NREL-Amoco CRADA Phase 3

Bench Scale Report 1.2

Continuous Fermentation of Pure Sugars by L1400 (pLNH33)

Project Title: Amoco-NREL CRADA with corn fiber

Researcher: Susan Toon @ (303) 384-6141

Research Manager: George Philippidis @ (303) 384-6888

Start Date of Experiment: August 4, 1995

Date of Draft Report: September 25, 1995

Date of Final Report: October 16, 1995

NREL Technical Record Book: No. 1472

Objective

To examine the capability of strain 1400 (pLNH33) to simultaneously ferment xylose and glucose at levels representative of pretreated corn fiber hydrolyzate and to demonstrate its ability to grow in continuous culture under conditions of interest to the NREL-Amoco CRADA.

Background

The compositional make-up of pretreated corn fiber hydrolyzate prepared by Amoco was ascertained to select the monomeric sugar loading of glucose and xylose for the present study. The levels of glucose and xylose were determined to be 2.4% w/v and 3.4% w/v, respectively. The pure sugar fermentation was planned at these levels of sugars to mimic the hydrolyzate composition, but without including any potential inhibitors present in the hydrolyzate.

Materials and Methods

Inoculum Preparation

A frozen (-70°C) stock vial of strain 1400 (pLNH33) was grown in 1% w/v corn steep liquor (CSL), 1% w/v yeast extract, 2% w/v peptone, and 2% w/v xylose at pH 5.0. The flask contained a total volume of 50 mL in a 250-mL baffled Erlenmeyer flask and was incubated at 30°C with an agitation of 150 rpm. After 28 hours of growth, 10% v/v was transferred to 2% w/v CSL, 1% w/v yeast extract, and 2% w/v xylose at pH 5.0 for inoculum growth. This flask contained a working volume of 100 mL in a 500-mL baffled Erlenmeyer flask and was incubated at 30°C at an agitation of 150 rpm.

Fermentation Conditions

The fermentation was started in batch mode with 2% w/v CSL, 1% w/v yeast extract, 2.4% w/v glucose, and 3.8% w/v xylose as medium. A 10% v/v inoculum was transferred to the fermentor vessel and was allowed to grow for 24 hours in batch before the fermentation was switched to continuous operation. The feed for continuous mode consisted of the same medium, but was made up in a 15-L batch with xylose and glucose being filter-sterilized and added after the yeast extract

and CSL solutions were autoclaved. The pH of the medium was adjusted to 5.0 in both the fermentor and the feed with 3N sodium hydroxide.

For the fermentation, a 1.7-L New Brunswick BioFlo III fermentor was employed. To minimize ethanol evaporation, the condenser was packed with 1-mm glass beads (to maximize the surface area) and equipped with 4°C water circulation. The working volume of the vessel was 1 L, agitation was controlled at 150 rpm, temperature was maintained at 30°C, and the pH was maintained at 5.0 with the addition of either 3N sodium hydroxide or 1% w/v sulfuric acid. Air was not supplied to the fermentation.

The feed, base, and acid addition vessels were placed on balances and their weights were recorded daily in order to calculate the dilution rate of the fermentation. The dilution rate was calculated by dividing the weight change over time of the feed (the base and acid additions were negligible) by the working volume of the fermentor (density of feed assumed to be 1.0 g/mL). The residence time is the inverse of the dilution rate.

Analysis

Duplicate samples were withdrawn at regular intervals and analyzed on the Yellow Springs Instrument (YSI) for ethanol and glucose. In addition, samples were analyzed on a Hewlett Packard 1090 HPLC for glucose, xylose, succinic acid, acetic acid, lactic acid, glycerol, and ethanol. Optical density at 600 nm (OD) and dry cell weight were obtained to monitor cell growth on every sample. The dry cell weight was determined by centrifuging 4 mL of the fermentation broth for 10 minutes at 5000 rev/min. The supernatant was decanted, and the pellet was washed with 10 mL of deionized water twice. The pellets were then transferred to weighed pans and let to dry in a 60°C drying oven for 24 hours.

