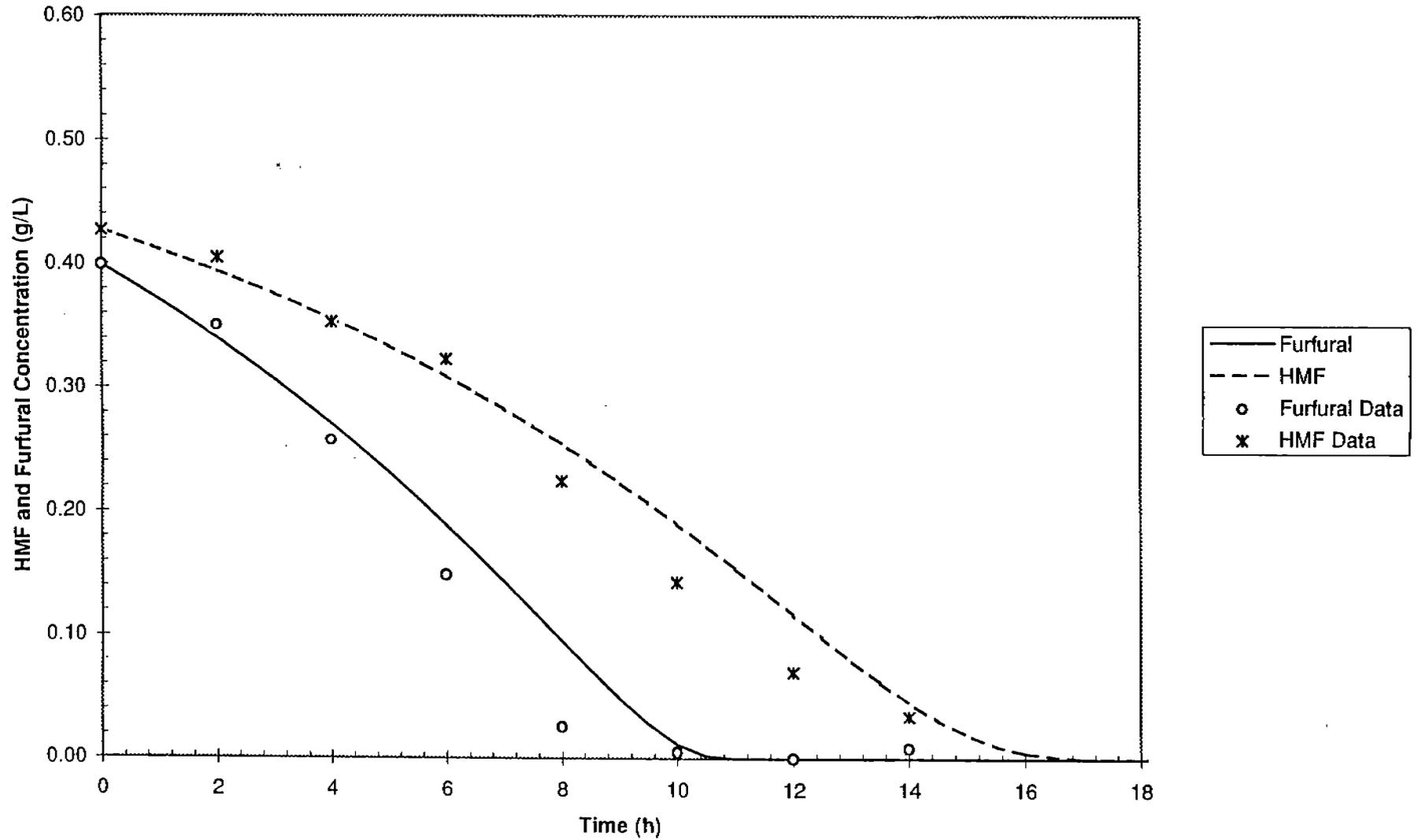


Figure F-15. Flask B (18% Equivalent Solids at Start)

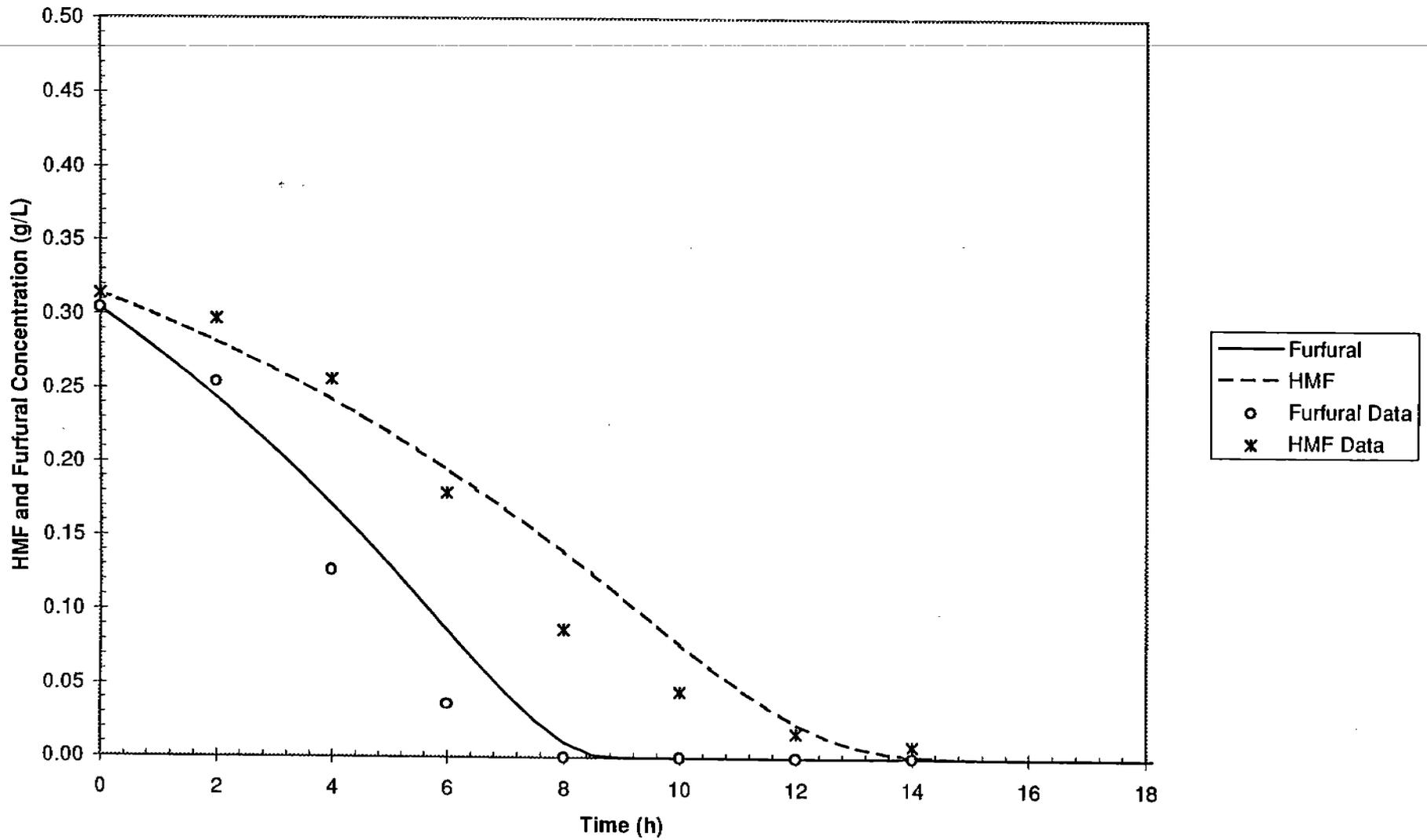


Figure F-16. Flask C (12% Equivalent Solids at Start)

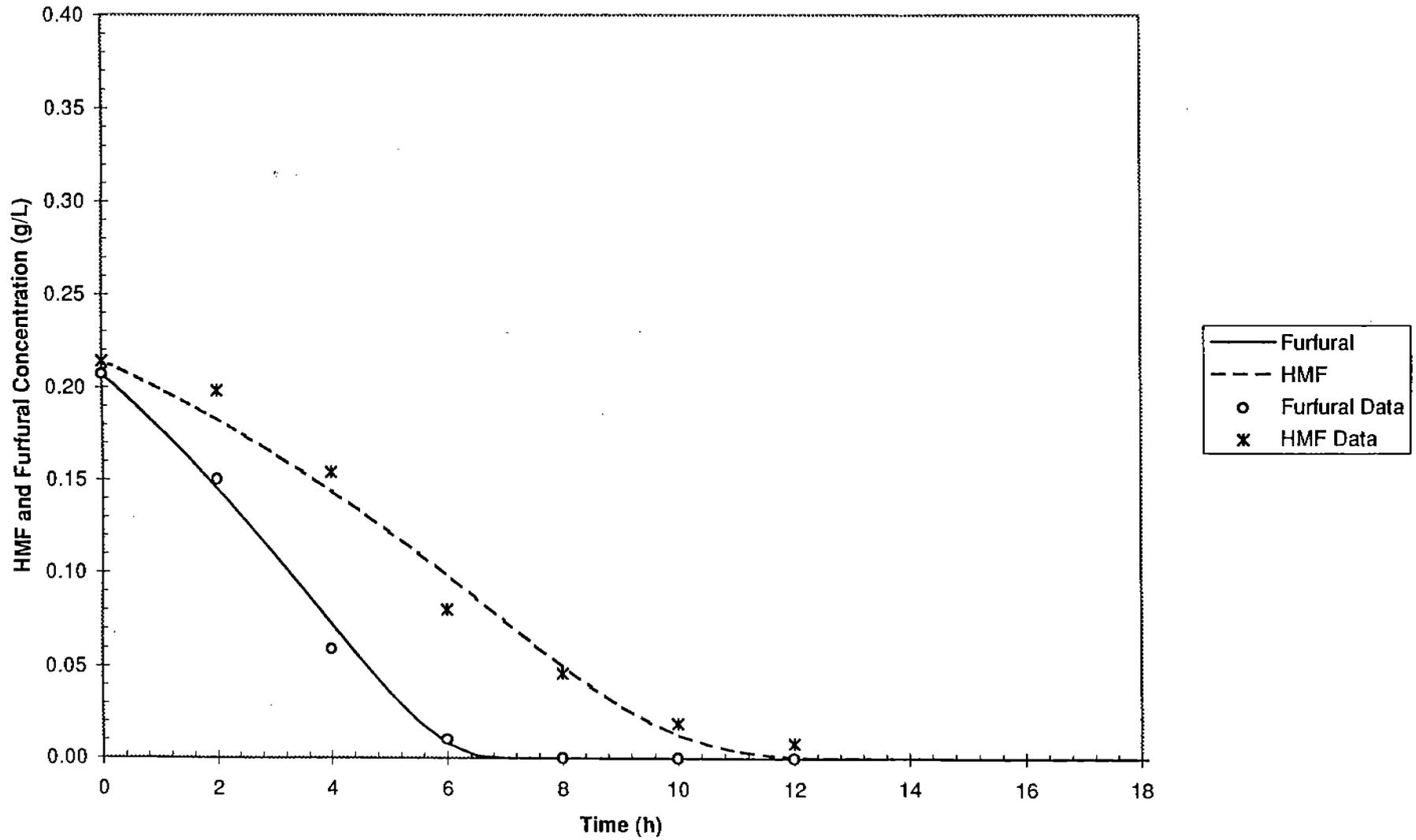


Figure F-17. Furfural versus Corrected Glucose Utilization

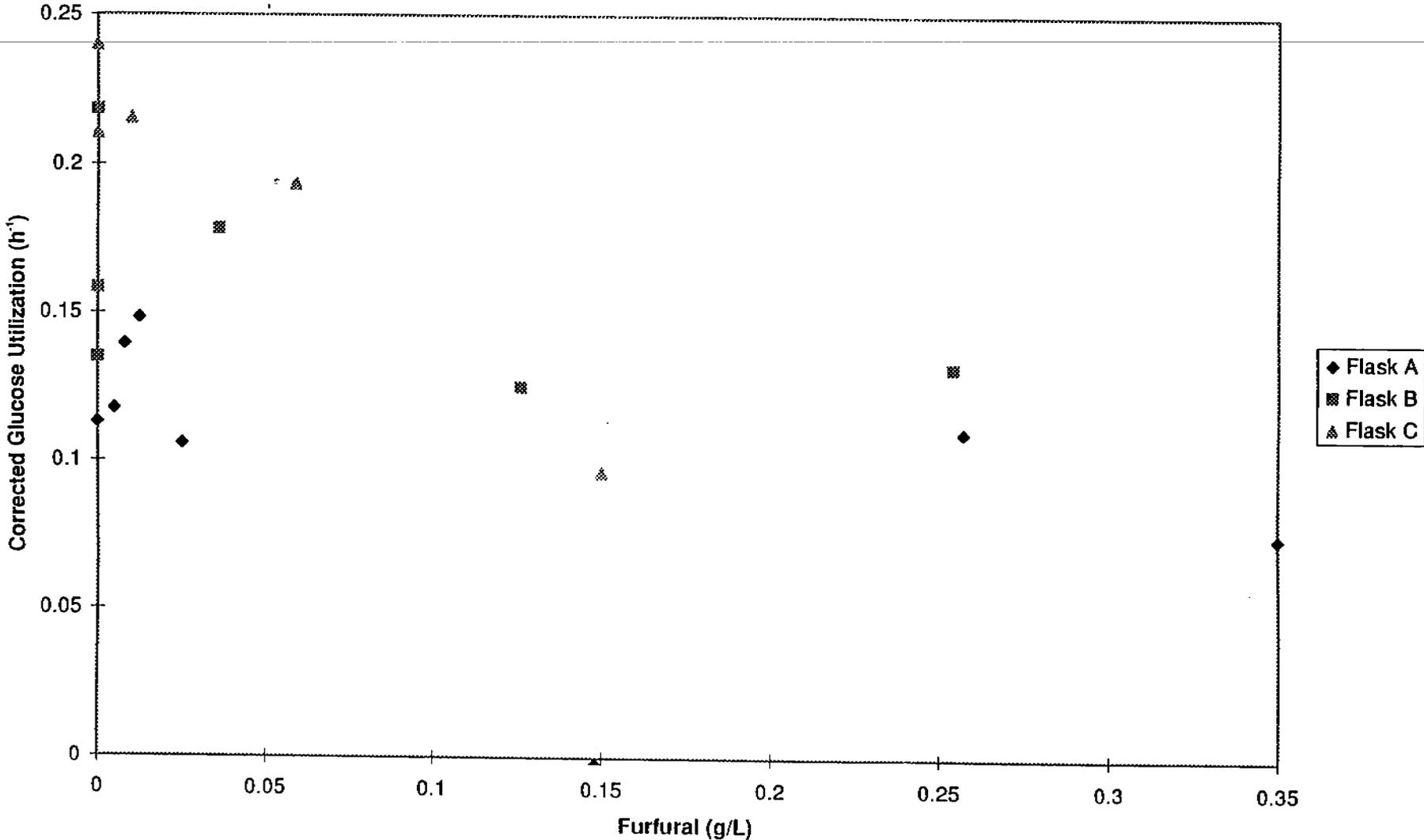


Figure F-18. HMF versus Corrected Glucose Utilization

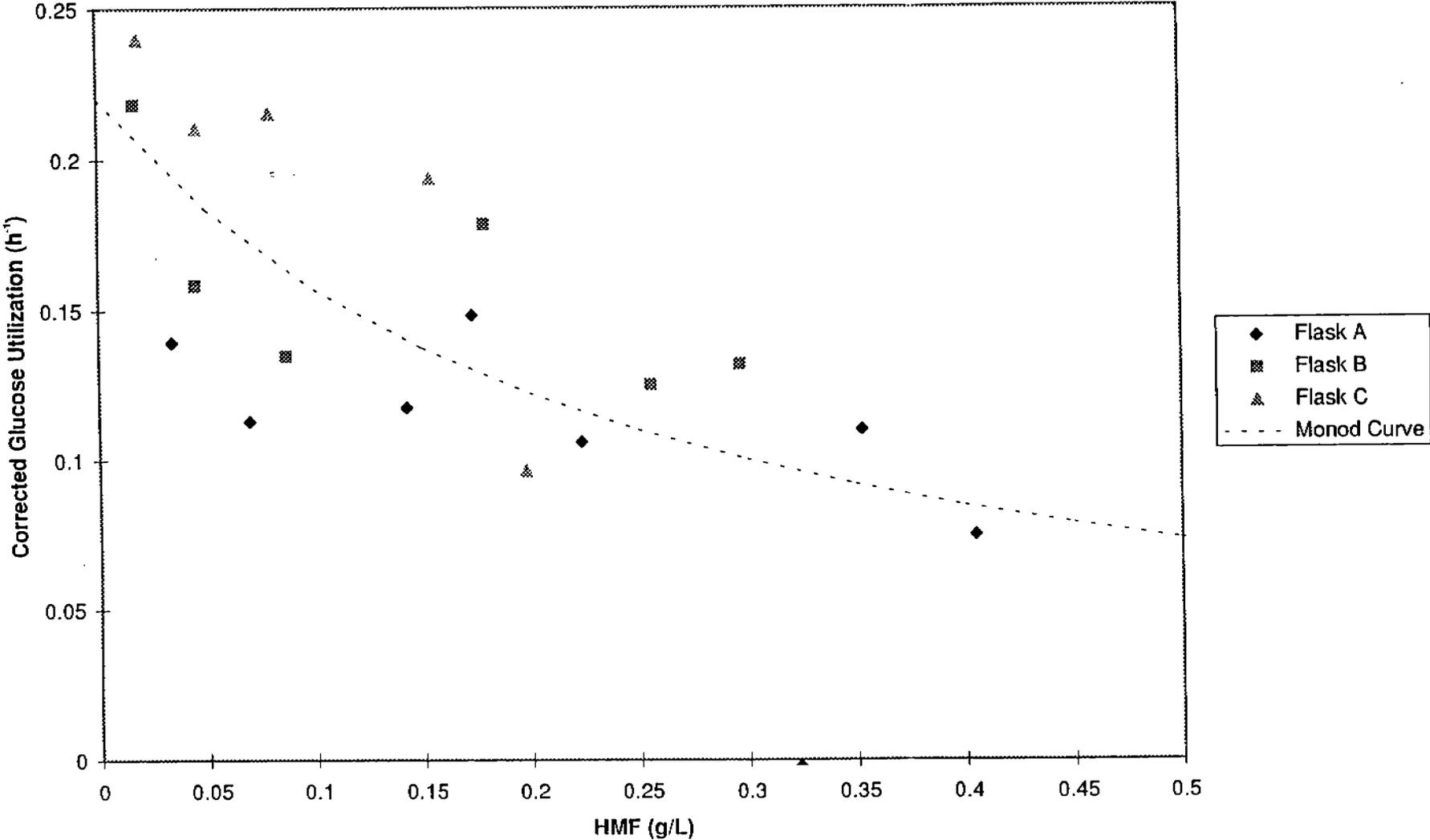


Figure F-19. Furfural versus Corrected Glucose Utilization with Correction for HMF Inhibition

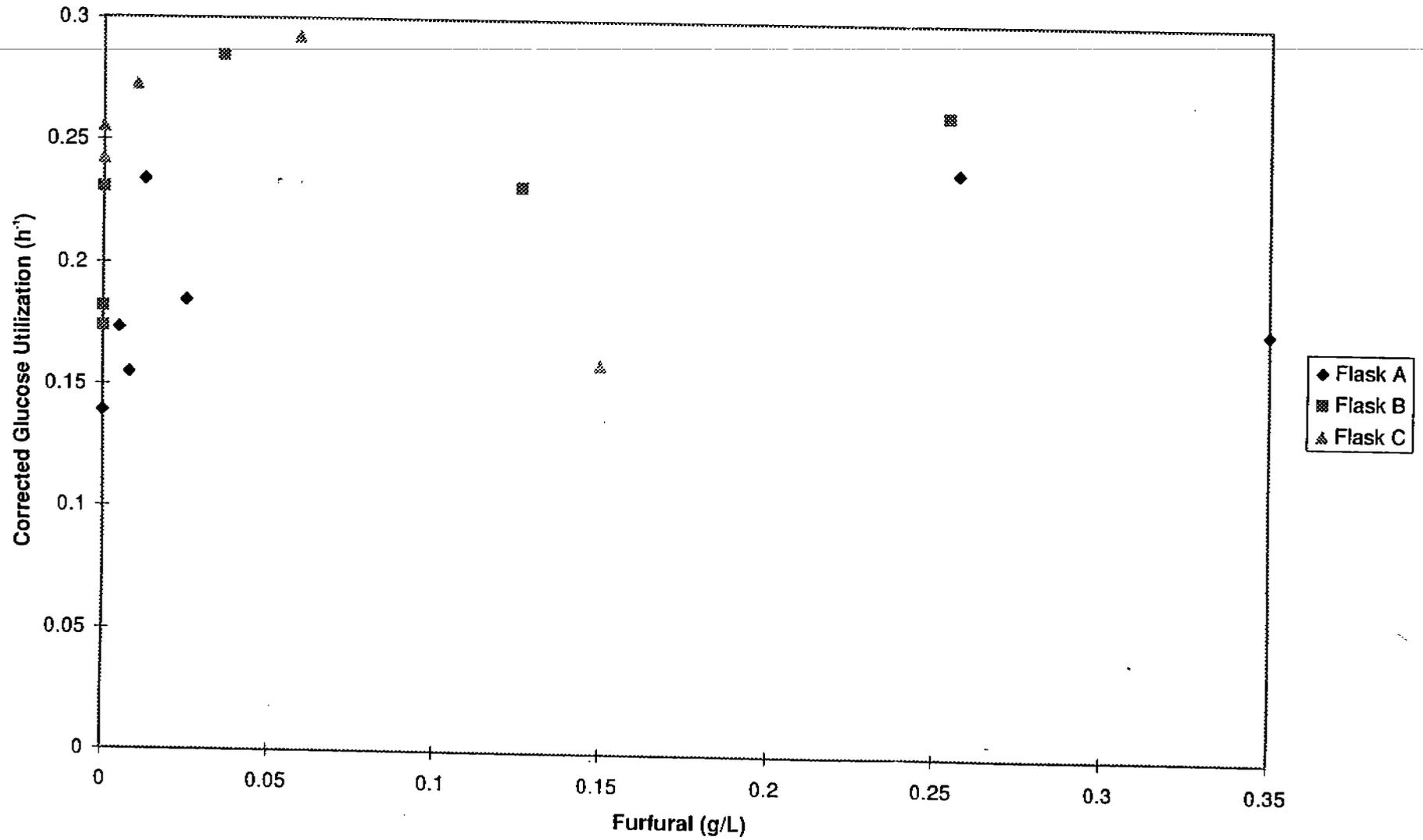


Figure F-20. Flask A (25% Equivalent Solids at Start)