The viability of the cells in the fermentor was determined on a daily basis. Plasmid stability was monitored occasionally. The viability was determined by doing serial dilutions in a 0.85% w/v sodium chloride solution and plating the appropriate dilution on three YPD (1% w/v yeast extract, 2% w/v peptone, 2% w/v glucose, pH 5) plates. The plates were incubated at 30°C until the colonies were of adequate size to be counted. The colony was then divided by the total number of cells counted by a hemacytometer to obtain the viability figure. The colonies that came up on the YPD plates were then transferred to YPX (1% w/v yeast extract, 2% w/v peptone, 2% w/v xylose, pH 5) plates using the replica plating technique. The number of cells that came up on the YPX plates divided by the number of cells that came up on the YPD plates is the plasmid stability.

Results and Discussion

The fermentation ran uncontaminated for 36 days during which three residence times were tested: 74, 60 and 44 hours. In batch mode (following inoculation), it appeared that the xylose was not metabolized until nearly all of the glucose was depleted (Figure 1 and Table 1). After 24 hours in batch, the fermentation was switched to continuous mode with a 74-hour residence time. At this point, all of the glucose had been consumed, while 20 g/L xylose still remained in the fermentor.

Interestingly, the same was not true for growth on xylose. When pure xylose was used (flasks 1 and 4), the growth rate was 0.131 h^{-1} (in YP) or 0.142 h^{-1} (in CSL). But when xylose became the sugar source after depletion of the glucose (flasks 2 and 5), the growth rate plummeted to 0.026 h^{-1} and 0.023 h^{-1} in YP and CSL, respectively. Despite the slow growth, however, the cells seem capable of producing significant amounts of ethanol from xylose, an indication that ethanol production may be non-growth associated. It is, therefore, imperative to take into account this value of growth rate rather than the one calculated on pure xylose, when glucose-xylose mixtures are used in the fermentation process with L1400 (pLNH33). This of course applies to the corn fiber hydrolyzate, too.

In the flasks containing just glucose, the glucose was consumed in 7 hours, whereas residual xylose was still present after 45 hours in the flasks containing just xylose. The results show that glucose is nearly depleted before xylose utilization commences (Figure 1) in the flasks containing the sugar mixture. The rate of xylose utilization seems to be higher in the flasks containing the sugar mixture (Figure 1). This could be due to the fact that the cell population is larger in those flasks than in the flasks with just xylose (Figure 2).

The process yields for the flasks containing only xylose were 71.1% in YP and 73.1% in CSL. In the mixture, those yields were 80.4% in YP and 76.3% in CSL, consistent with the average of the yields observed on the individual sugars (Table 2, flask 2 vs. flask "1+3" and flask 5 vs. flask "4+6"). The process yield in the flasks with only glucose were the greatest observed in this experimental study: 83.9% in YP and 79.9% in CSL (see Table 2). The increase in the process yield in the flasks with glucose can probably be best explained by examining the by-product formation as presented in Table 3. This table clearly shows that a significant fraction of the utilized xylose is being converted into xylitol in the flasks containing xylose or the sugar mixture. The data also indicate that the nutrient source may play a role in ethanol and other by-product formation: the ethanol yield is greater in YP containing glucose and the sugar mixture than in the same CSL flasks. More glycerol and xylitol is produced in the flasks with CSL than YP. In addition, more cell mass is produced in the YP flasks containing glucose and the sugar mixture than the same CSL flasks.

Table 3: Fermentation product distribution.

			Product Yield (100 g/g C5+C6 Utilized)					
Flask #	Residual Glucose (g/L)	Residual Xylose (g/L)	Ethanol	Cell Mass	Carbon Dioxide	Glycerol	Xylitol	**Total**
1	0.0	1.8	38.5	12.0	36.8	3.3	11.3	101.9
2	0.0	0.9	41.8	9.6	40.0	4.0	6.3	101.7
3	0.0	0.6	44.1	11.9	42.1	2.3	0.0	100.4
4	0	1.5	39.3	12.0	37.6	4.1	12.8	105.8
5	0	1.3	40.0	8.4	38.2	5.7	7.0	99.4
6	0	0.4	41.6	10.4	39.7	3.5	0.0	95.3