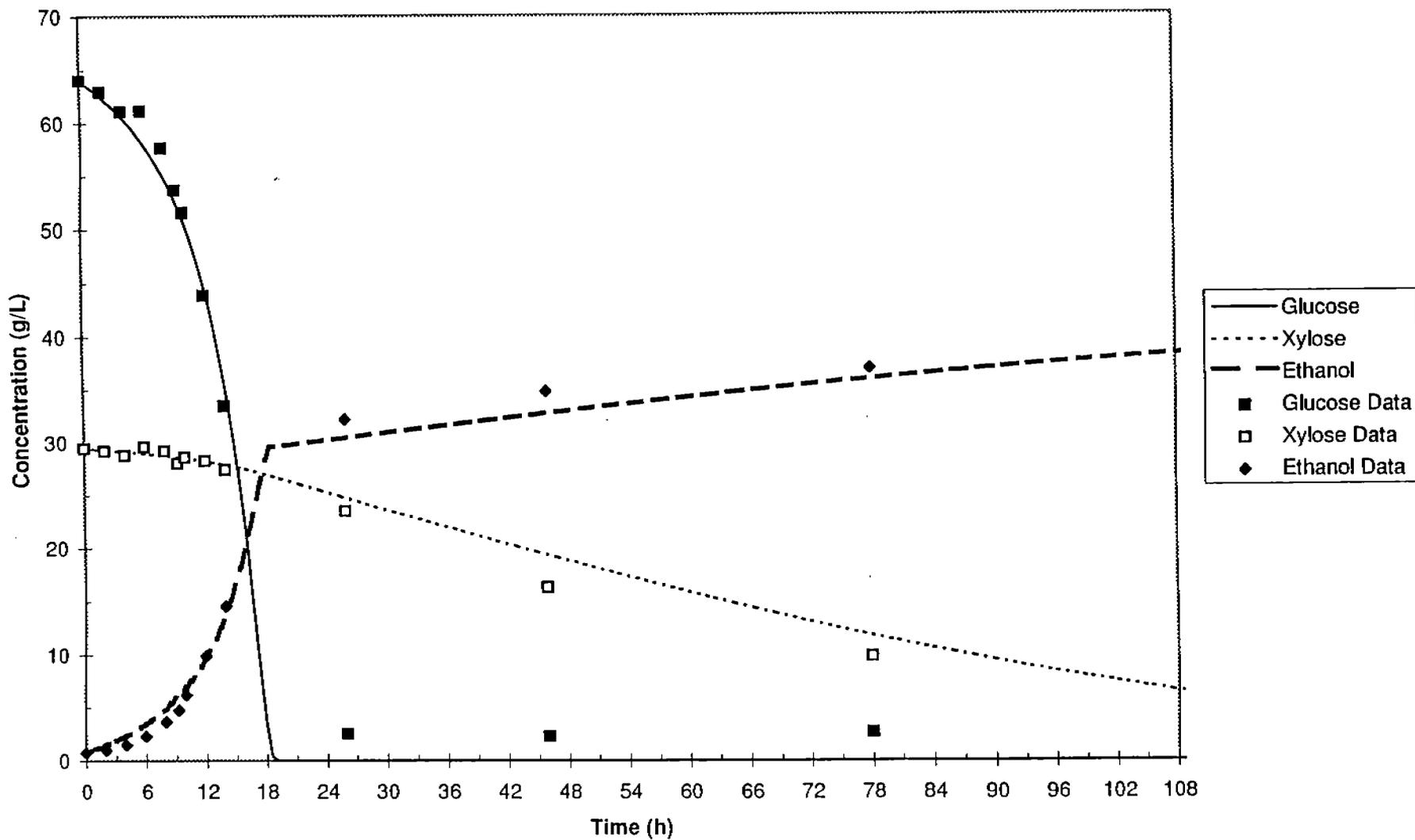


Figure F-21. Flask A (25% Equivalent Solids at Start)

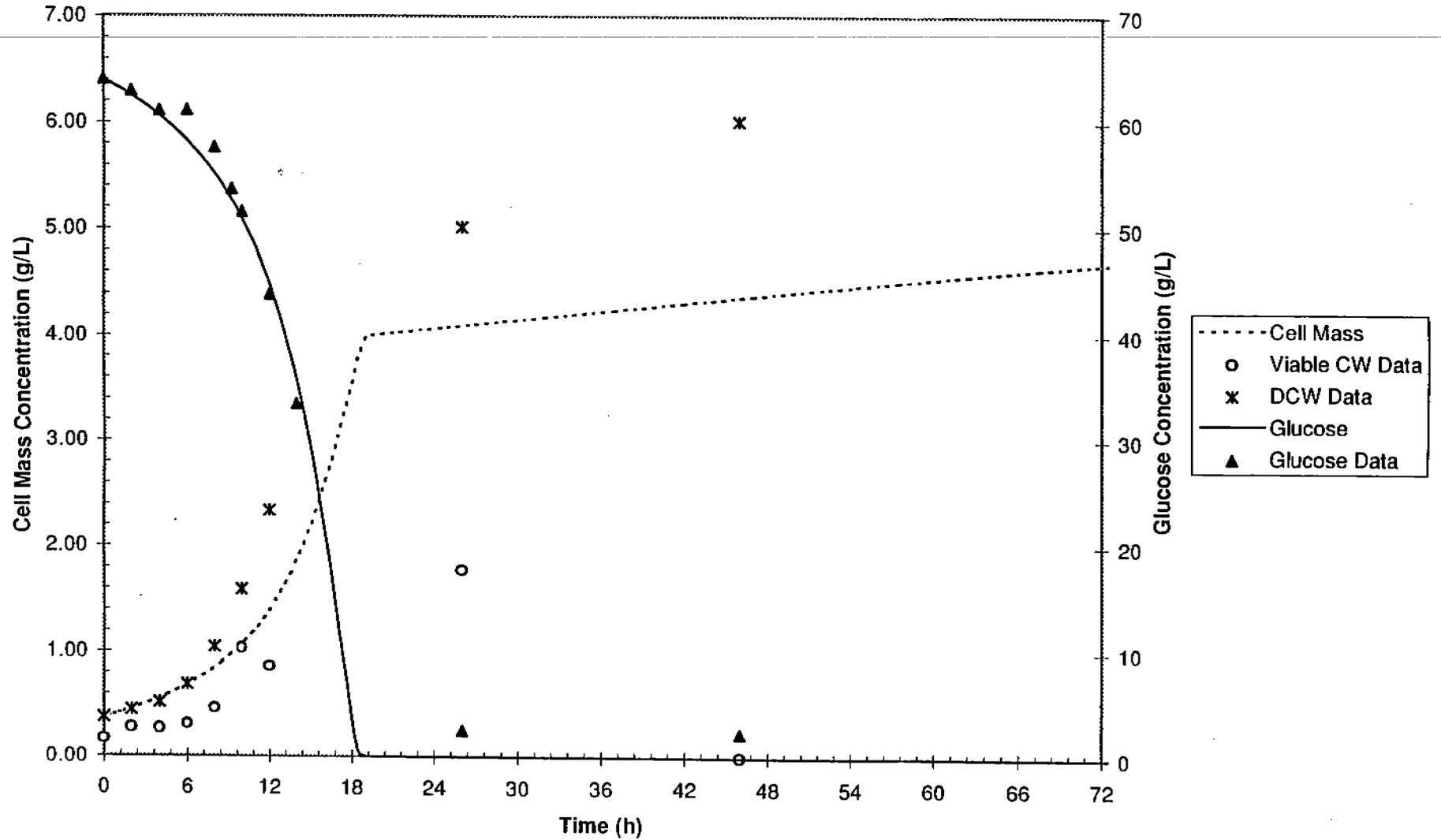


Figure F-22. Flask B (18% Equivalent Solids at Start)

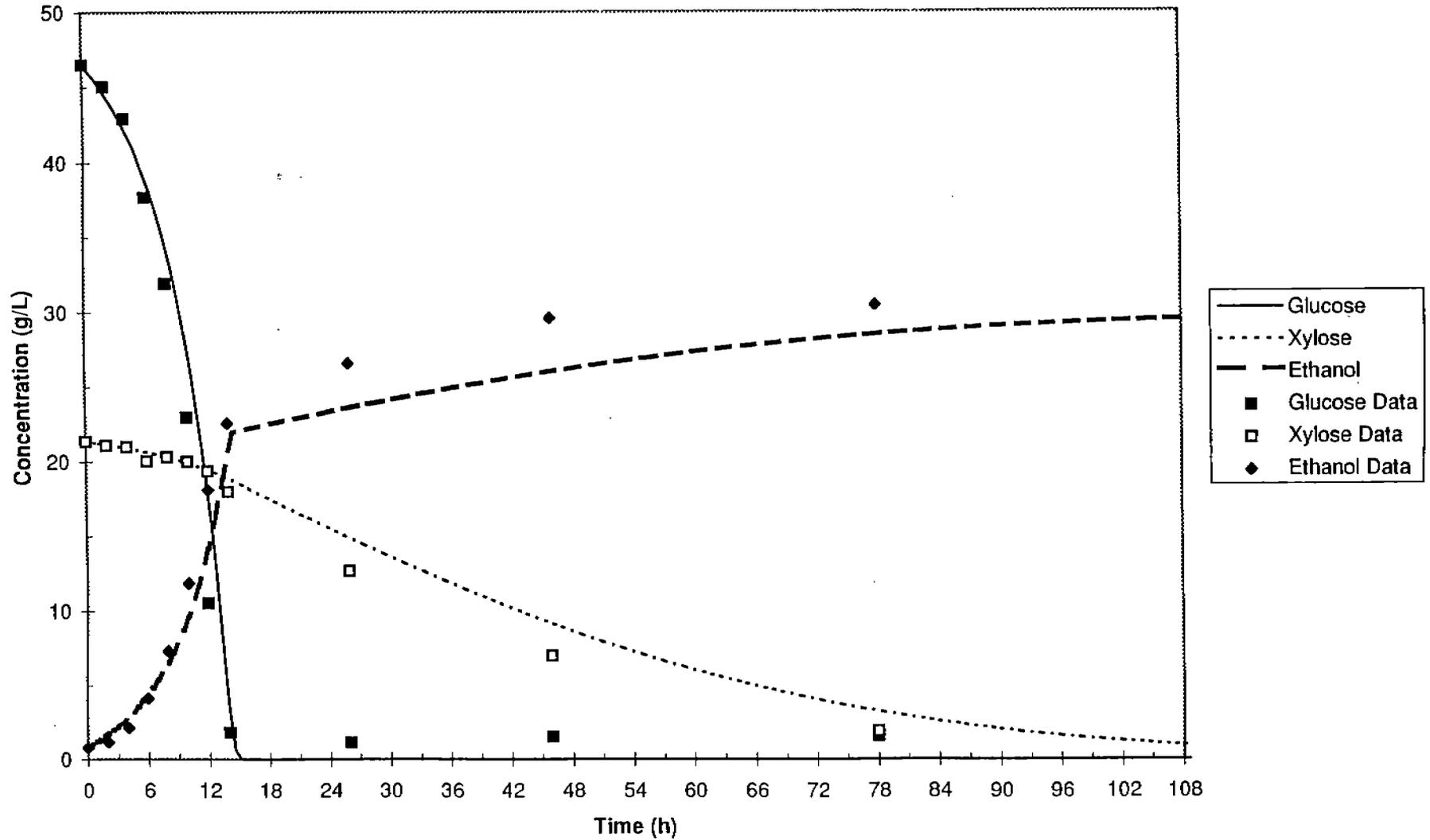


Figure F-23. Flask B (18% Equivalent Solids at Start)

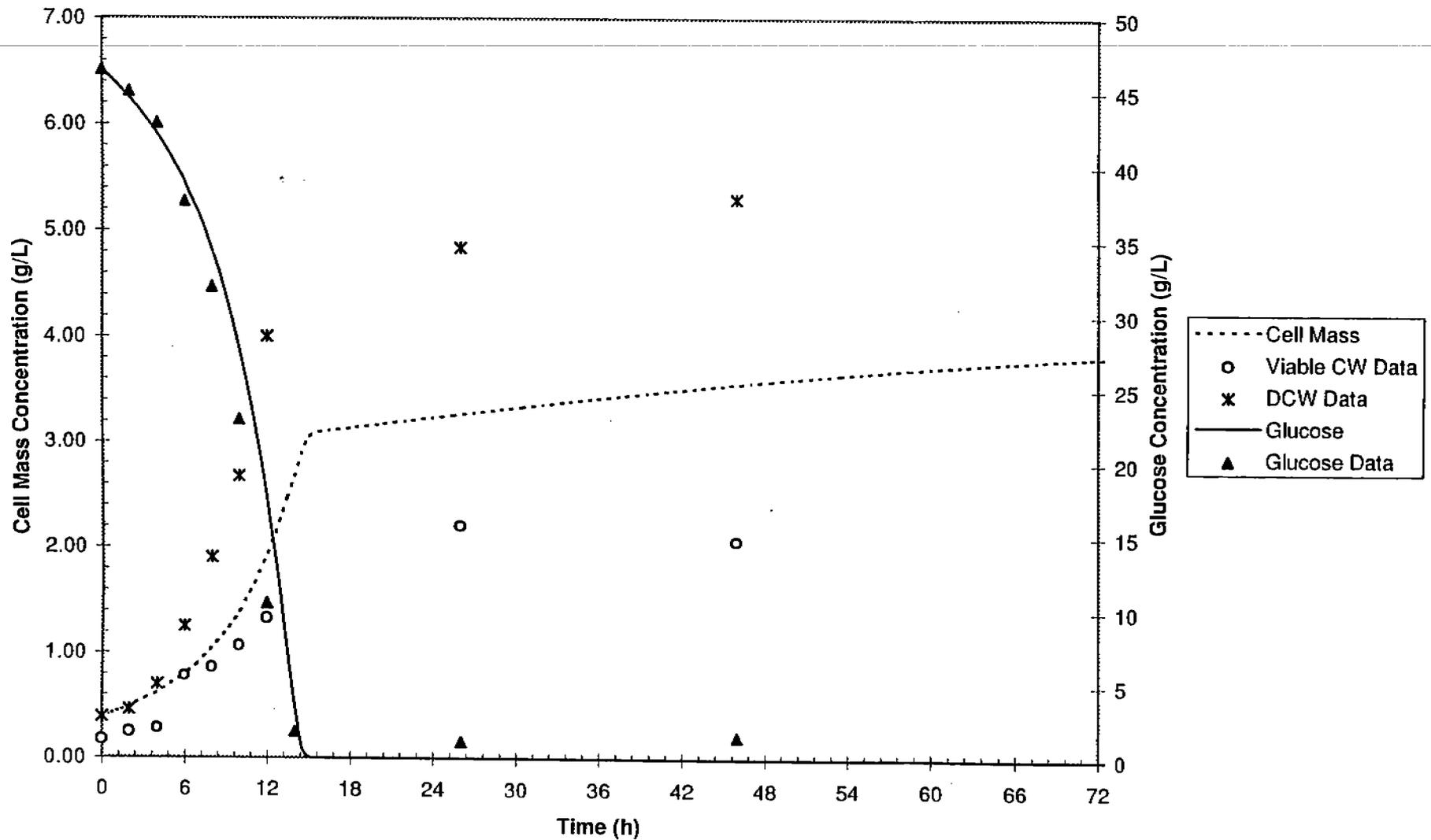


Figure F-24. Flask C (12% Equivalent Solids at Start)

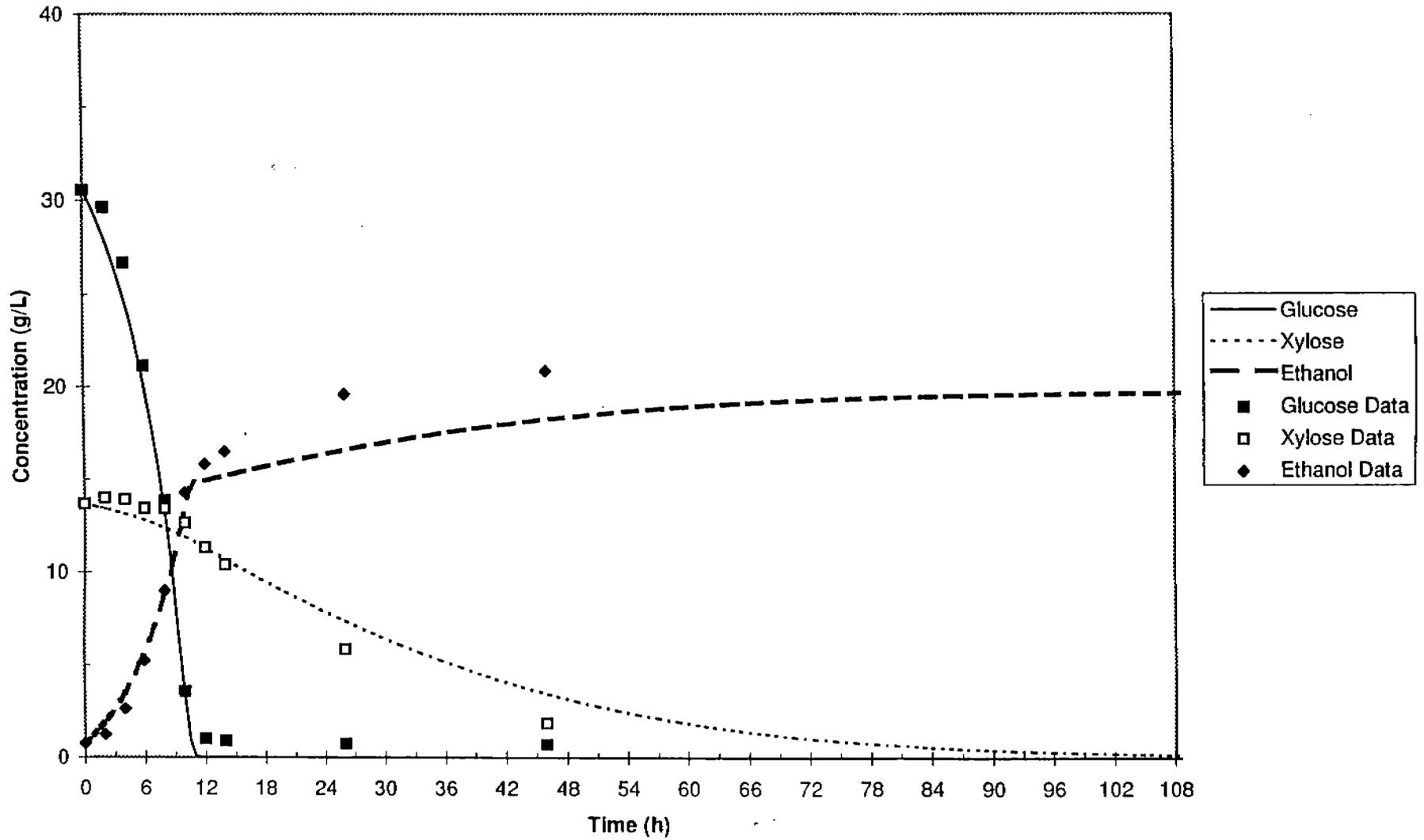


Figure F-25. Flask C (12% Equivalent Solids at Start)

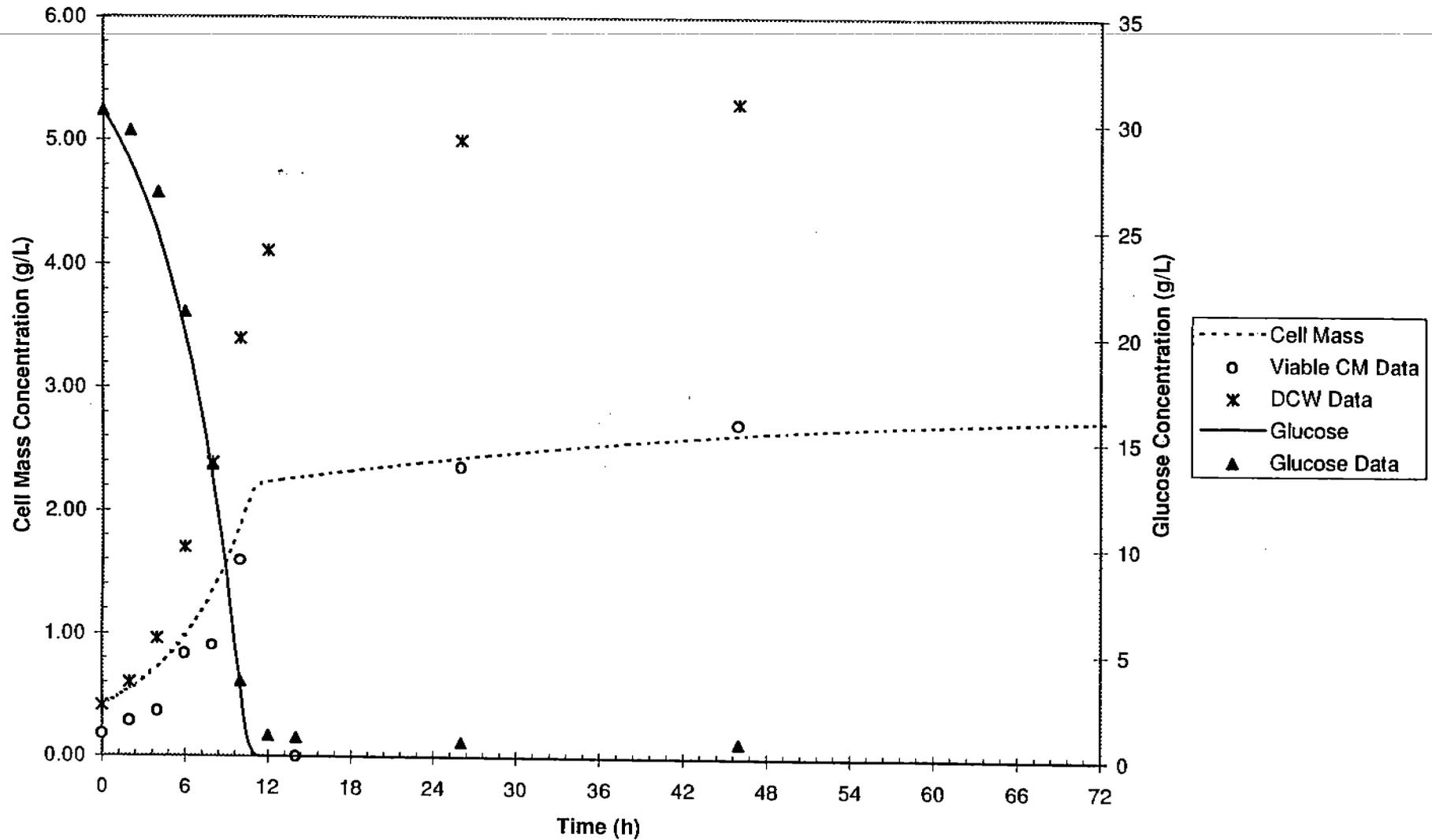


Figure F-26. Initial Furfural Levels versus Cell Mass Reduction

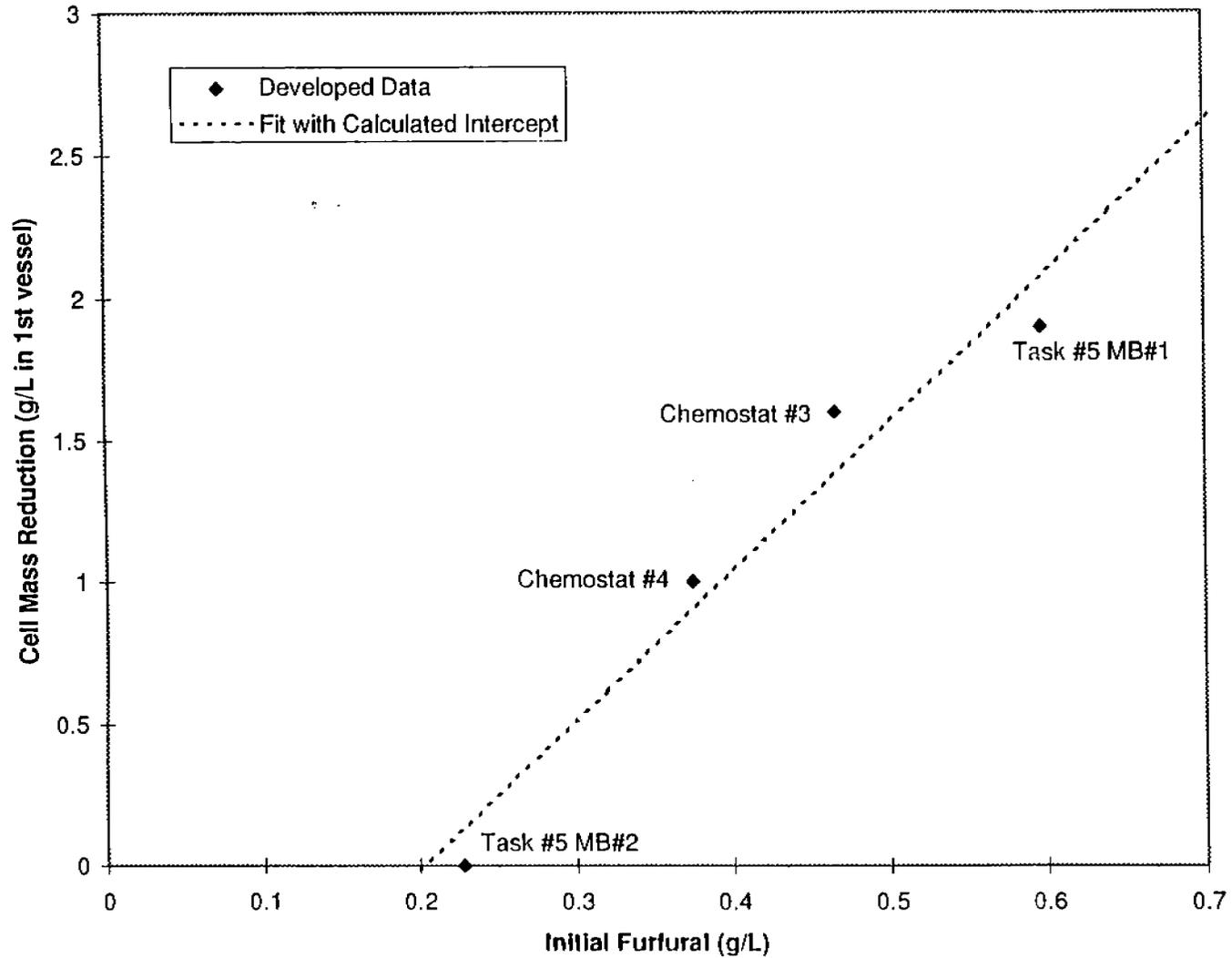


Figure F-27. Initial HMF Levels versus Cell Reduction

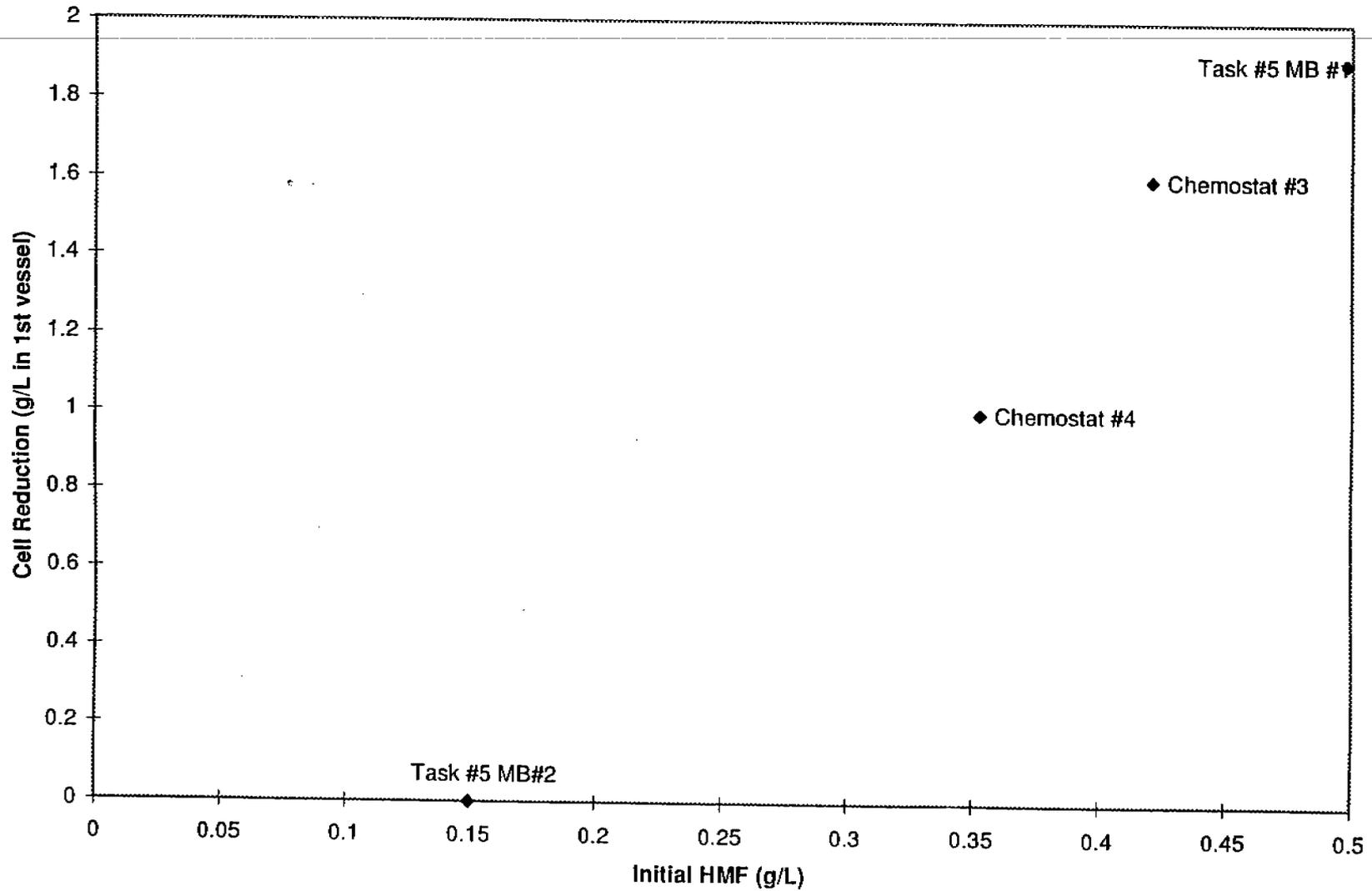


Figure F-28. Chemostat Run #3 Ethanol Concentrations at Steady State

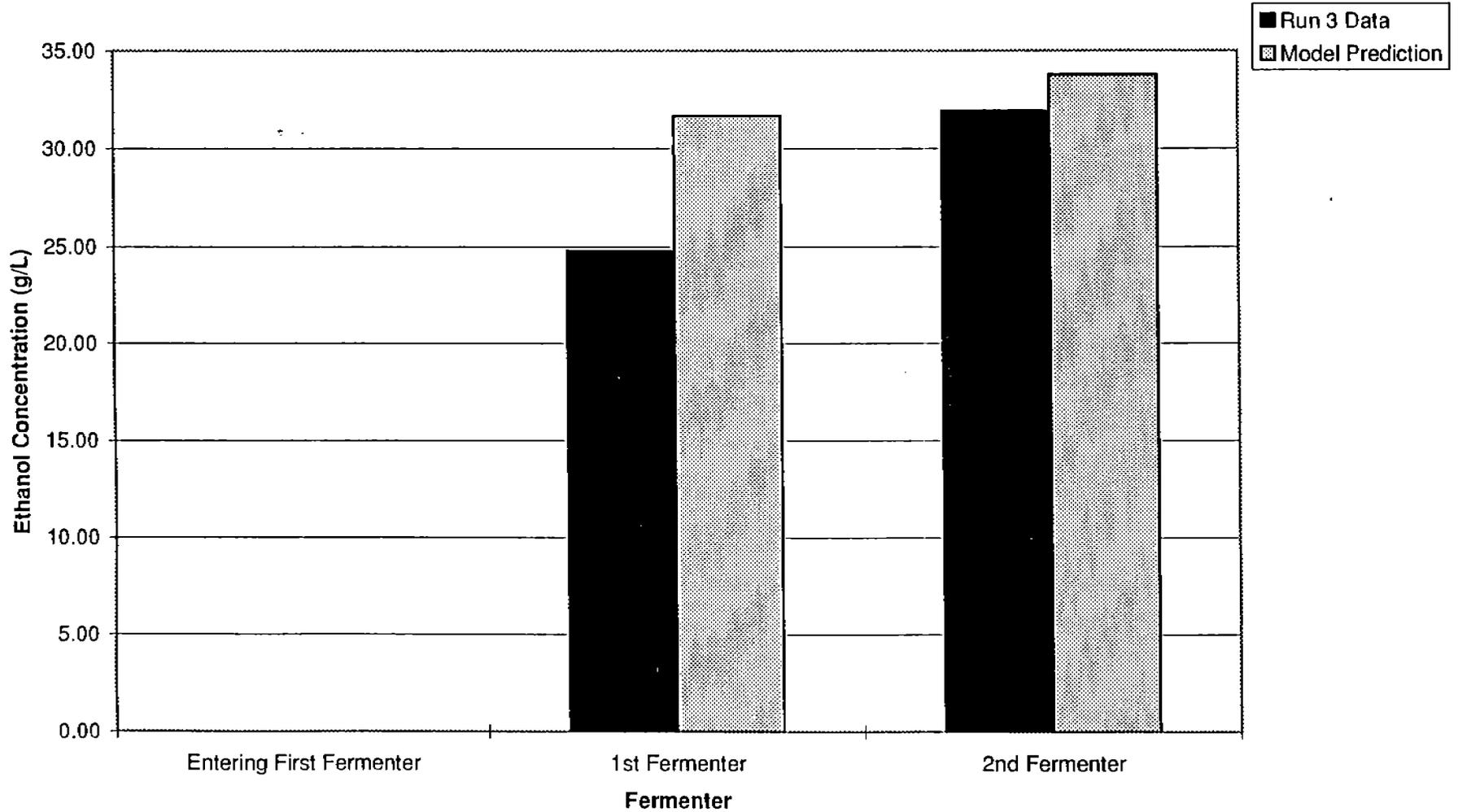


Figure F-29. Chemostat Run #3 Xylose Concentrations at Steady State

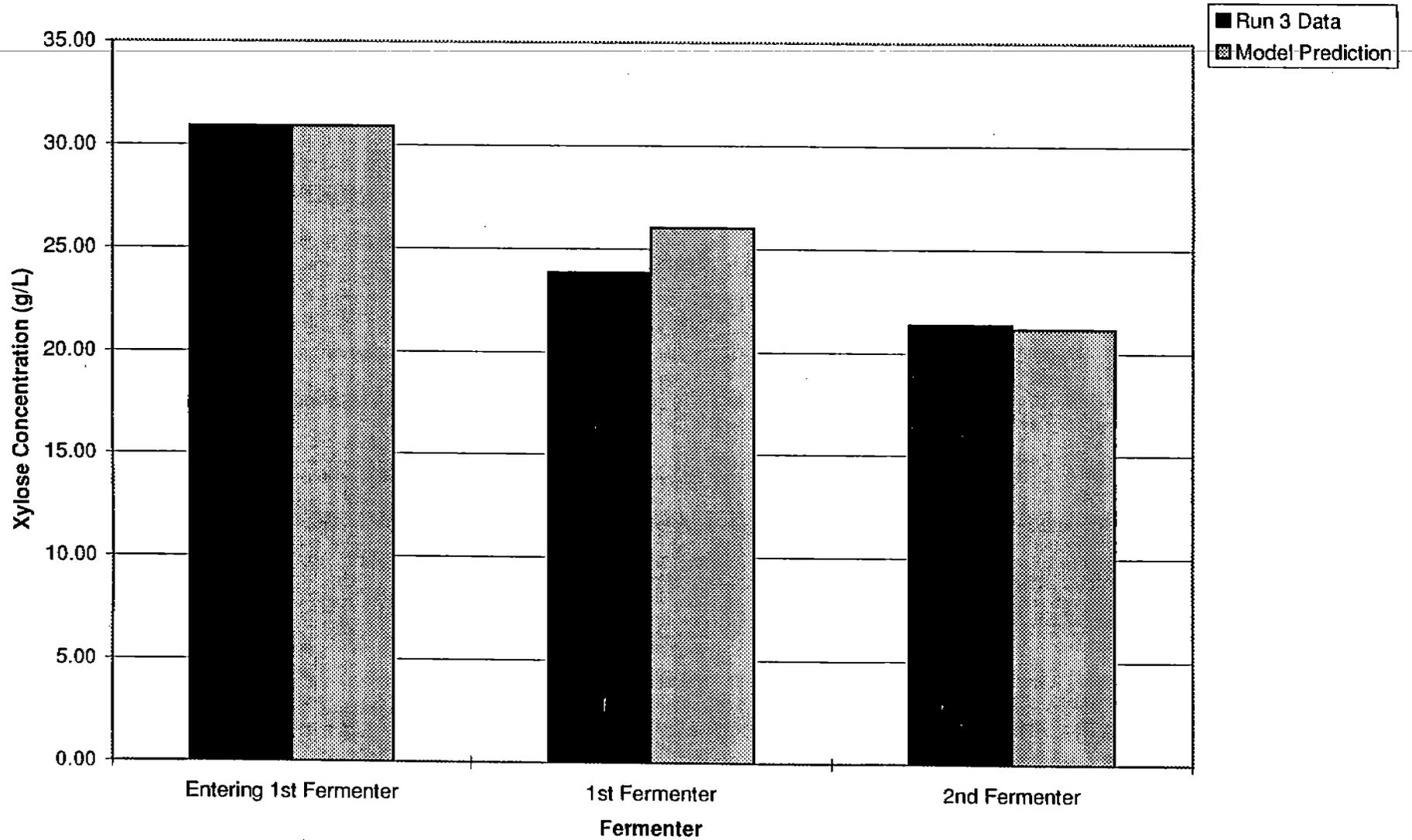


Figure 30. Chemostat Run #3 Glucose Concentrations at Steady State

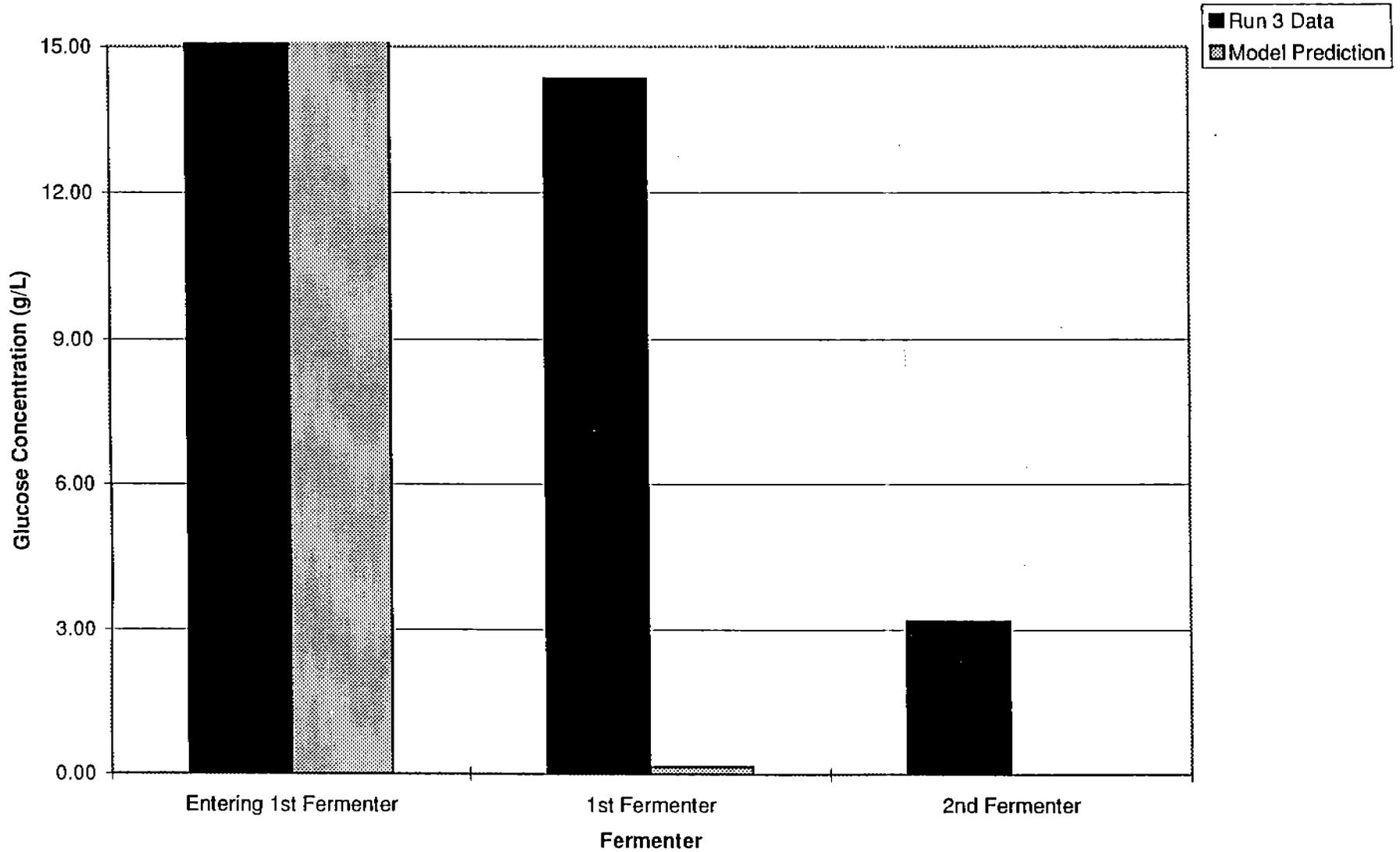


Figure F-31. Chemostat Run #4 Ethanol Concentrations at Steady State

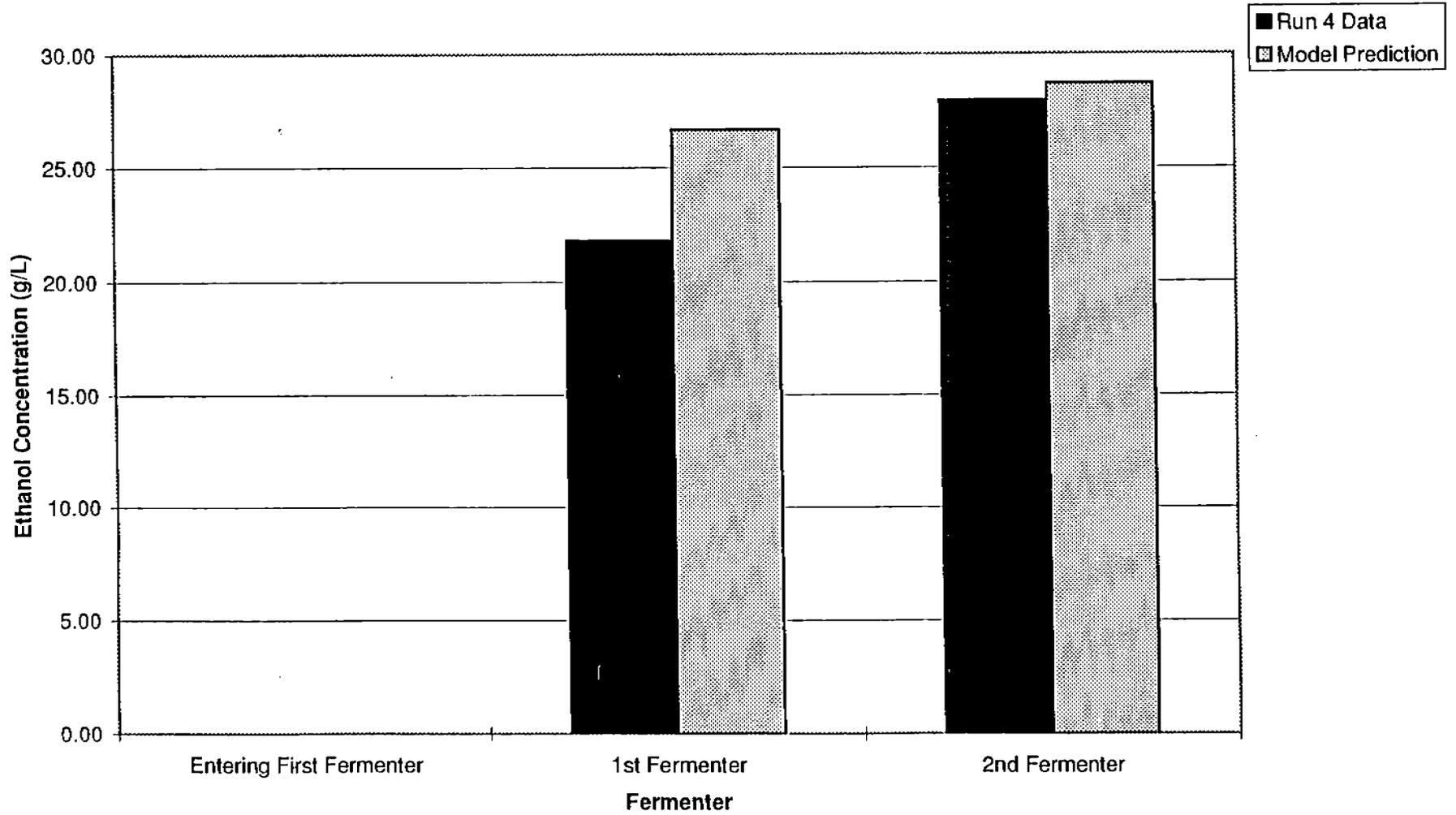


Figure F-32. Chemostat Run #4 Xylose Concentrations at Steady State

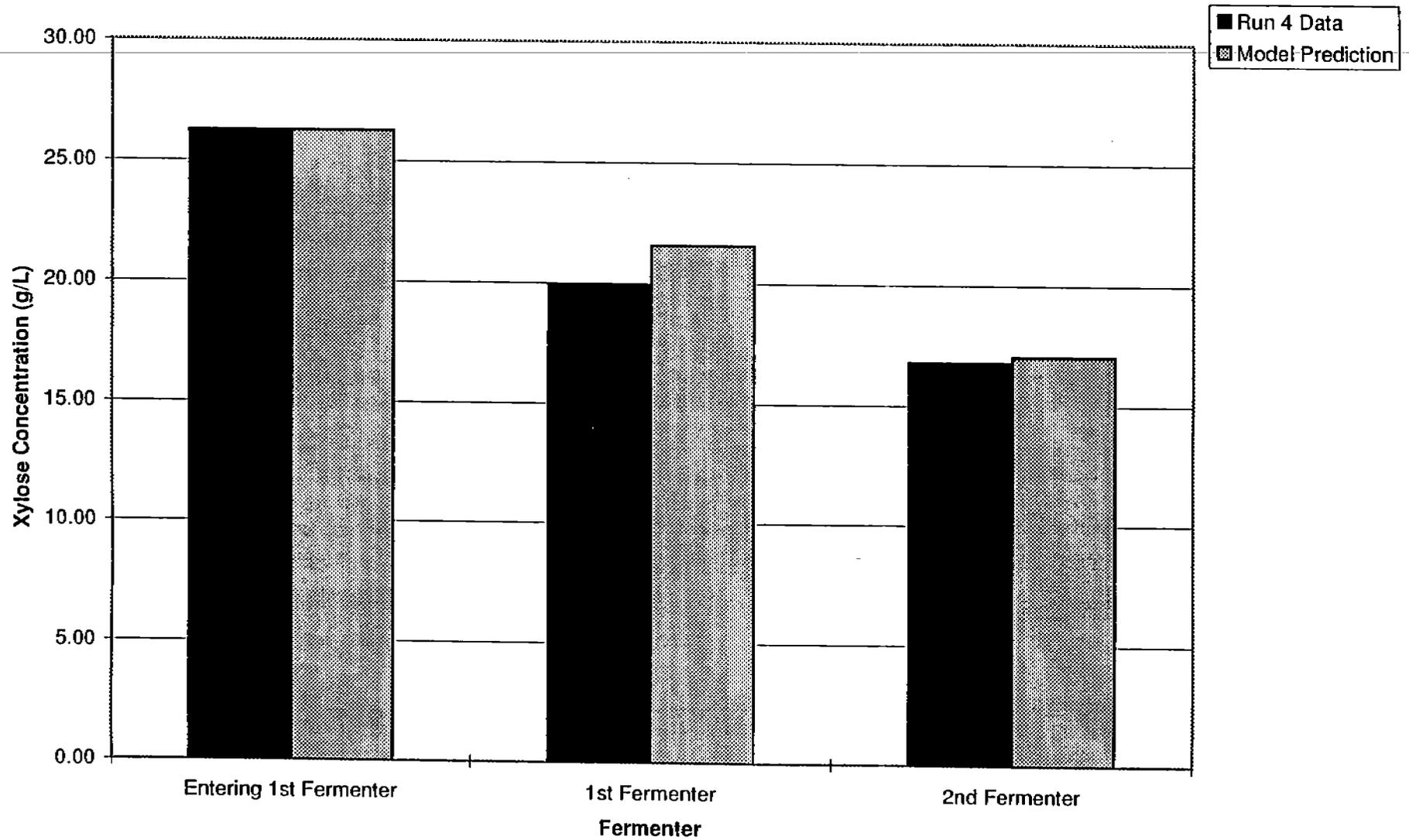


Figure F-33. Chemostat Run #4 Glucose Concentrations at Steady State

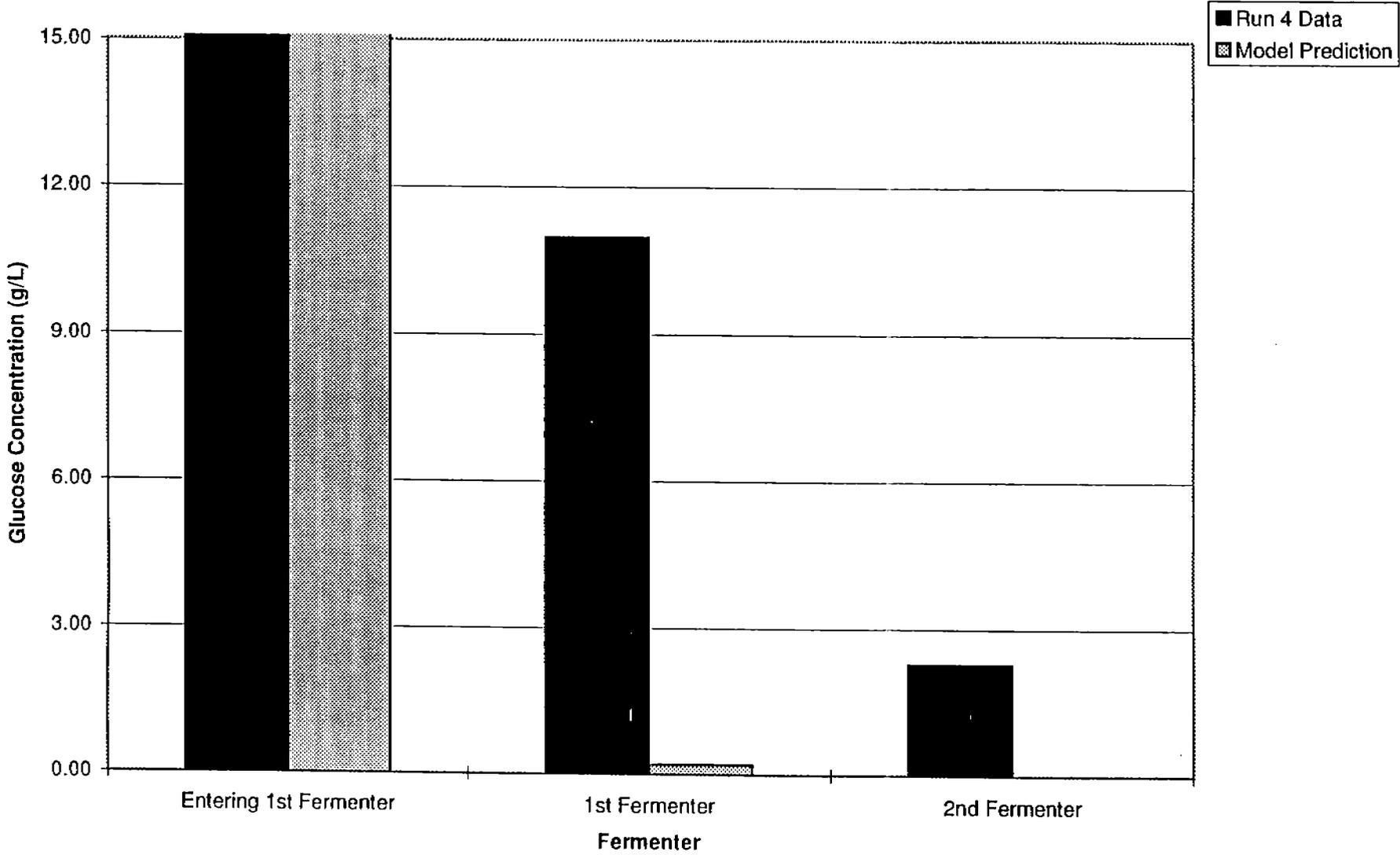


Figure F-34. Task 5 Mass Balance #1 Ethanol Concentrations at Steady State

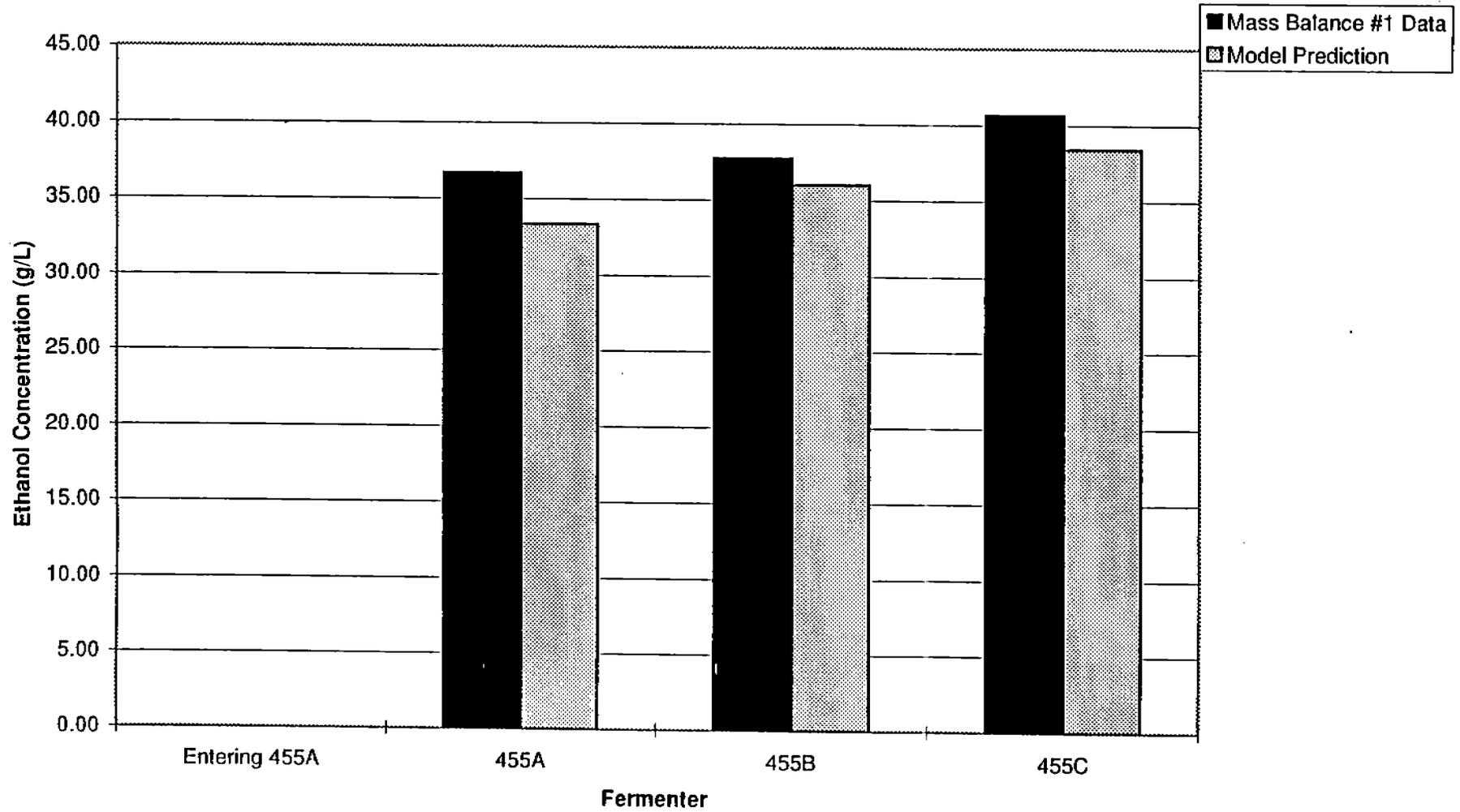


Figure F-35. Task 5 Mass Balance #1 Xylose Concentrations at Steady State

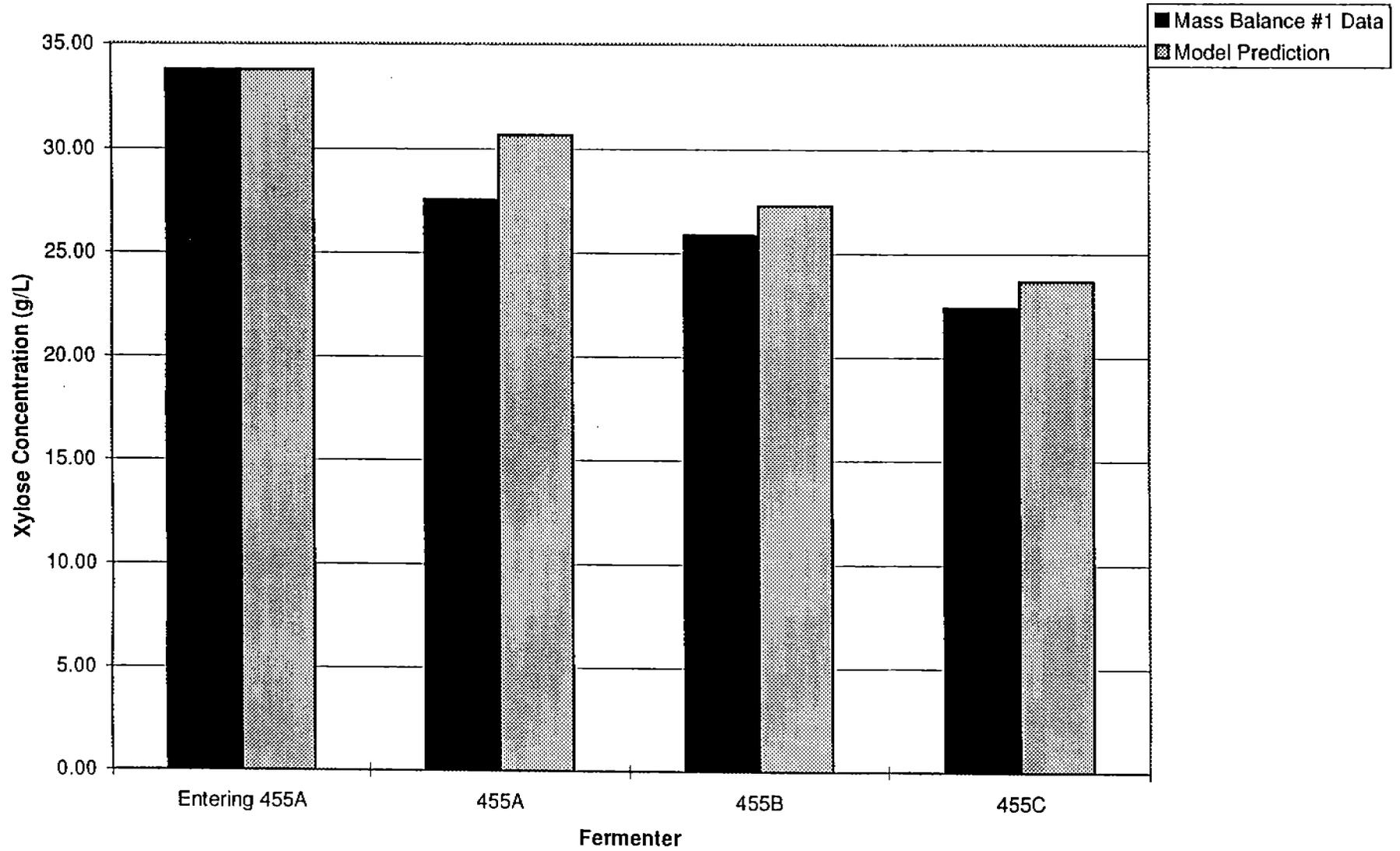


Figure F-36. Task 5 Mass Balance #1 Glucose Concentrations at Steady State

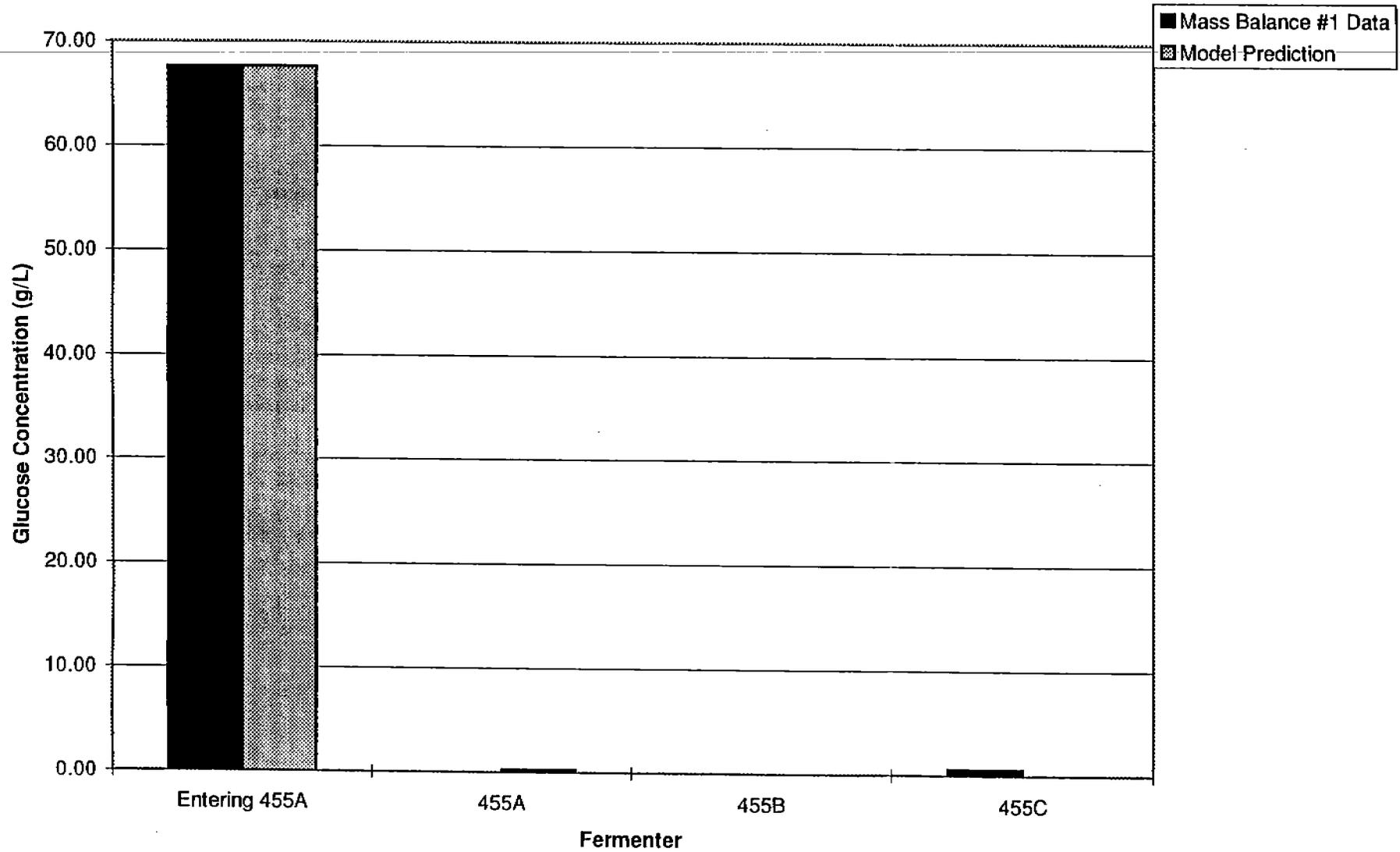


Figure F-37. Task 5 Mass Balance #1 Cellulose Concentrations at Steady State

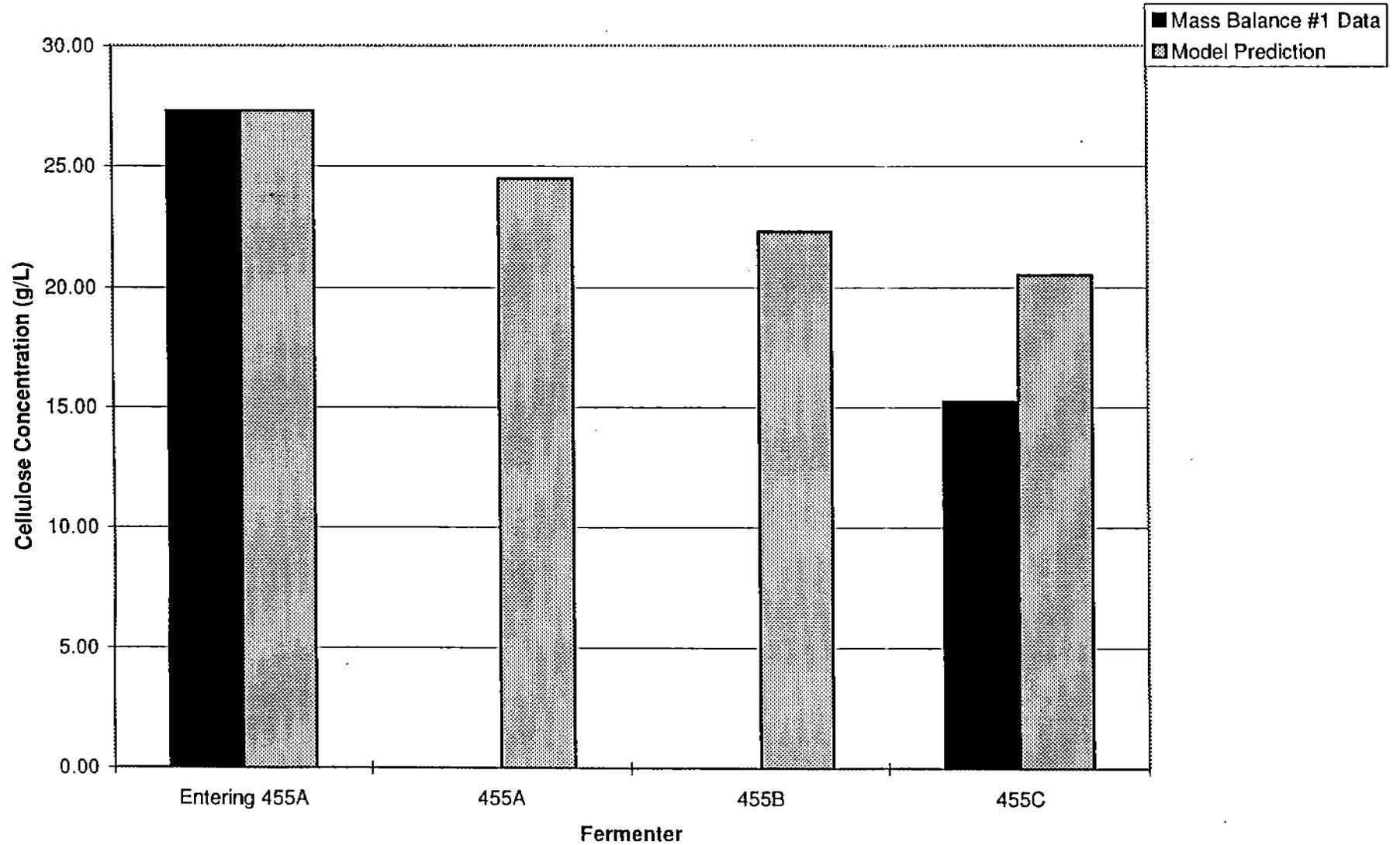


Figure F-38. Task 5 Mass Balance #2 Ethanol Concentrations at Steady State

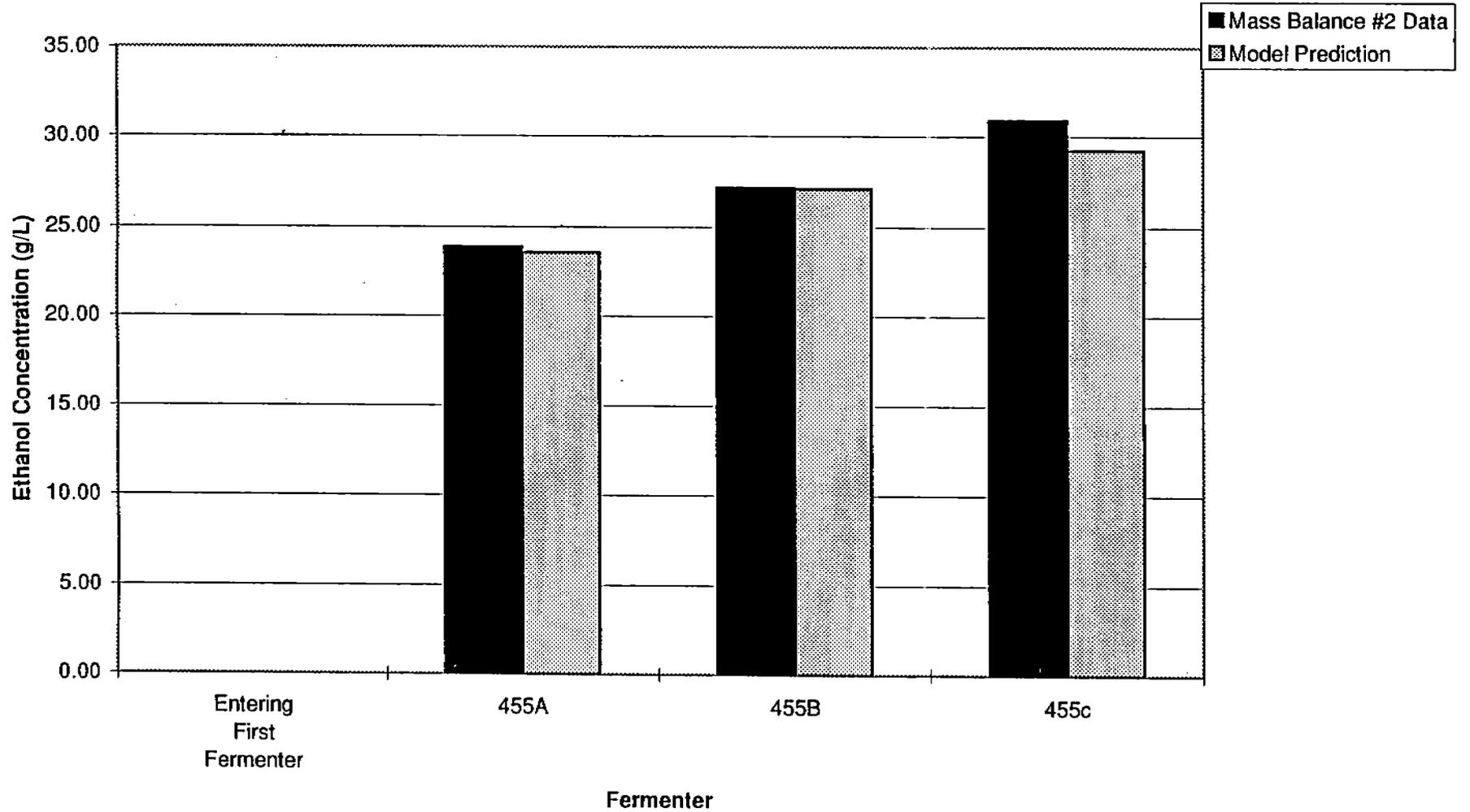


Figure F-39. Task 5 Mass Balance #2 Xylose Concentrations at Steady State

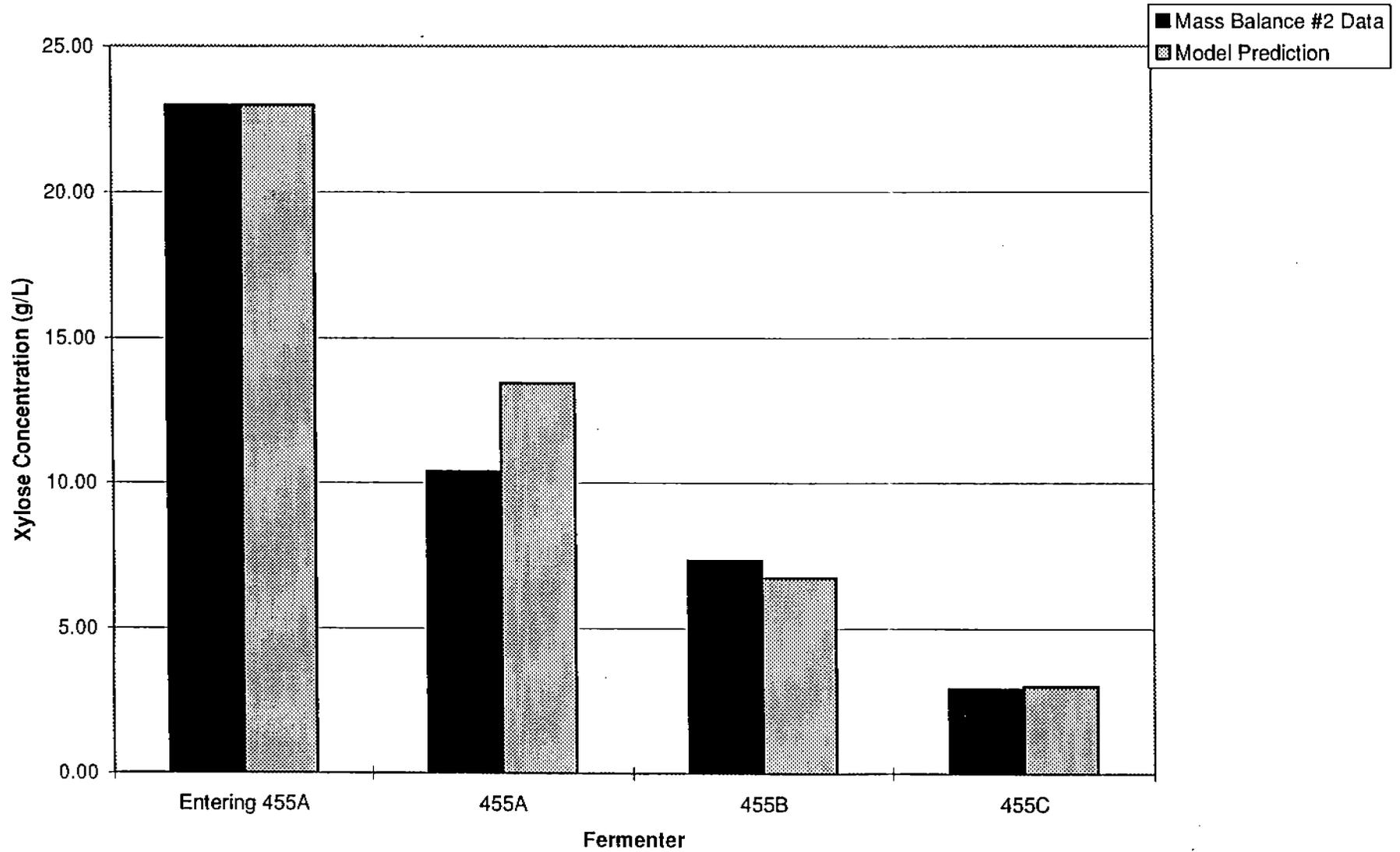


Figure F-40. Task 5 Mass Balance #2 Glucose Concentrations at Steady State

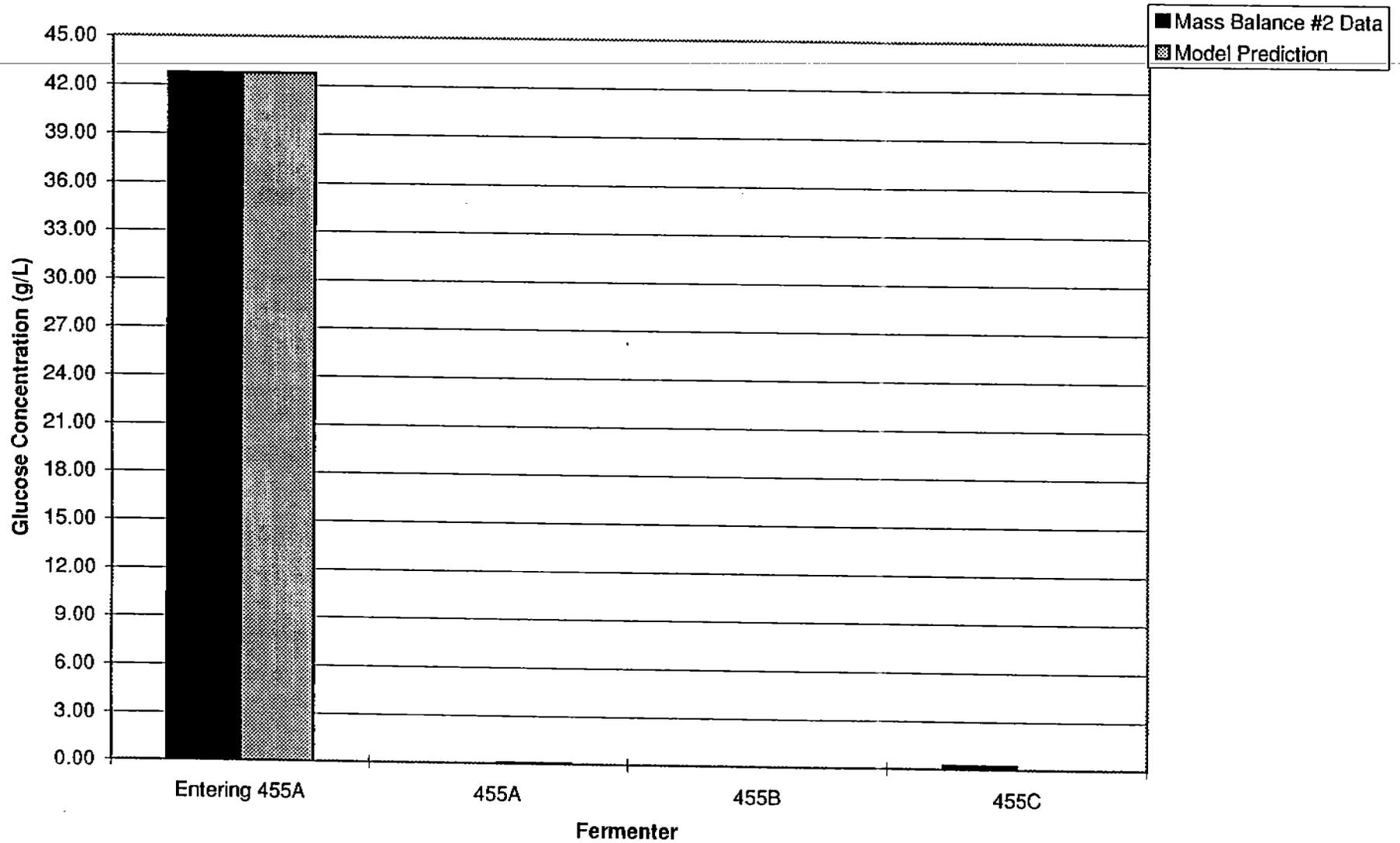
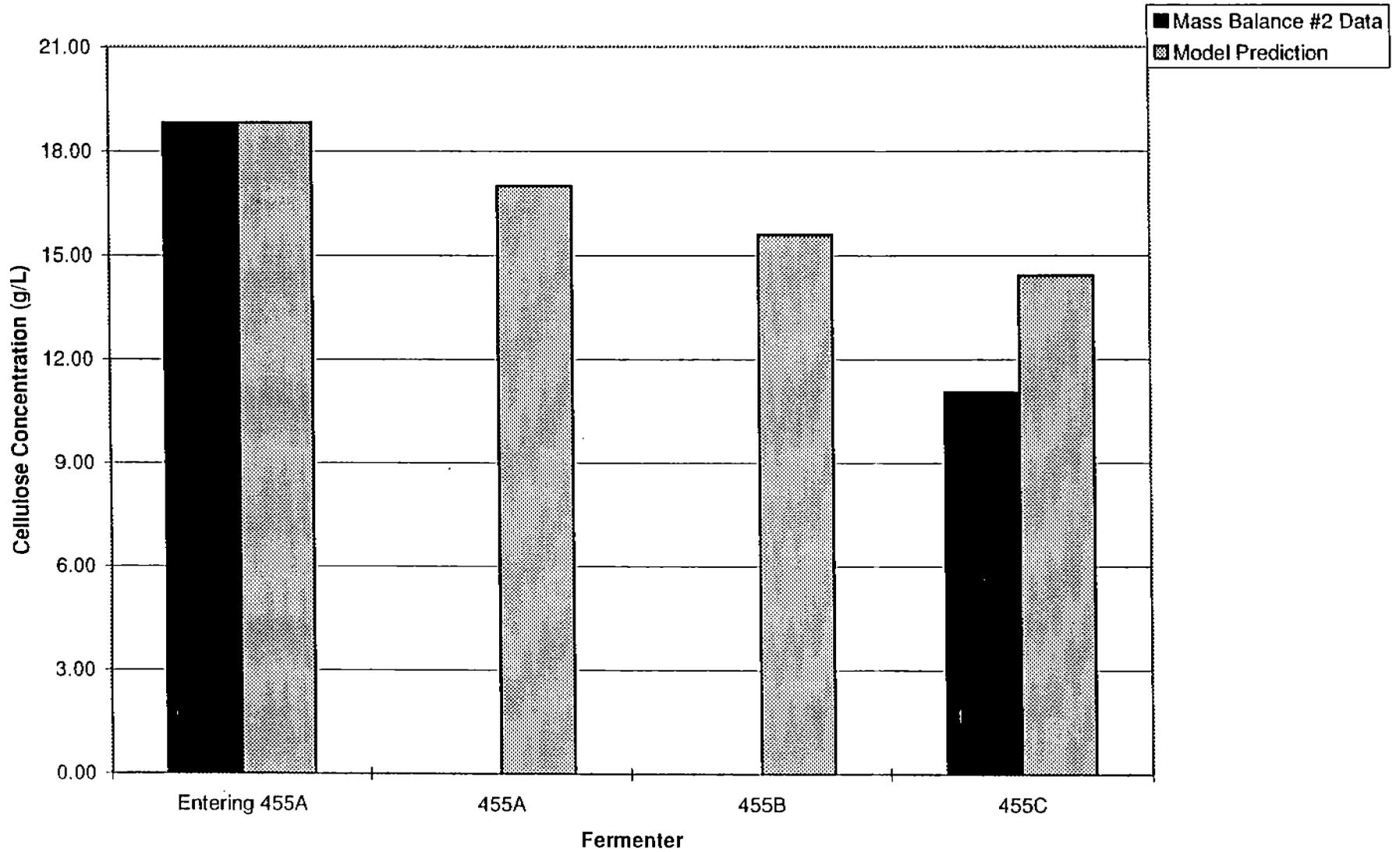


Figure F-41. Task 5 Mass Balance #2 Cellulose Concentrations at Steady State



**Report on Progress in Membrane Introduction Mass Spectrometry
Fermentation Monitoring at the National Renewable Energy Laboratory -
June 1996 and Associated Preparatory Work**

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Abstract: Recent experiments at the National Renewable Energy Laboratory (NREL) have demonstrated the stability and usefulness of the membrane introduction mass spectrometry (MIMS) system under pilot plant conditions. The work employed a Finnigan ITS-40 ion trap instrument, adapted to MIMS experiments by addition of an external membrane/jet separator interface and packaged to operable in a rugged environment. The broth of a 9000 liter fermentation reactor was continuously monitored, on-line, and yielded quantitative data on the ethanol concentration. The only sample pretreatment was filtration using a tangential stainless steel filter. This filter was capable of withstanding the temperature and pressure of 30 psig steam sterilization as well as the 1-20 psig pressure and being capable of operating with a sample stream consisting of the 15 percent solids. The filtrate was sampled using a flow injection analysis system (FIA) which allowed quantification using external standards. Calibration experiments established that the system displayed a linear response to ethanol at concentrations between 1 and 10 percent, by volume. Subsequent experiments alternated injections of ethanol standards and sample streams, using standard solutions to quantitate the response of the sample stream and reduce errors associated with long term instrumental drift. Ethanol concentrations were found to be approx. 3 percent and were quantitatively in agreement with offline HPLC data.

Objectives