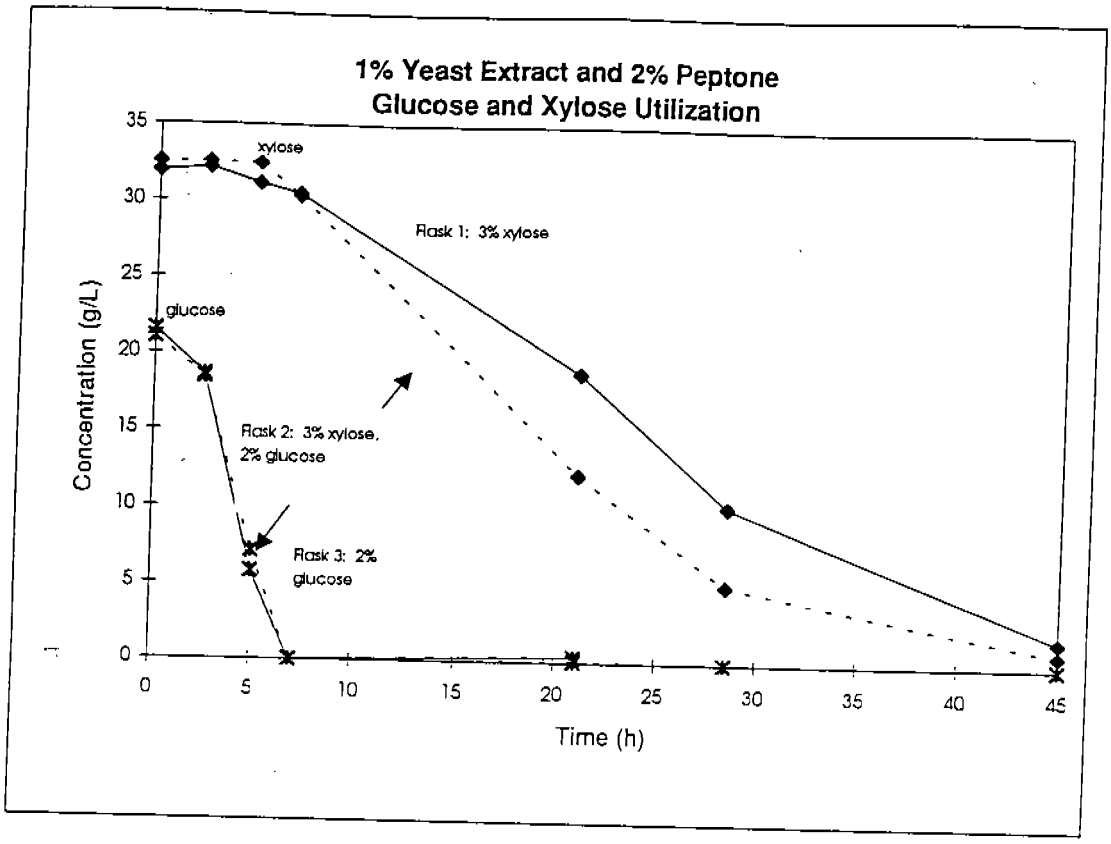
As seen in Table 3, carbon closure in all flasks was satisfactory with a mean value of 100.8 % ± 3.4 %. It should be noted that the carbon dioxide was calculated on the basis of equimolar formation with respect to ethanol production. The detailed carbon balance spreadsheets are attached at the end of this report.

Conclusions

Overall, the effect of nutrient source on the performance of the organism was rather minor. It is, therefore, recommended that CSL be used in the PDU and commercial applications, in light of its lower cost. In contrast, growth on the two sugars (glucose and xylose) was markedly different. The doubling time of L1400 (pLNH33) is 2.15 to 2.5 times faster on pure glucose than on pure xylose. The data show that the xylose fraction is not utilized until nearly all of the glucose is depleted in the sugar mixture flasks. Nevertheless, the overall ethanol yield in the sugar mixture is quite satisfactory (76-80%) within 45 hours.

This study also provides useful information for the development of a CRADA SSCF (simultaneous saccharification and cofermentation) kinetic model based on the NREL SSF and cofermentation models. Using the data presented in Table 2, values for the growth rate of the cells (μ_m) on each sugar were calculated. Furthermore, the information on by-product formation contained in Table 3 allows us to determine the values of the yield coefficients of cell mass on glucose (Y_{XG}) and xylose (Y_{XZ}), as well as those of xylitol on xylose (Y_{TZ}) and glycerol on glucose (Y_{RG}) and xylose (Y_{RZ}).