The objective of this work was to modify a membrane introduction mass spectrometer so that it could be adapted from the laboratory environment to that of the pilot plant. A further objective was to test the performance of the system for continuous quantitative determination of ethanol in the fermentation medium. Objectives for later work included the determination of other components of the broth, including lactic and acetic acid, and possibly furfural and other higher molecular weight compounds. The initial experiments described here focused upon modification of the mass spectrometer so as to prepare it for the rugged pilot

plant environment, the set-up of the instrumental system within the pilot plant, construction and interfacing of a filtration system to one of the three reactors (reactor #1), and finally, utilization of the system to monitor ethanol in the fermentation broth.

Topics Covered in this Report:

Background Information on MIMS and Fermentation Monitoring

- A. Operation of MIMS and Comparison to Chromatography Systems*
- B. Types of Membrane Interfaces*
- C. Choice of Membrane*

Experimental

- A. Apparatus*
- B. Flow Injection System*
- C. Filtration System*
- D. Chemicals*

Data Analysis

- A. Example of Calculations*
- B. Comparison of MIMS to HPLC Data*

Discussion

Further Experiments

Conclusions

Background Information on MIMS and Fermentation Monitoring:

A. Operation of MIMS and Comparison to Chromatography Systems

Membrane introduction mass spectrometry (MIMS)¹ is a mass spectrometric method in which the analyte is introduced via a semipermeable membrane. In the case of a fermentation broth, this membrane presents a barrier to biomass and to water while allowing ethanol and other compounds to be monitored rapidly by the sensitive and specific method of mass spectrometry.²

Fermentation broths typically require extensive filtration and relatively long separation processes are required for analysis by techniques such as HPLC or GC; MIMS requires only filtration (to the one micron level). The mass spectrometer records spectra continuously and the abundances of all the ions in each spectrum are conveniently summed and plotted as a function of time. Such a total ion chromatogram records the total concentration of all the compounds passing through the membrane. More interesting is a selected ion chromatogram, a plot of

the abundance of particular ions against time. These abundances represent the concentrations of the compounds for which these ions are characteristic, and the way in which they change with time reflects the changes concentration in the medium. The separation of the total ion chromatogram into separate into single ion chromatograms is normally done after data acquisition and this allows one to monitor several compounds at once. The fermentation broth is interrupted by standard solutions (separated by pure water plugs) in order to maximize the quantitative accuracy of the method.

If one wishes to compare the separation process of MIMS with that of a chromatography system, one would see that differentiation of the individual compounds in MIMS is achieved in the gas phase, after ionization, and after the analyte has passed through the membrane. Chromatography systems typically require long elution times from their chromatography columns prior to introduction to their detectors. In addition, chromatography detectors are typically “blind” to the composition of their samples. “Blind” refers to the fact that the detectors used for chromatography typically cannot differentiate between two compounds which elute simultaneously. *The advantages inherent in MIMS are rapid sample introduction with the selectivity of mass spectrometry combined with the simplicity and ruggedness of a semipermeable membrane introduction system.*

B. Types of Membrane Interfaces

There are various methods of performing membrane sampling with a mass spectrometer; some of these considered for this project are indicated below. The membrane can be introduced directly in the mass spectrometer ion source via a direct insertion membrane probe or it can be mounted externally. In the latter case pneumatic transport of the permeate is employed and a jet separator, a form of momentum separator, is used to remove the helium transport gas (**figure 1**). This was the scheme chosen for these experiments. The jet separator was from a Finnigan 4500 series GC/MS as supplied by SGE. The direct insertion membrane probe (DIMP) has seen numerous applications with both Silastic© vulcanized nonreinforced sheeting, 0.005" thick, or a capillary Silastic© medical grade tubing with a wall thickness of 0.022". External configurations use the same types of membranes that are used with the DIMP.

As already noted, the membrane functions as a semipermeable membrane to the sample stream. In the case of the Silastic sheeting above, these membranes present a hydrophobic barrier to the sample stream. Ideally, the membranes would reject all of the water from the mass spectrometer, only allowing the relatively

volatile compound of interest to pervaporate from the membrane. In fact, some water pervaporates across the membrane. The mass spectrometer is normally operated so that the resulting water ions are not normally detected but the water vapor itself can be used as a reagent gas in the chemical ionization (CI) mode to enhance the sensitivity of the instrument. Typically the membrane is heated to 70-95 C in order to achieve an optimal balance between the quantity of analyte crossing the membrane and the quantity of water crossing the membrane. As the temperature is decreased below 70 C, the sensitivity drops sharply, and above 95 C, the separation efficiency of the membrane is compromised due to the interference from the large amount of water permeating the membrane. The DIMP heats the sample stream before it is introduced to the ion source while in the external configuration the whole MIMS system is heated by being mounted in a small oven.

The choice between the use of the DIMP and external configuration is one that depends on the particular application. The DIMP has the advantage of having the membrane placed directly into the ion source, thereby giving it an efficient mass transfer from the solution to the gas phase, but suffers in other areas. The membrane area using the DIMP is limited by the space available within the mass spectrometer ion source. In addition, there is a concern for the ruggedness of the system. Under typical laboratory conditions, the DIMP is placed within the mass spectrometer source when needed and removed when not. One does not wish to introduce foreign objects into an ion source under plant conditions however, due to the introduction of contaminants from the environment. Once the DIMP has been placed in the instrument it is commonly left untouched until required to be removed.

The external jet separator (or momentum separator) configuration has the further advantage of having a relatively unlimited and easily accessible space for new membranes. Note that in this configuration the jet separator has two purposes: the first is an enrichment stage for the removal of the helium carrier gas from the gas phase analyte stream. Fortunately, the process of the analyte transfer across the membrane and the jet separator, the efficiency of analyte transport is very high. In the comparison of an identical sheet Silastic membrane used within a DIMP configuration versus an external configuration, the DIMP typically has lower limits of detection (LLD) which are an order to magnitude lower than the external configuration. However, this limited LLD for the external configuration can be compensated for by simply increasing the membrane size.