Figure 1


1% Yeast Extract and 2% Peptone
Glucose and Xylose Utilization
xylose
glucose
Flask 1: 3% xylose
Flask 2: 3% xylose, 2% glucose
Flask 3: 2% glucose
Concentration (g/L)
Time (h)
0
5
10
15
20
25
30
35
40
45

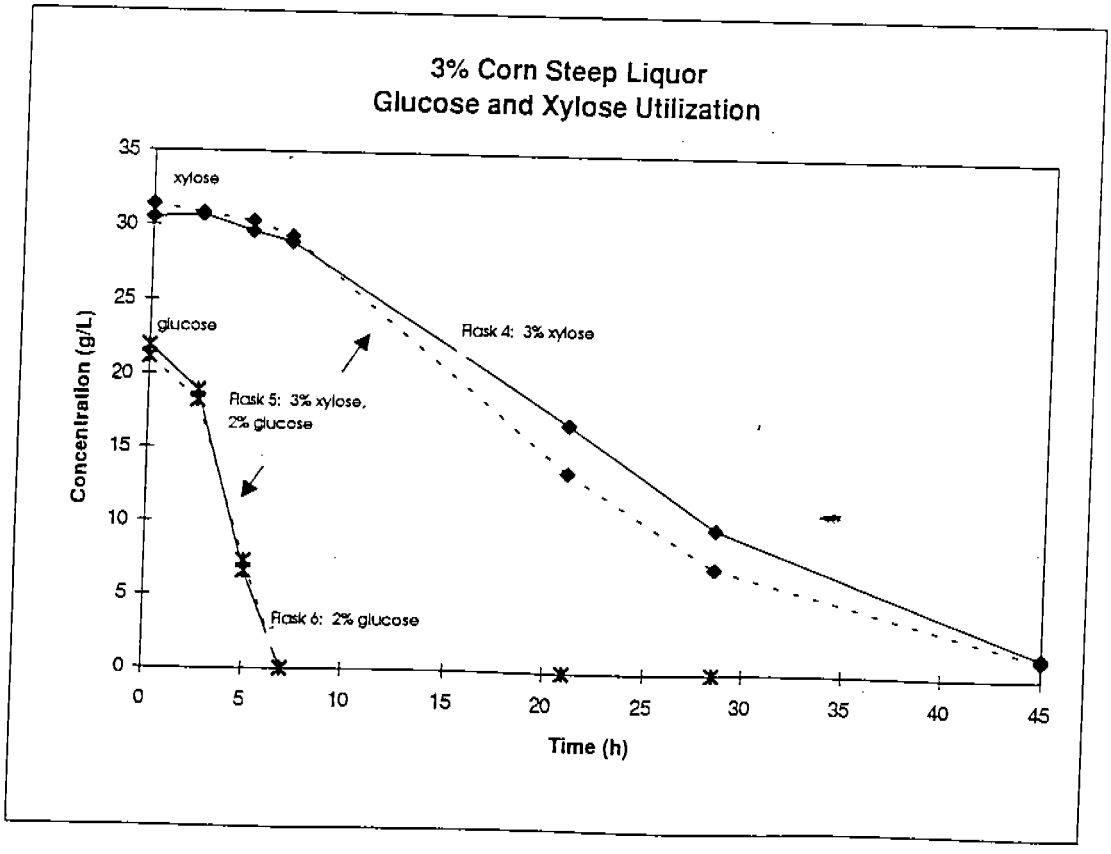

3% Corn Steep Liquor
Glucose and Xylose Utilization
xylose
glucose
Flask 4: 3% xylose
Flask 5: 3% xylose, 2% glucose
Flask 6: 2% glucose
Concentration (g/L)
Time (h)
0
5
10
15
20
25
30
35
40
45

Figure 2: Growth Profile of L1400(pLNH33)