The second purpose of the jet separator is to provides a type of safety

barrier to any leaks or impurities introduced through the membrane connection. This last point was an important one in the design of this system due to the fear of the rupture of the membrane, or some particle being sucked into the manifold through the use of the DIMP. While neither of these is a common occurrence, they should be avoided at all cost due to their potential impact on the instrument. *One can think of the comparison of the two membrane introduction source as being analogous to a comparison of two methods for introducing a drug into the human heart. It is feasible to inject a drug in the close proximity of the heart, but it is preferred to do so through a person's arm. In the case of the DIMP, one is injecting the sample into the heart of the instrument, the source. The dangers inherent in this have been reduced by using an external configuration using a jet separator.*

C. Choice of Membrane:

There are several types of established membranes that may be used for MIMS. These include sheet and capillary membranes that may be used with either membrane interface (direct insertion probe or external membrane/jet separator). Vulcanized nonreinforced sheeting from Silastic© is the workhorse of MIMS research, with highly reproducible results from laboratory to laboratory. Silastic membranes present a barrier to sample streams based on hydrophobicity.

Microporous membranes are also well established and consist of polytetrafluoroethylene (PTFE) or polypropylene, typically with a 1 micron pore size. These microporous membranes present semipermeable barriers to sample streams, based on steric separation. Microporous membranes allow more rapid sample introduction but it is accompanied by a larger quantity of solvent. The advantage of these membranes is their rapid elution times; the high solvent flux can be used as an ionizing reagent. Due to their high solvent flux, microporous membranes are typically operated at a lower temperature than Silastic membranes.

In order to develop a system that would be compatible with the fermentation system and considering the high concentration of ethanol in the sample stream, the sheet Silastic membrane was used in the external jet separator configuration. By using an external membrane of small area one could decrease analyte permeation into the source so that dimeric and higher oligomeric ions were not observed. A short ionization period was also used to limit that analyte flow to allow this very sensitive instrument to be used to monitor percent concentrations in the sample stream. In addition, the quantity of water that permeated through the sheet

membrane, at the operating temperature of 91 C, was great enough to be used as a water chemical ionization reagent gas.

Experimental:

A. The membrane used was a vulcanized, nonreinforced sheeting from Dow Corning. This was used on a membrane interface previously developed for affinity membrane work (see figure 2)³. The jet separator originated from a Finnigan 4500 series GC/MS, and was originally built for Finnigan by SGE. The helium carrier gas used was 99.99% pure and was transferred to the instrument via 50 feet of polyethylene 1/4" tubing due to the remote location of the helium tank. The helium carrier gas was filtered through a gas filter purchased from Alltech.

The membrane was operated at 91 C, the jet separator at 110 C, the transfer line at 110 C and the manifold at 50 C. The membrane, jet separator and transfer line were differentially heated and controlled with Omega CN 76020 units. The membrane and jet separator were placed in an oven measuring 18"x12"x4" and insulated with 1" thick insulation purchased from Zircar. The transfer line was the original transfer line that accompanied the Finnigan ITS-40 GC/MS, and was heated with a cartridge heater. T-type thermocouples were used in these three aspects of the apparatus. The heating and control of the manifold band heater was performed through the software of the instrument control station.

The instrument used was a Finnigan ITS-40 Ion Trap Mass Spectrometer, originally built for use as an GC/MS with a solids inlet port (see figure 3, of instrument system). Any use of the DIMP would have been performed via the solids inlet port. The GC was removed and replaced with a simple oven and membrane interface. The system was controlled with a Compaq 386 computer with the standard Finnigan Magnum software. The vacuum system consisted of a Balzers TPH-050 turbomolecular pump backed a General Electric rotary vane pump. The jet separator was differentially pumped from the vacuum manifold by an additional rotary vane pump. Both rotary vane pumps were vented to atmosphere via 50 foot of flexible tubing purchased from McMaster Carr and were operated with molecular sieves to prevent backstreaming. Operating pressures were 100 microtorr in the jet separator and 4 millitorr in the manifold, both pressures measured at their respective rotary vane pumps. The manifold pressure was measured with a Granville-Phillips Convector Gauge, for pressures between 1 millitorr and 1 atmosphere, and the jet separator pressure was measured with a thermocouple gauge purchased from Kurt Lesker.

B. Flow Injection System

The flow injection system used was designed around a Waters Filter Acquisition Module (FAM) which operated with a Rheodyne 7000 injector valve with a 20 microliter sample loop. Sample and standard streams were alternately switched into the injector valve via a three way valve. The FAM was controlled by a custom-built FIA controller, built by Mark Carlson and Mark Hayward in 1989.⁴ This unit can be controlled by a personal computer via an IEEE-488 interface card or manually by an automated cycling switch. This FIA unit can be used for automated feedback control studies, but requires an additional computer beyond the one required to control the instrument. These experiments were performed using the simple cycling circuit placed in the system which loaded the sample for 50 seconds and injected it for two minutes and ten seconds, followed by the same procedure for the standard solution. All sample lines were 1/16" with 0.030" ID and used a peristaltic pump flowing at 1.5 mL/min. Three types of transfer lines were used, PTFE (Upchurch Scientific), 316 stainless steel tubing and Masterflex tubing for the peristaltic pump and connections between these other types of tubing.

C. Filtering System

The interface to the reactor was created by feeding the sample broth through a 1" flexible hose to the tangential filter via a 1" peristaltic pump (see figure 4). Once filtered the sample broth was returned to the reactor. Sample flow rates to the filter were on the order to 5-10 gallons per minute (GPM). The Filter consisted of a 1 inch O.D. sintered stainless steel tube with a 1/16" wall thickness, 48" long, purchased from Newmet Krebsoge. Two 316 SS cuffs (short pieces of tubing) were welded to either end of the sintered stainless steel tubing and placed within a 1 1/4" OD 316 SS tube with a wall thickness of 1/16". The 1" sintered stainless steel tube was six inches longer on either side of the 1 1/4" tubing. The sintered stainless steel tube was secured within the 1 1/4" tubing by a series of two Swagelock fittings. One was a 1 1/4"-1 1/4" Swagelock union to a 1 1/4"- 1" reducing union. The 1 1/4" end of the reducing union was swaged into the 1 1/4" union while the 1" end of the reducing union was swaged to the cuff of the sintered stainless steel filter. This provided a seal between the larger tube, the housing of the filter, and the inner tube, which served as the filter. Initially, PTFE ferrules were used with the filter in order to remove the filter if necessary, but they were later replaced with stainless steel when they failed under the temperature of steam sterilization. A 1/4" tube was silver soldered to the housing, 1 1/4" tube, to

collect sample permeate. A regulating valve was placed at the end of the 1/4" line in order to seal system during sterilization and isolate the filter in the case of leaks. Beyond the regulating valve three lines were placed, one for a 3/8" recirculation of the filtered broth back to the reactor, a 1/4" input for sterilization with ethanol prior to use, and a 1/16" PTFE line that led to the instrument. This was termed the crown of the filter. The ethanol was used to sterilize the crown to avoid contamination from the crown as the permeate was recirculated back to the reactor.

There were four peristaltic pumps used with the filtering scheme. The first one, used for circulating the broth, was the 1" peristaltic pump previously discussed. The second was a Masterflex pump to transfer the permeate from the filtering system to a deairating container, a small vial, followed by a Gilson pump which empowered the FIA system, pumping sample from the bottom of the deairating container to the Rheodyne injector. A fourth pump was used simply to increase the sampling frequency of the filtering system due to the relatively large volume of permeate between the sintered stainless steel filter and the 1 1/4" housing as compared to the 3 ml/min flow rate to the deairating container. This additional pump was a Masterflex pump equipped to pump a 3/8" thick walled Viton tubing at a flow rate between 100-500 mL/min. The 100-500 mL/min was reintroduced to the one inch flexible hose (to the reactor) through a 3/8" Nupro check valve and a Triclamp 1" to 1/2" reducing tee. This additional Masterflex pump insured that the sample that reached the FAM was representative of the sample stream through the filter and not simply a slow sampling rate of the permeate contained in the filter housing.

D. Chemicals

Standards for ethanol and acetic acid were provided by the National Renewable Energy Laboratory (NREL). Denaturated ethanol was used to create standards for quantification. HPLC grade ethanol (Aldrich) was used in some experiments with no discernable difference in the performance of the MIMS system. The mobile phase for the MIMS system was DI water, stored in a 10 Liter Carboy© container with a rubber stopper. On-line pH changes were made by addition of HCl (Aldrich) using solutions of 1% in water.

Data Analysis:

Initial experiments with the sample broth were performed off-line with a manually filtered broth using a 0.2 micron disposable filter. Ions noted were m/z

45 and 47 {(ethanol-H)⁺; and (ethanol+H)⁺, respectively}; as well as m/z 61 and 89. Standards of ethanol, between 1 and 15 percent in water, were analyzed for response and the results plotted for linearity using the external sheet membrane configuration. The response of the membrane interface was found to be linear from 1 to 10 percent. These findings were consistent with previous studies by Dejarme and Wong.⁵ Once the dynamic range of the calibration was established, 20 microliters of sample was injected, eluted for two minutes and ten seconds, and repeated for a standard injection. Due to the fact that the response of the system was linear between 1 and 10 percent, one could mix a standard that was between 1 and 10 percent and directly compare the standard response to that of the sample. Assuming the sample response was within the dynamic range of the standard calibration, a simple proportion could be used to determine the concentration of the sample.

A. Example of Calculation (proportion):

$$\frac{5\% \text{ Ethanol}}{10000 \text{ counts}} = \frac{\text{unknown concentration}}{5000 \text{ counts}}$$

The unknown concentration can then be established as 2.5% ethanol.

This calculation can only be performed once the linear dynamic range has been established.

Figure 5 is a typical selected ion chromatogram recorded using MIMS, in which one can visually note the difference between the larger standard and the smaller sample peak. The standard that was used was a 5% solution of ethanol in water. On-line pH change was possible with the use of the custom-built FIA system, by the mixing of two sample streams, one consisting of either the sample or standard and the other of a solution of HCl in water. Previous studies have indicated that the response of organic acids, and to a lesser extent, ethanol, can be increased by an adjustment in pH. The response may have been increased due to the change in pH, but the overall response was subsequently decreased by 50% due to the mixing of the two sample streams. As a result, it was decided to sample at the fermentation pH of 5.0 while the standard solution was sampled with no on-line pH adjustment.

B. Comparison of MIMS to HPLC Data:

Comparison of three Typical Data points of MIMS to One of HPLC; Using HPLC as Reference

<u>Date:</u>	<i>Ethanol Concentration (%)</i>		
	<u>HPLC</u>	<u>MIMS</u>	<u>% Error</u>
Day 1			<u>MIMS</u>
	3.01	2.94	2.33
	3.00	0.33	
	2.98	0.99	

The intensity of the both peaks was calculated manually through the use of the Magnum software, using background subtraction. In this process, the intensity of the analyte peak is subtracted from the background, measured directly before the analyte elutes. Peak height is measured by the software, not area as is often the case in chromatography.

Discussion:

Figure 6 displays a typical MIMS selected ion chromatogram for m/z 45 + m/z 47, for an alternating sample and standard over a period of three and one half hours. Problems with instrumental drift were addressed by the rapid cycling of the sample and standard solutions, though drift did not seem to play a major role in the experiment, as may be seen in a sample chromatogram of **figure 6**. Data was collected for four day continuously during working hours. There was very little change in the concentration of the sample broth due to the fact that the fermentation had reached a steady state, and the frequency of sampling was much greater than needed to accommodate the changes in concentration. Deviations of ion abundances of up to 5% can be attributed to the FIA system. Commercial systems are available today which could reduce this error. **Figure 7** displays a typical mass spectrum from the sample broth while **figure 8** displays a mass spectrum from a standard injection. Note the clean sample spectrum from the MIMS system. This spectrum was scanned from mass 30 to 300 with no peaks noted above 100 m/z .

Ions present at m/z 61 and 89 appear to originate from acetic acid and lactic acid. The ability of MIMS to monitor these two types of organic acid would greatly enhance the on-line data provided by the system. Acetic and lactic acid are two compounds which are indicative of contamination or other problems within

the fermentation reactor. On-line data from the mass spectrometer could greatly aid in detecting the onset of contamination in an ethanol fermentation.

Initial experiments were performed in order to monitor semivolatile compounds such as 2-furaldehyde. These experiments simply consisted of removing the mobile phase, water, from the membrane and heating the membrane to 150 C. This experiment appeared to be successful in that the desorption of some semivolatiles into the gas phase, including ions of m/z 97 which may be the protonated form of 2-furfuraldehyde. Further investigation must be performed in order to identify the source of these ions as well as the feasibility for such a technique in online experiments.

Recall that MIMS is designed to be an on-line monitoring system in which an operator can rapidly gauge the status a process, in this case a fermentation. With comparison to HPLC which requires many hours to prepare and run the standards alone, MIMS can quantitate ethanol and many other relevant compounds with two to three minute cycles, once the sample is delivered to the instrument. This delivery time period includes the amount of time required to move the sample through the filtering device to the deairating container, to the FIA system, on to the membrane and elute into the mass spectrometer, and has been estimated to be on the order of fourteen minutes. *MIMS is not designed to compete with HPLC for the number of compound that may be quantitated, but rather to supplement HPLC data with smaller data sets of desired information that may be used as indicators within a process. In this case, it is desirable to monitor ethanol, and in the future, acetic acid and lactic acid will be monitored. These compounds are currently used as indicators for problems such as contamination. MIMS can provide a rapid method of testing for such contamination.*

There was a minimum amount of maintenance daily during these online experiments. The only maintenance required with the MIMS system was the need for a daily or diurnal flushing of the FIA lines with a 10-20 % solution of ethanol or in order to remove any particles that may have begun to build up within the sample lines. Specifically, it was found that the Masterflex tubing in for the FIA/FAM system had a high tendency to attract particulates in the solution.

Further Experiments

Further experiments with MIMS will include the quantitation of lactic and acetic acids, furfural and, if possible, polyols in sample broths. Thermal

desorption experiments such as those previously described could be used in an on-line manner in order to thermally desorb compounds from the membrane. Such an experiment would be very useful in conjunction with chemical ionization, to stabilize the molecular ion of higher molecular species. Besides additional experiments with on-line standard addition, we will explore analyte addition to the analyte stream as a method of quantitation.

Microporous membranes will be investigated for this type of monitoring to supplement experiments performed with Silastic membranes. Microporous membranes composed of PTFE, or polypropylene, are very rugged and allow for the monitoring of compounds that typically do not desorb from the Silastic sheeting. Recall that microporous membranes present a barrier to a sample stream based more on sterics than hydrophobicity (as is the case with the Silastic sheeting). Compounds of a more semivolatile nature could potentially be monitored with such a system.

The use of MIMS with multiple sampling ports is also desirable, especially in a plant environment with many reactors. Such an experiment could be implemented in future. Two filtering systems have been built and will be used in future experiments.

Conclusion

The ultimate goal of these experiments was to create and test a system that can provide accurate on-line information to gauge the status of a given fermentation process. At the moment, such information may be obtained from monitoring ethanol, lactic acid and acetic acid. MIMS proved to be a system that could monitor these compounds, in addition to potentially monitoring others such as 2-furaldehyde.

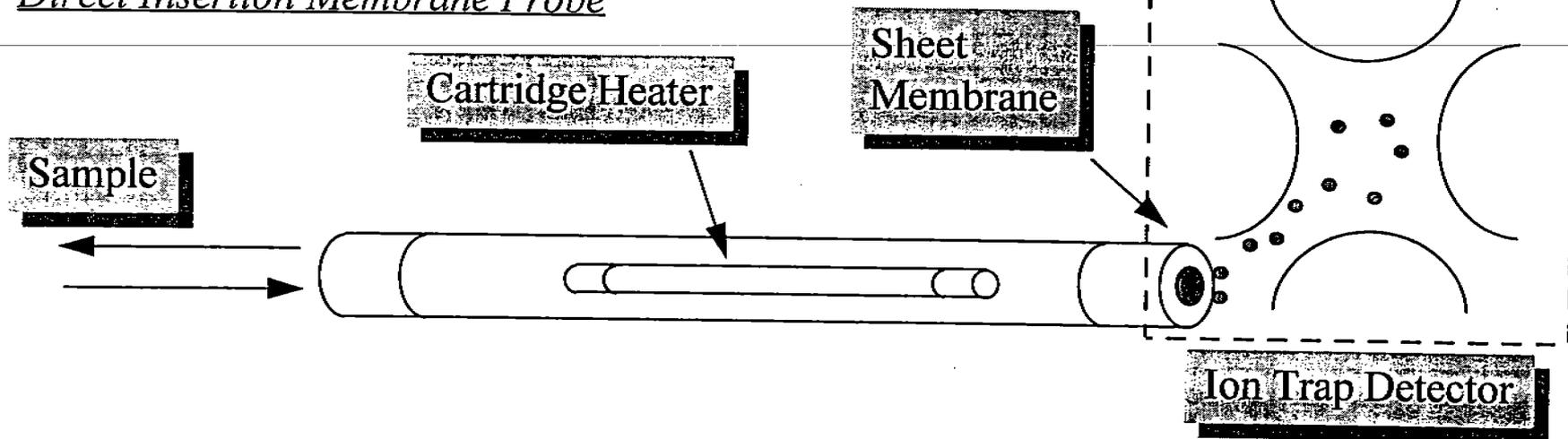
These experiments have established that MIMS can:

- a) Withstand operating conditions within a pilot plant,*
- b) Accurately determine the concentration of ethanol online,*
- c) Monitor acetic acid and lactic acid,*
- d) Significantly increase data about the status of a large fermentation process, and*
- e) On a plant scale, the value of such data would be far more valuable than the initial cost of such an instrument.*

1. P.S. Wong, R.G. Cooks, M.E. Cisper, P.H. Hemberger. *Environmental Science and Technology*. Vol 29, 1995. pp. 215A-218A.
2. B.K. Teeter, L.E. Dejarme, T.K. Choudhury, R.G. Cooks. *Talanta*. Vol 41, No. 8, 1994. pp. 1237-1245.
3. C. Xu, J.S. Patrick, R.G. Cooks. *Analytical Chemistry*. Vol 34. 1995. pp. 724-728.
4. Mark Hayward, *Ph.D. Thesis, Purdue University*. 1989.
5. P.S. Wong, L.E. Dejarme. Unpublished data.

Figure 1: Membrane Interfaces

Direct Insertion Membrane Probe



External Membrane/Jet Separator Interface

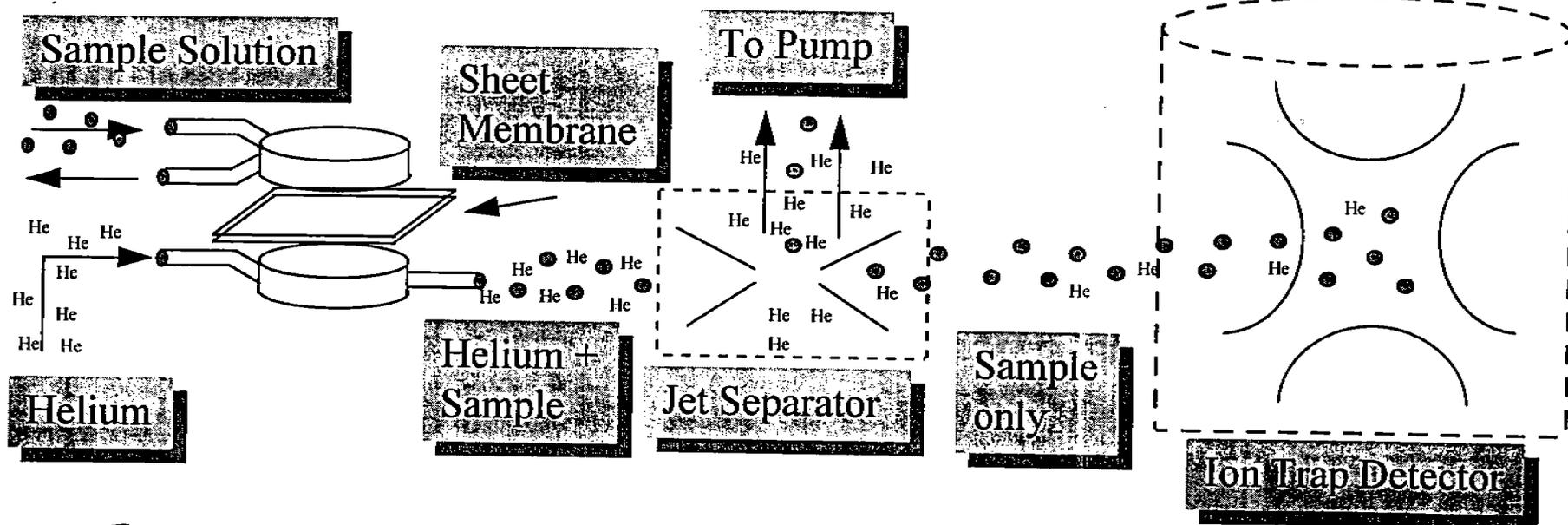
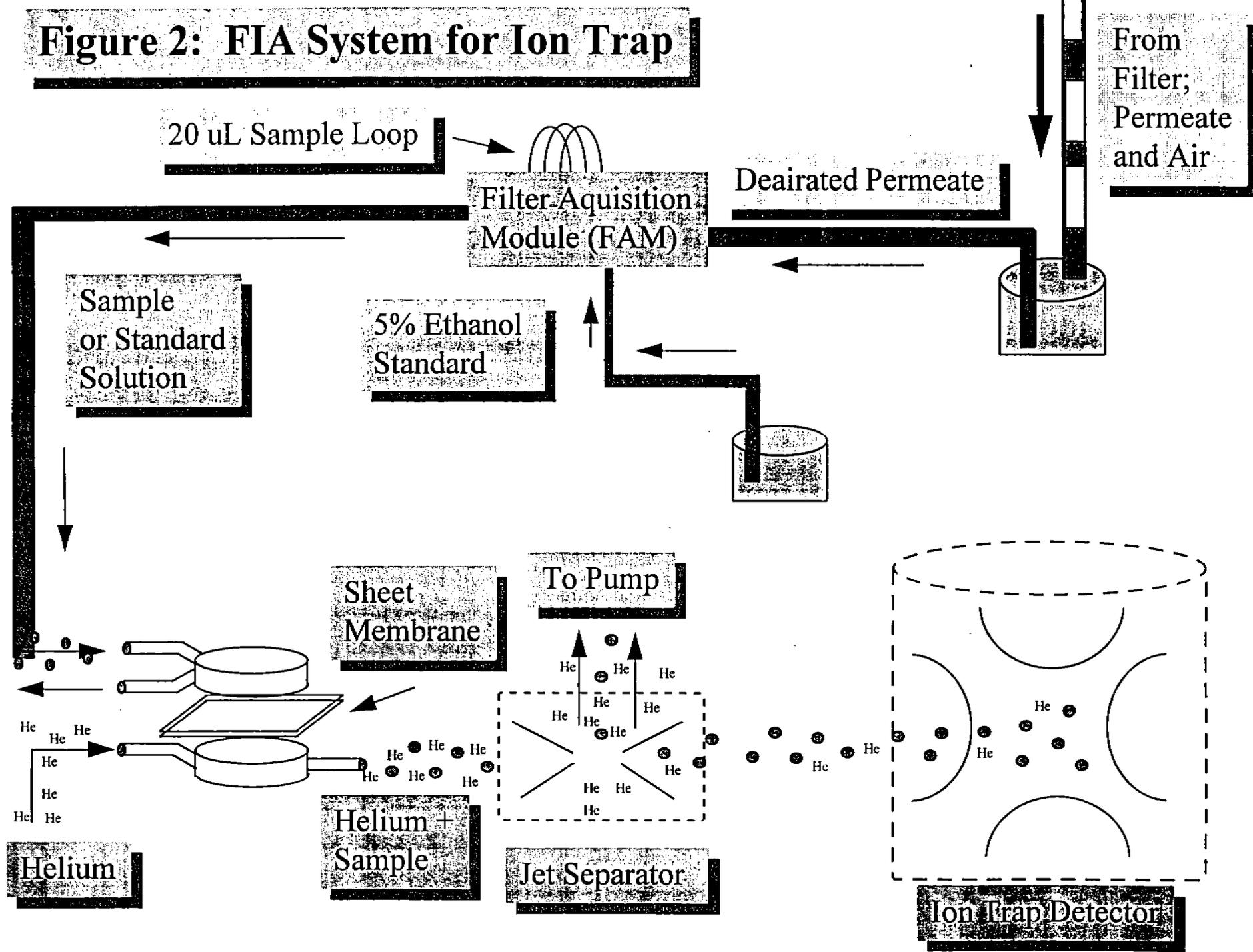