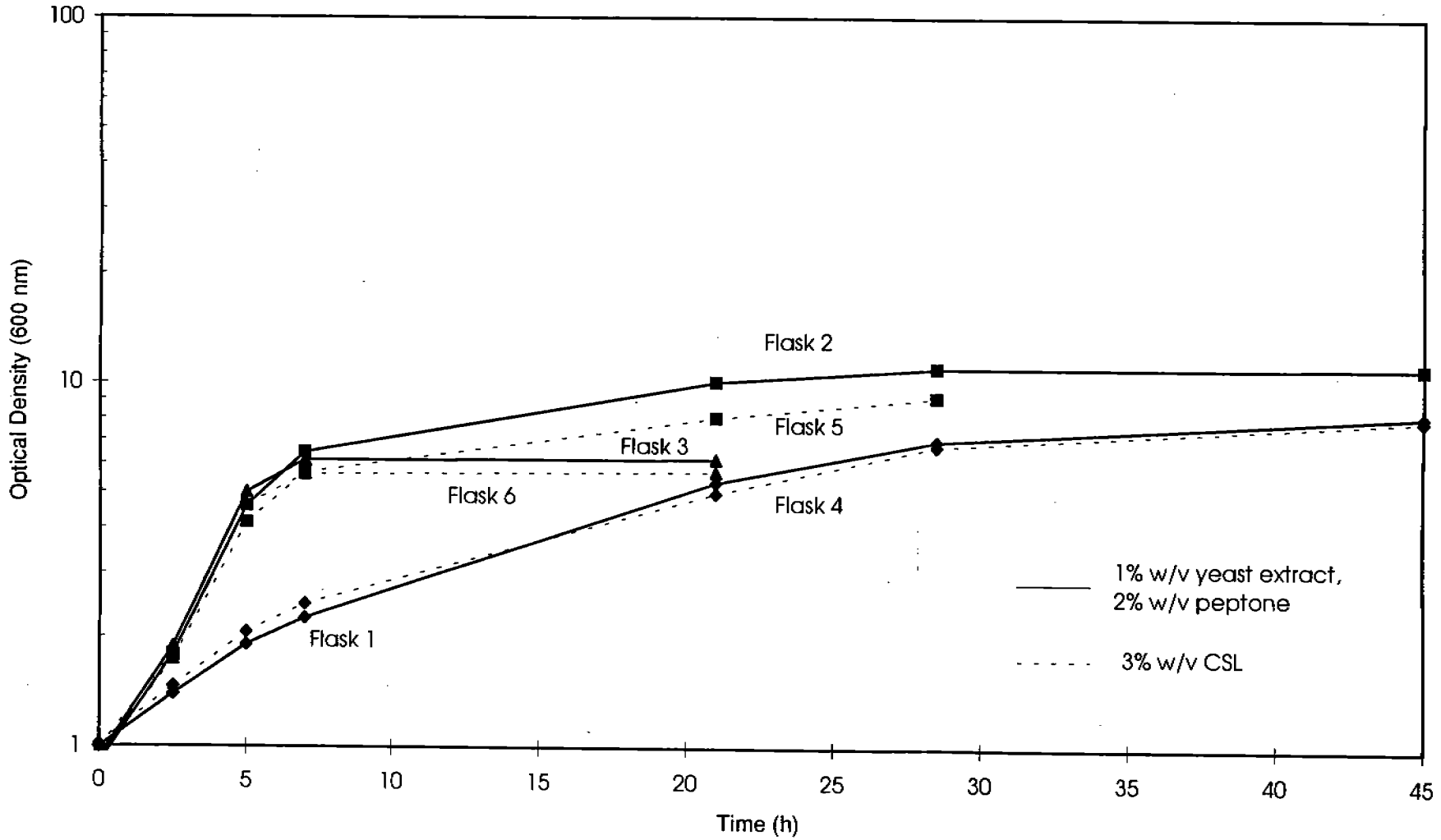
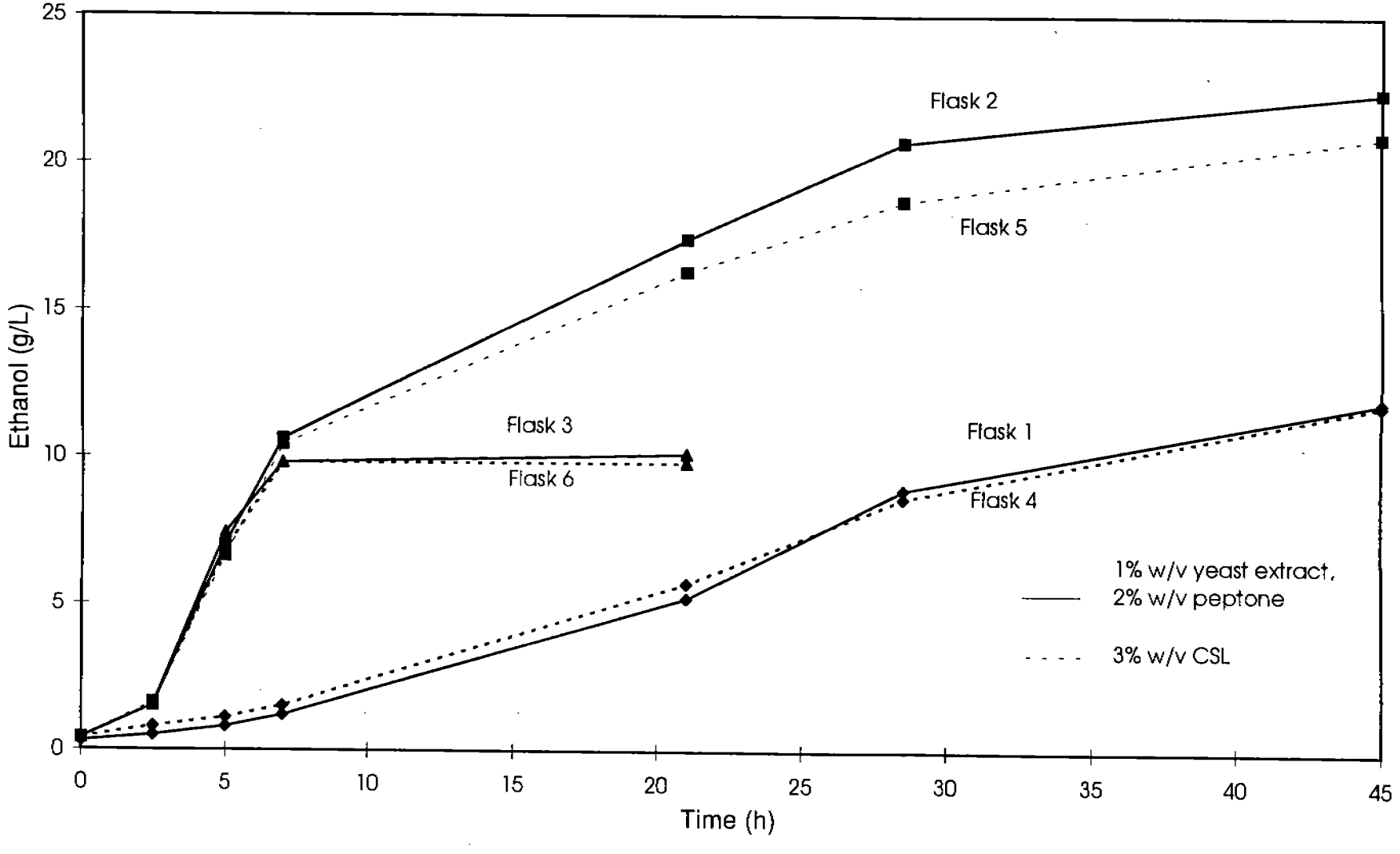