Figure 2: FIA System for Ion Trap



**Figure 3: Instrument Platform
Protected with Plexiglas and Steel Plate**

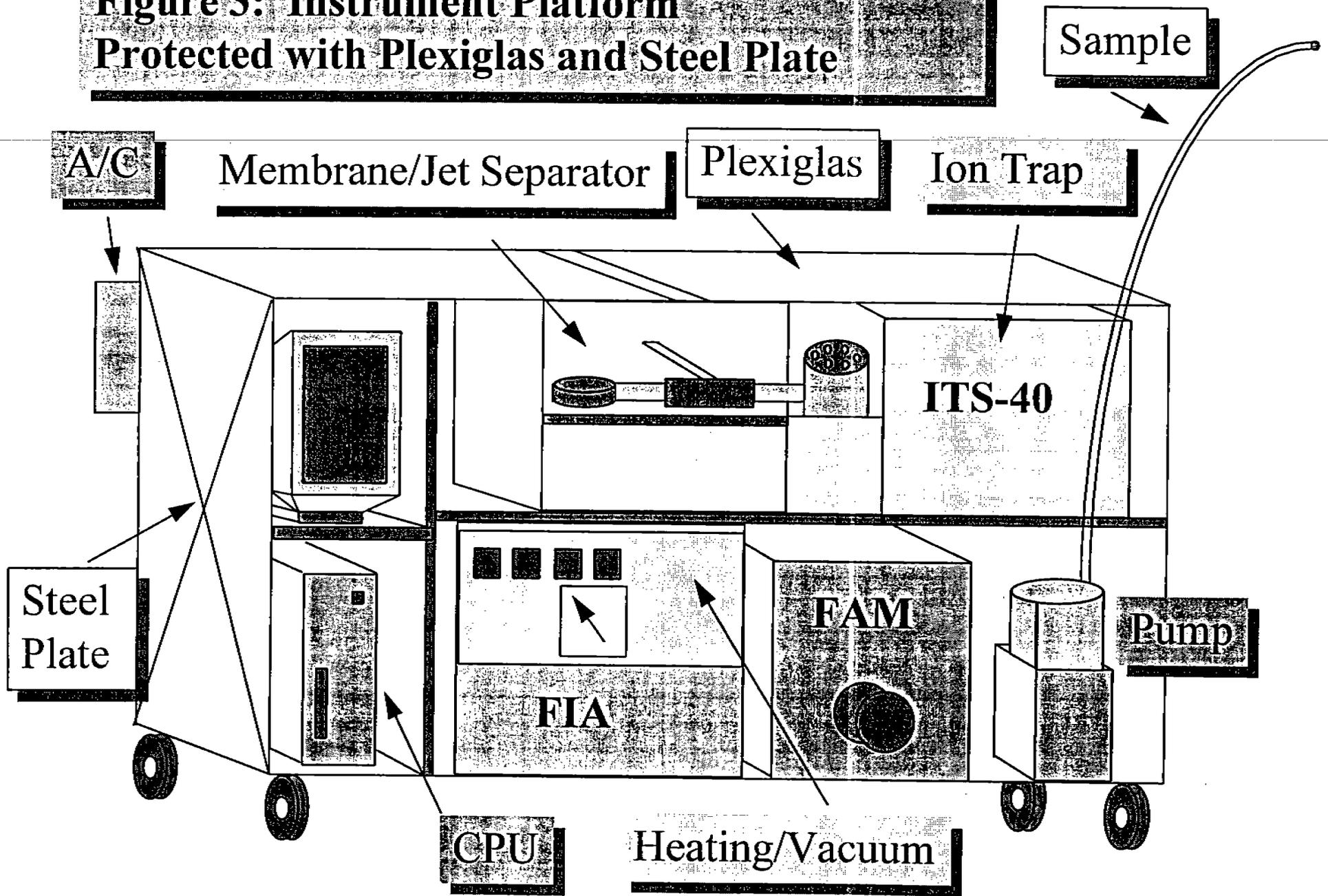


Figure 4: Filtering System from Reactor

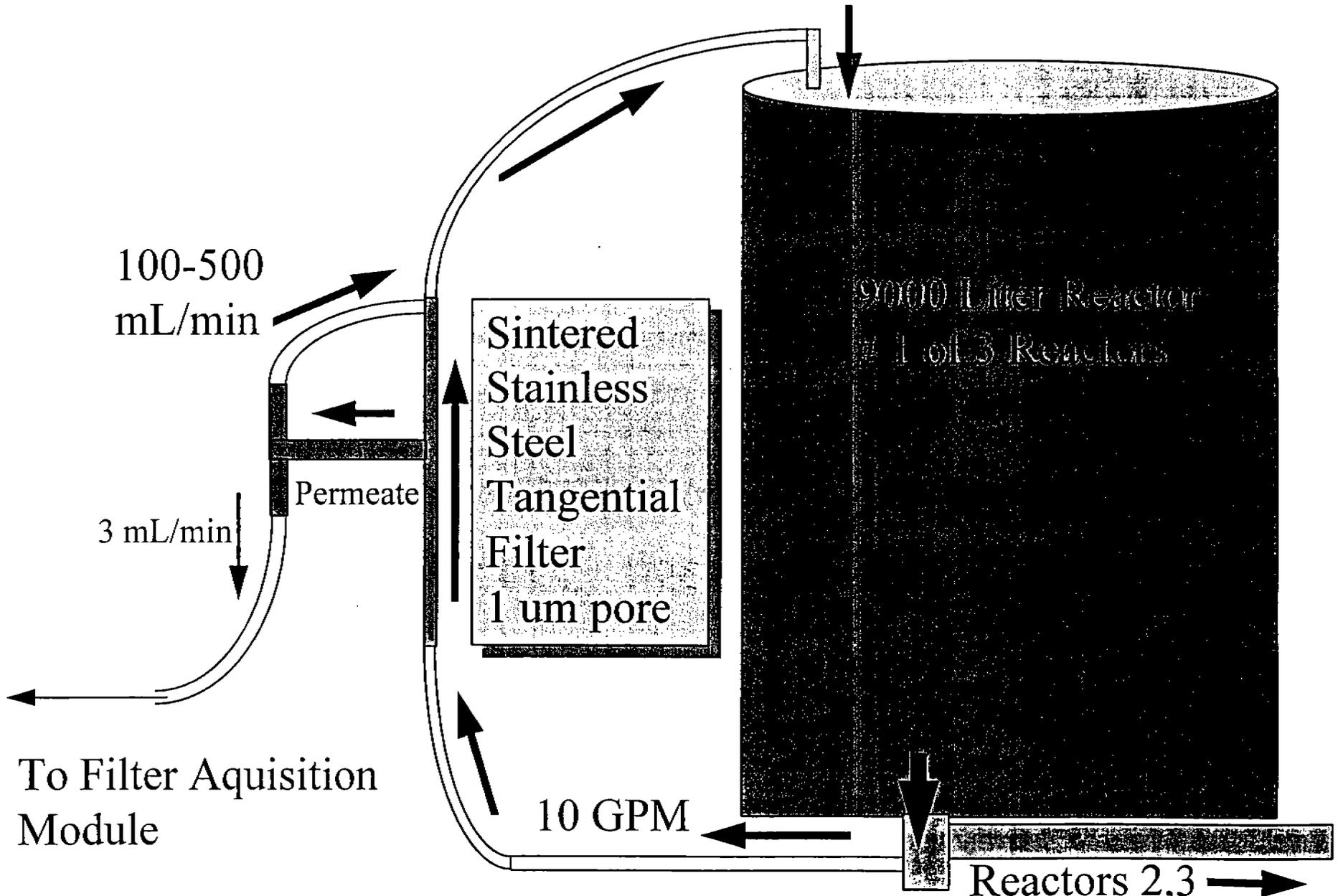


Figure 5: Typical Chromatogram in MIMS

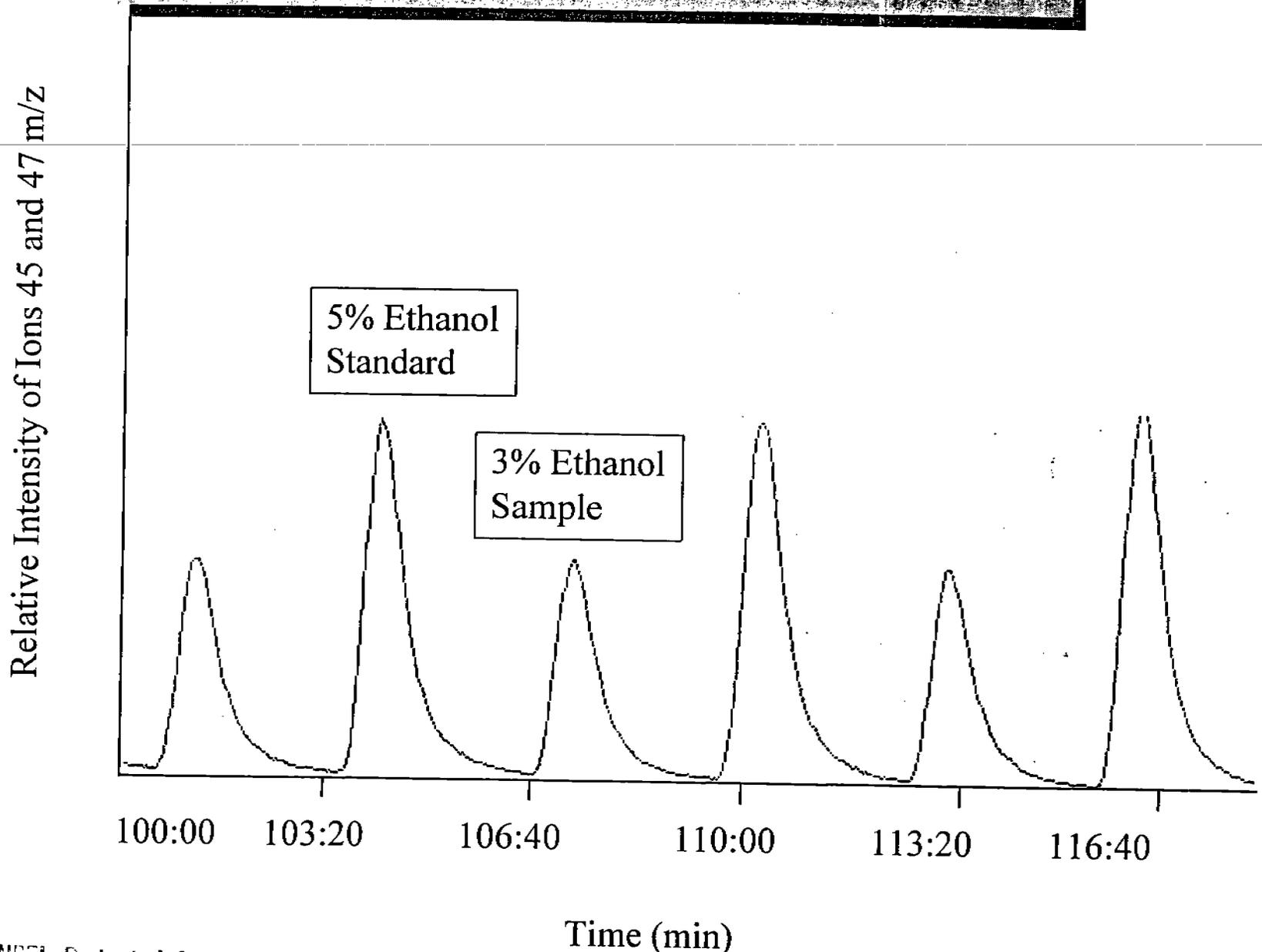


Figure 6: Online Monitoring Over 3.5 Hours

Relative Intensity of Ions 45 and 47 m/z

Note: Taller peaks are 5% standard;
smaller peaks are sample concentrations of ethanol

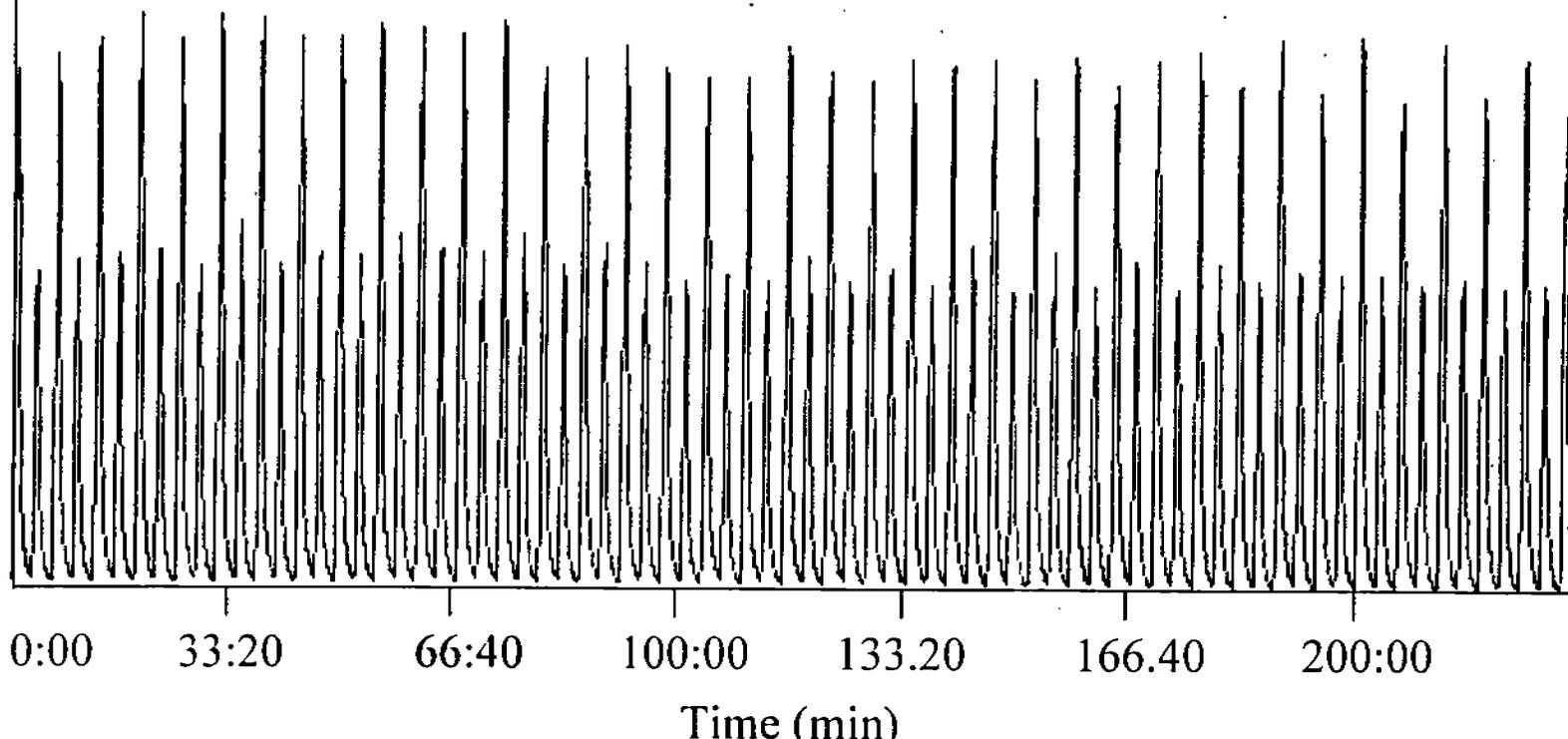


Figure 7: Sample Mass Spectrum

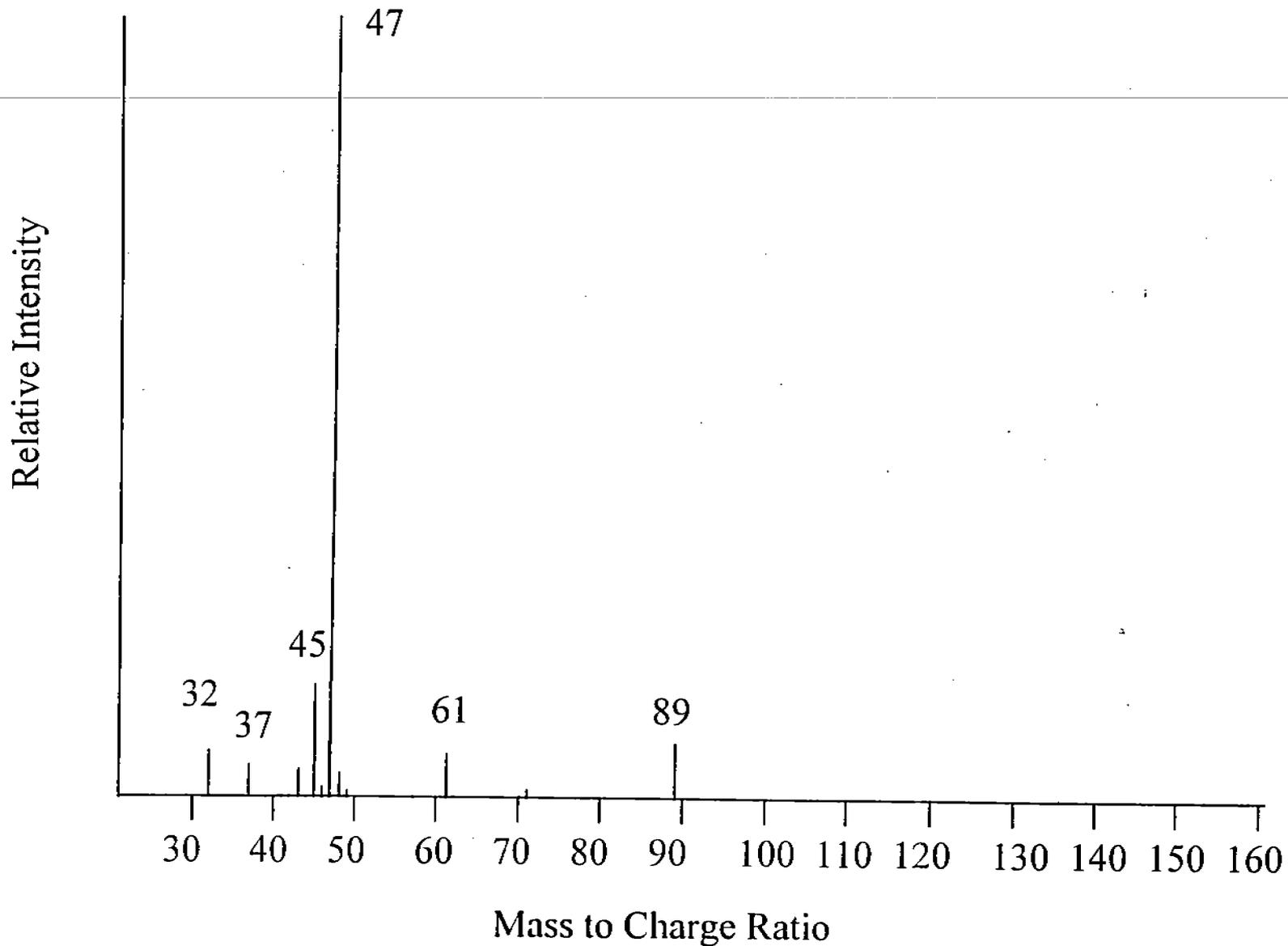
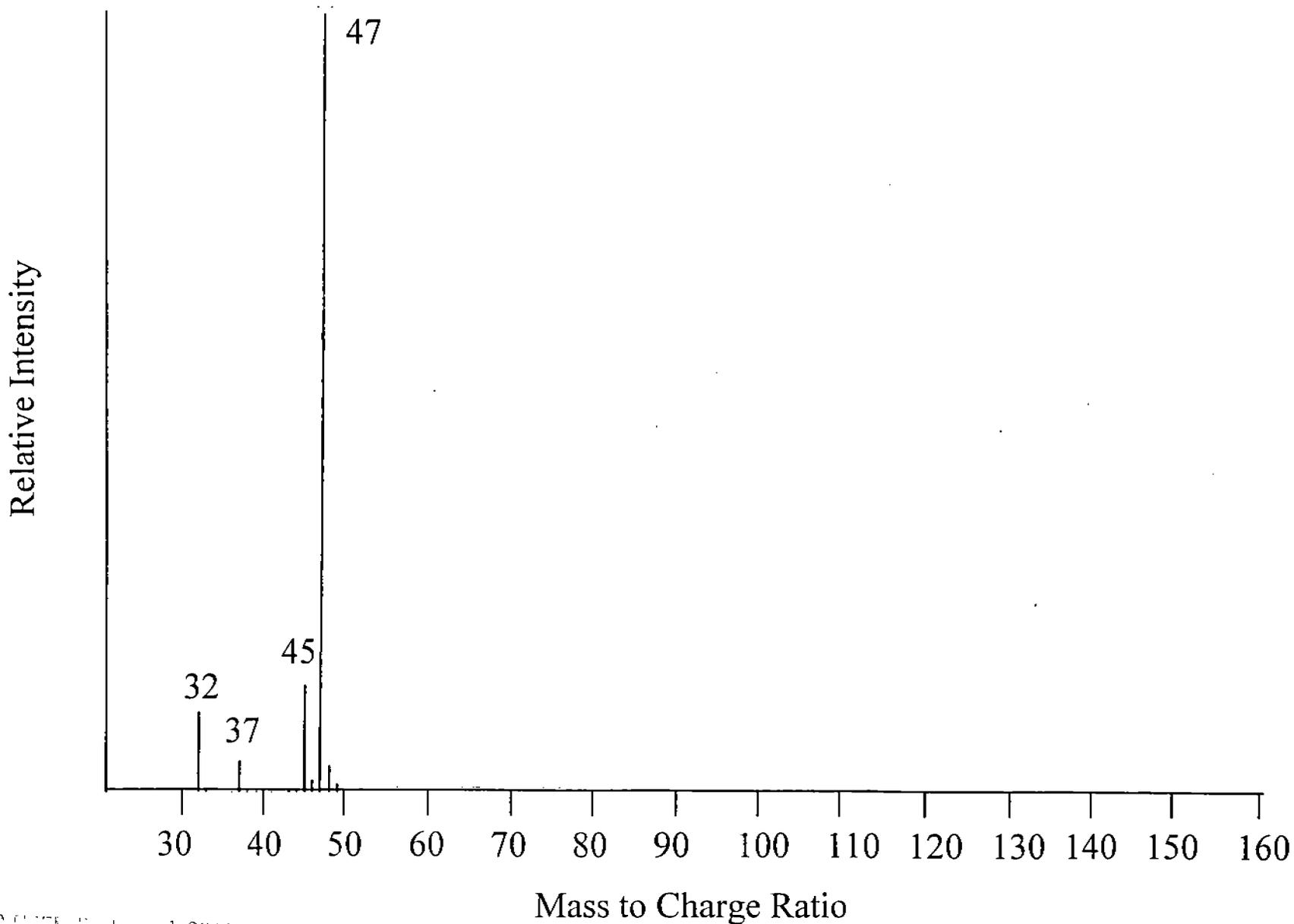


Figure 8: 5% Ethanol Standard Mass Spectrum



Calculations

Pretreatment Conversions (Based on raw feedstock composition)

Feedstock Conversions	
Fraction Starch Hydrolyzed	99.09%
Fraction Cellulose Hydrolyzed	16.73%
Fraction Galactan Hydrolyzed	92.59%
Fraction Xylan Hydrolyzed	93.89%
Fraction Arabinan Hydrolyzed	91.75%

Sugar Yields	
Starch to Total Soluble Glucose	99.07%
Cellulose to Total Soluble Glucose	14.50%
Galactan to Total Soluble Galactose	61.47%
Galactan to Monomeric Galactose	57.07%
Cellagalulose to Total Soluble Glucose	24.74%
Glucan to Glucose	72.88%
Xylan to Total Soluble Xylose	85.00%
Xylan to Monomeric Xylose	67.21%
Arabinan to Total Soluble Arabinose	76.09%
Arabinan to Monomeric Arabinose	62.27%
C6 to Sugars	71.98%
C5 to Sugars	81.50%

Other Product Yields	
Starch to HMF	0.93%
Xylan to Furfural	3.95%
Glucose to HMF	0.61%
Glucan to HMF	0.64%
Xylose to Furfural	2.84%
Acetate to Acetic Acid	63.66%
C6 to HMF	0.59%
C5 to Furfural	2.40%

Unconverted	
Glucan	26.41%
Galactan	7.41%
Xylan	6.11%
Arabinan	8.25%
C6	24.90%
C5	6.95%

Mass Balance Closure

Glucan	99.94%
Galactan	68.88%
Xylan	95.06%
Arabinan	84.34%
C6	97.47%
C5	90.85%

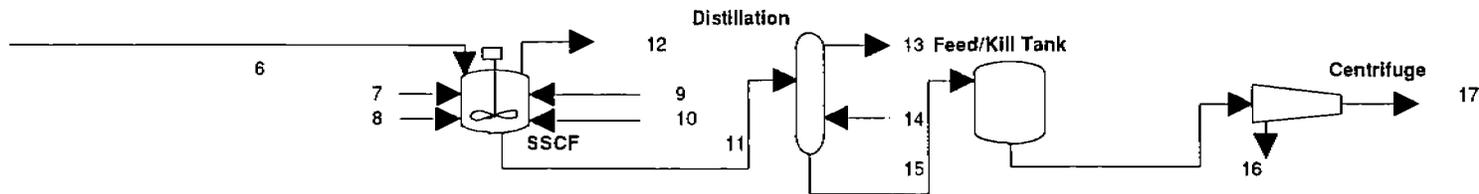
Concentrations (wt.% or g/L)	6 Hydrolyzate	7 Enzyme	8 CSL	9 Sterile Water	10 Caustic	11 Beer	12 Vent Gas	13 Distillate	14 Steam	15 Bottoms	16 Centrate	17 Cake
Water												
Starch						1.65%				1.52%		
Cellulose						32.28%				30.61%		
Galactan						0.97%				1.00%		
Xylan						1.91%				3.74%		
Arabinan						2.72%				2.57%		
Lignin						30.91%				33.37%		
Acid Soluble Lignin						8.12%				5.75%		
Acetate						1.00%				1.00%		
Ash						2.05%				3.14%		
Protein						28.24%				26.72%		
Feedstock Solubles												
Glucose (Oligomeric)						17.64				14.8		
Glucose (Monomeric)						0.55				0.52		
Galactose (Oligomeric)						1.52				1.8		
Galactose (Monomeric)						4.38				3.02		
Xylose (Oligomeric)						8.89				7.49		
Xylose (Monomeric)						21.84				16.9		
Arabinose (Oligomeric)						4.7				3.18		
Arabinose (Monomeric)						17.47				14.45		
Sulfuric Acid												
Lactic Acid						2.64				1.96		
Acetic Acid						3.96				4.41		
Furfural						0				0		
HMF						0				0		
CSL												
Cellulase												
Glucoamylase												
Sodium Hydroxide					50.00%							
Cells						1.49				1.49		
Glycerol						3.93				3.4		
Xylitol						2.51				2.74		
Ethanol						37.37		587.7		2.63		
Carbon Dioxide												
Density (g/cc)		1.1	1.02	1	1.5	1.072		0.84	1.00	1.082	1.052	1.086
Insoluble Solids (%)						4.40%				8.12%	0.17%	14.14%

Flowrates (kg/h)	Vent Stream Data			Composition (mole %)			Centrifugation Data		
	Flowrates (mole/m)	Ethanol	CO2						
Cellulase (7)	0.25			1st Fermenter	0.091	1.08%	81.40%	Insoluble Solids Recovery (Cake)	65.00%
Glucoamylase (7)	0.042			2nd Fermenter	2.51	1.24%	14.35%		
CSL (8)	5.52	CSL Dilution	18.00%	3rd Fermenter	5	1.02%	2.47%	Fractional Liquid Recovery (Cake)	35.00%
Sterile Water (9)	25.09			4th Fermenter	0	0.00%	0.00%		
Distillate (13)	5.77								
Steam (14)	15.68								
Beer (11)	107.67	Bottoms	117.58	Total (moles/m)	7.60	0.08	0.56		

Calculations

SSCF Conversions (based on pretreated feed composition)

Pretreated Feedstock Conversions	
Fraction Starch Hydrolyzed	-16.88%
Fraction Cellulose Hydrolyzed	44.27%
Fraction Galactan Hydrolyzed	32.50%
Fraction Xylan Hydrolyzed	66.55%
Fraction Arabinan Hydrolyzed	45.44%
Glucose Product Yields	
Total Soluble Glucose to Cell Mass	3.08%
Total Soluble C6 to Cell Mass	2.89%
Glucan to Cell Mass	2.62%
C6 to Cell Mass	2.47%
Glucose to Glycerol	7.97%
Total Soluble C6 to Glycerol	7.49%
Glucan to Glycerol	6.76%
C6 to Glycerol	6.38%
Xylose Product Yields	
Xylose (monomeric) to Xylitol	7.54%
Unconverted	
C6	34.71%
C5	77.45%
Total Soluble Xylose	73.69%
Ethanol Yields	
Total Soluble C6 to Ethanol	67.10%
Xylose to Ethanol	26.22%
Total Process Yield	46.92%
Total Metabolic Yield	84.57%



Streams (kg/h)	6	7	8	9	10	11	12	13	14	15	16	17
	Hydrolyzate	Enzyme	CSL	Sterile Water	Caustic	Beer	Vent Gas	Distillate	Steam	Bottoms	Centrate	Cake
Water	45.50		4.92	93.75	0.87	152.42		7.59	30.44	157.54	127.61	29.93
Starch	0.09					0.09				0.07	0.00	0.07
Cellulose	3.15					1.85				1.68	0.10	1.57
Galactan	0.01					0.03				0.03	0.00	0.03
Xylan	0.24					0.13				0.12	0.01	0.12
Arabinan	0.15					0.07				0.04	0.00	0.03
Lignin	1.27					1.80				1.93	0.12	1.81
Acid Soluble Lignin	0.47					0.46				0.36	0.29	0.07
Acetate	0.07					0.06				0.05	0.00	0.05
Ash	0.02					0.04				0.11	0.01	0.11
Protein	1.48					1.89				1.79	0.11	1.68
Feedstock Solubles	3.27					3.27				3.27	2.65	0.62
Glucose (Oligomeric)	4.09					1.73				1.58	1.28	0.30
Glucose (Monomeric)	4.80					0.04				0.06	0.05	0.01
Galactose (Oligomeric)	0.36					0.27				0.08	0.06	0.01
Galactose (Monomeric)	0.45					0.21				0.20	0.16	0.04
Xylose (Oligomeric)	2.42					0.99				1.03	0.83	0.20
Xylose (Monomeric)	2.42					0.49				0.46	0.38	0.09
Arabinose (Oligomeric)	1.00					0.73				0.31	0.25	0.06
Arabinose (Monomeric)	1.88					0.96				0.16	0.13	0.03
Sulfuric Acid	1.07											
Lactic Acid	0.16					0.54				1.57	1.27	0.30
Acetic Acid	0.35					0.61				1.27	1.03	0.24
Furfural	0.03					0.00				0.00	0.00	0.00
HMF	0.02					0.00				0.00	0.00	0.00
CSL			1.08			1.08				1.08	0.57	0.51
Cellulase		0.25				0.25				0.25	0.20	0.05
Glucosylase		0.04				0.04				0.04	0.03	0.01
Sodium Hydroxide					0.87							
Cells						0.23				0.25	0.02	0.24
Glycerol						0.56				0.55	0.44	0.10
Xylitol						0.64				0.68	0.55	0.13
Ethanol						5.05	0.13	4.86		0.00	0.00	0.00
Carbon Dioxide							1.61					
Density (g/cc)	1.15	1.10	1.02	1.00	1.50	1.05		0.90	1.00	1.06	1.05	1.08
Total Solids (%)	39.10%	100.00%	9.00%	0.00%	50.00%	10.38%				9.42%	7.42%	21.40%
Insoluble Solids (%)	8.71%					3.32%				2.80%	0.07%	13.60%
Total (kg/h)	74.76	0.29	6.00	93.75	1.75	176.55	1.74	12.45	30.44	176.55	138.14	38.40
Total (L/h)	65.01	0.27	5.88	93.75	1.16	167.50		13.82	30.44	166.55	131.31	35.56

Calculations

SSCF Conversions (based on pretreated feed composition)

Pretreated Feedstock Conversions	
Fraction Starch Hydrolyzed	1.32%
Fraction Cellulose Hydrolyzed	41.25%
Fraction Galactan Hydrolyzed	-294.68%
Fraction Xylan Hydrolyzed	45.61%
Fraction Arabinan Hydrolyzed	54.40%
Glucose Product Yields	
Total Soluble Glucose to Cell Mass	4.39%
Total Soluble C6 to Cell Mass	4.08%
Glucan to Cell Mass	3.64%
C6 to Cell Mass	3.41%
Glucose to Glycerol	10.65%
Total Soluble C6 to Glycerol	9.88%
Glucan to Glycerol	8.82%
C6 to Glycerol	8.27%
Xylose Product Yields	
Xylose (monmeric) to Xylitol	26.33%
Unconverted	
C6	33.33%
C5	41.77%
Total Soluble Xylose	30.75%
Ethanol Yields	
Total Soluble C6 to Ethanol	79.52%
Xylose to Ethanol	53.21%
Total Process Yield	55.11%
Total Metabolic Yield	82.22%

PDU SSF Material Balance

Run #: P960506CF
 Date: 6/8/96
 Time: 10:00

Run Conditions:	Hydrolyzer Temp (C):	Flash Tank Temp (C):	%
	Hydrolyzer Residence Time (min):		
	Hydrolyzer Acid Concentration (%):	#DIV/0!	

Input Data		Exhaust Gas Flow Rate		Ethanol		CO2	
		(Corrected mole/min)		(mole%)		(mole%)	
Feed Flow Rate (SA-150) (kg/h):	59.1	Feed Solids Concentration (%):	45.82	Caustic Flow (kg/h):	1.74	γ-455A:	0.071
Water Inlet (kg/h):	10	γ-201 Acid Concentration (%):	8	Enzyme Flow Rate (FE-455A-B) (kg/h):	0.25	γ-455B:	1.68
Acid Flow Rate (kg/h):	13.29	Urea Concentration (%):	#DIV/0!	CSL Flow Rate (FE-455A-B) (kg/h):	5.52	γ-455C:	1.24
APR Steam (kg/h):	9.85	Hydrolyze Insoluble Solids (%):	8.03	Steele Water (kg/h):	25.69	γ-455D:	5
		Fermenter Insoluble Solids (%):	4.4				102
Flash Vapor (kg/h):	17.21						0

Xylan Conversion:	67.5%
Overall C6-Sugar Conversion:	65.5%
Overall C5-Sugar Conversion:	19.7%
Ethanol Process Yield (% theor):	46.6%
Ethanol Metabolic Yield (% theor):	83.7%

Carbon Balance: SSF

Component	Carbon In										Carbon Out					Conversion (%)	Yield (g product / 100 g C6 cons)					
	In Preheated Feed		In Feed Liquid		In Inoculum		In Enzyme		Total		In Solids		In Vapor		In Exhaust Gas			Total (C-mole/h)				
	(% d.f./wt)	(C-mole/h) (% Total In)	(g/L) (C-mole/h) (% Total In)	(g/L) (C-mole/h) (% Total In)	(g/L) (C-mole/h) (% Total In)	(C-mole/h)	(% d.f./wt)	(C-mole/h) (% Total Out)	(g/L) (C-mole/h) (% Total Out)													
Cellulose			0.00	0.000	0.000	0.000	#DIV/0!		0.000								0.000					
Glucose	51.78	103.898	26.5	124.76	286.717	73.0	0.0	0.000	0.0	300.0	2.135	0.5	392.750	37.66	59.397	48.8	18.19	62.333	51.2	121.730	69.01	
Galactose	1.25	2.508	10.7	9.08	20.867	89.9	0.0	0.000	0.0	23.375	1.08	1.703	7.8	0.000	0.000	5.90	20.218	92.7	0.000	21.921	6.22	
Mannose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.0	0.000	0.0	0.000	0.000	#DIV/0!	0.0	0.000	0.000	#DIV/0!	0.000	#DIV/0!	0.000	0.000	#DIV/0!	
Xylose	5.12	10.273	6.7	52.20	142.545	93.9	0.0	0.000	0.0	153.218	2.12	3.344	3.1	30.73	105.305	63.9	22.17	75.972	64.0	108.649	29.09	
Arabinose	4.47	0.033	0.0	35.99	82.710	100.0	0.0	0.000	0.0	82.743	3.10	4.889	4.0	22.17	75.972	64.0				90.864	2.27	
Lignin	26.92	77.513	22.0	9.15	30.175	28.0		0.0	107.689	39.03	88.336	80.5	4.36	21.440	78.5					109.776	-1.93	
Ethanol							0.000	0.000	#DIV/0!	0.000				37.37	166.929		9.973	5.637	176.902			42.80
Cell Mass							0.000	0.000	#DIV/0!	0.000				1.49	6.119				6.119			1.61
Carbon Dioxide																33.466	100.000		33.466			15.47
Glycerol			0.00	0.000	#DIV/0!	0.000	0.000	#DIV/0!	0.000					3.93	13.172				13.172			4.25
Acetic Acid			5.74	13.191	100.0	0.000	0.000	0.0	13.191					3.96	13.570				13.570			0.12
Lactic Acid			2.20	5.056	100.0	0.000	0.000	0.0	5.056					2.64	9.047				9.047			1.26
Succinic Acid			0.00	0.000	#DIV/0!	0.000	0.000	#DIV/0!	0.000					0.00	0.000				0.000			0.00
Other			0.00	0.000	#DIV/0!	0.000	0.000	#DIV/0!	0.000					2.51	8.487				8.487			2.71
Total	89.54	194.226	25.0	581.662	74.8				778.029	82.89	157.656	27.7		494.106	71.7				696.213			68.23

C-RECOVERY: 89.36%

Component	% Carbon In	% Carbon Out
Glucose	50.5%	17.5%
Galactose/Mannose	3.0%	3.2%
Total C5 Sugars	30.3%	27.3%
Lignin	13.8%	15.8%
Ethanol	0.0%	25.4%
Byproducts	2.3%	10.8%

Material Flows:

Total Insoluble Solids in Hydrolyzate (kg/h)	6.02
Total Liquids in Hydrolyzate (kg/h)	69.00509
Total Flow from Pretreatment (kg/h)	75.03
Total Flow from Fermentation (kg/h)	107.63

Exhaust Gas:

	Ethanol (C-mole/h)	CO2 (C-mole/h)
γ-455A:	0.117936	4.44444
γ-455B:	3.3488	21.6111
γ-455C:	6.12	7.41
γ-455D:	0	0
Total	9.972816	33.46554

PDU SSF Material Balance

Run #: P960506CF
 Date: 6/21/96
 Time: 15:00

Run Conditions: Hydrolyzer Temp (C): Flan Tank Temp (C): 96
 Hydrolyzer Residence Time (min):
 Hydrolyzer Acid Concentration (%): #C11.0

Input Data:		Feed Solids Concentration (%)		Enzyme Flow Rate (FE-455A-B) (g/min)		Exhaust Gas Flow Rate (Corrected) (mole/min)		Ethanol (mole/h)	CO2 (mole/h)
Feed Flow Rate (34-150) (g/min)	59.1	45.82	1.25	0.25	0.25	0.085	0.72	93.1	
Acid Flow Rate (g/min)	10	8	0.25	0.25	0.25	2.51	0.56	14.28	
APR Steam (g/min)	10.65	8.71	93.75	6	6	5	0.65	3.45	
Flash vapor (g/min)	18.36	1.32				0	0	0	

Overall Cell Conversion:	45.6%
Overall C6-Sugar Conversion:	67.9%
Overall C5-Sugar Conversion:	57.5%
Ethanol Process Yield (% Invert):	56.2%
Ethanol Metabolic Yield (% Invert):	82.7%

Carbon Balance: SSF

Component	Carbon In										Carbon Out				Conversion (%)	Yield (g product / 100 g C6 cons)							
	In Feedstock					In Enzyme					In Solids		In Gas										
	In Pretreated Feed (% dry wt)	In Feed (g/min)	In Feed (% Total In)	In Inoculum (g/L) (C-mole/h)	In Enzyme (g/L) (C-mole/h)	Total (C-mole/h)	In Solids (% dry wt)	In Solids (C-mole/h)	In Solids (% Total Out)	In Gas (g/L) (C-mole/h)	In Gas (% Total Out)	Total (C-mole/h)											
Cellulose		0.00	0.000	0.000	0.000	#DIV/0!					0.000												
Glucose	48.4	104.951	26.0	130.32	296.210	73.4	0.0	0.000	0.0	300.0	2.135	0.5	403.307	31.59	61.653	52.9	10.48	59.561	49.7	121.214	49.94		
Galactose	0.13	0.282	7.0	11.87	26.990	69.0	0.0	0.000	0.0	27.262	0.57	1.182	6.6	3.79	15.856	93.4					16.959	37.76	
Mannose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.0	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!					0.000	#DIV/0!	
Xylose	3.74	8.111	4.8	20.84	161.036	95.2	0.0	0.000	0.0	169.126	2.26	4.411	8.2	8.71	49.502	91.8					53.913	68.12	
Arabinose	2.29	0.010	0.0	42.16	95.827	100.0	0.0	0.000	0.0	95.838	1.16	2.264	3.9	9.92	56.319	96.7					58.643	38.81	
Glycerin	26.68	83.028	61.9	14.76	46.838	36.7				129.866	38.67	108.301	74.0	4.66	38.005	26.0					146.306	-12.66	
Ethanol							0.000	0.000	#DIV/0!	0.000					29.57	219.066		5.660	2.519	224.726			42.30
Cell Mass							0.000	0.000	#DIV/0!	0.000					1.33	9.058				9.058			1.85
Carbon Dioxide																		36.604	700.000	36.604			13.16
Sucrose		0.09	0.000	#DIV/0!	0.000	0.000	0.000	0.000	#DIV/0!	0.000					3.29	18.289					18.289		4.59
Acetic Acid		0.24	0.591	100.0	0.000	0.000	0.000	0.000	0.0	0.591				3.59	20.403					20.403			4.86
Lactic Acid		1.42	3.228	100.0	0.000	0.000	0.000	0.000	0.0	3.228				3.19	18.130					18.130			3.66
Succinic Acid		0.00	0.000	#DIV/0!	0.000	0.000	0.000	0.000	#DIV/0!	0.000				0.00	0.000					0.000			0.00
Pyruvate		0.00	0.000	#DIV/0!	0.000	0.000	0.000	0.000	#DIV/0!	0.000				3.77	21.142					21.142			5.26
Total	81.24	196.392	23.7	430.690	76.1					829.217	74.25	177.741	24.5	504.249	69.6					724.264			75.67

Overall Conversion: 87.34%

Component	% Carbon In	% Carbon Out
Glucose	48.6%	16.7%
Galactose/Mannose	3.3%	2.3%
Total C6 Sugars	32.0%	15.5%
Glycerin	15.7%	20.2%
Ethanol	0.0%	31.0%
Byproducts	0.5%	14.2%

Material Flow:

Total Insoluble Solids in Hydrolyzate (g/min)	6.51
Total Invert in Hydrolyzate (g/min)	49.2484
Total Flow from Pretreated Feed (g/min)	74.76
Total Flow from Fermentation (g/min)	176.51

Exhaust Gas:

	Ethanol (C-mole/h)	CO2 (C-mole/h)
Y-455A	0.07344	4.7481
Y-455B	1.68672	21.50568
Y-455C	3.9	10.35
Y-455D	0	0
Total	5.68016	36.60378