Figure 3: Ethanol Production by L1400(pLNH33)



CARBON BALANCE: Flask 1:1% w/v YE, 2% w/v Peptone

Enzyme:
Organism: LNH33C
Feedstock: Pure Sugars
Pretreatment: N/A
Run: Shake Flask
Sample: 45 h

Researcher : Susan Toon
Run Date : 14-Sep-95
CAT Reports : 95-082 PDU

SOLIDS BALANCE	In	Out
Lignin (%)	0.00	0.00
Insoluble Solids (%)	0.00	0.00

PERFORMANCE PARAMETERS

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	#DIV/0!
Overall C5-Sugar Conversion:	94.4%
Ethanol Process Yield (% theor):	71.1%
Ethanol Metabolic Yield (% theor):	75.4%

Carbon Balance: SSF

Component	Carbon In							Carbon Out							Conversion	Product Yield	
	In Solids			In Liquor			Total	In Solids			In Liquor			Total	100 (In-Out)/In	100 g /	100 g /
	(% dry wt)	(C-mole/Kg Sln)	(% Total In)	(g/L)	(C-mole/Kg Sln)	(% Total In)	(C-mole/Kg Sln)	(% dry wt)	(C-mole/Kg Sln)	(% Total Out)	(g/L)	(C-mole/Kg Sln)	(% Total Out)	(C-mole/Kg Sln)	(%)	g C6 cons	g (C5+C6) cons
Cellobiose				0.00	0.000		0.000				0.00	0.000		0.000			
Glucose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Galactose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Mannose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Xylose	0.00	0.000	0.0	31.90	1.062	100.0	1.062	0.00	0.000	0.0	1.80	0.060	100.0	0.060	94.4		
Arabinose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Lignin	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Ethanol				0.30	0.013		0.013				11.90	0.517		0.517		#DIV/0!	38.5
Cell Mass				0.50	0.020		0.020				4.10	0.163		0.163		#DIV/0!	12.0
Carbon Dioxide												0.252		0.252		#DIV/0!	36.8
Glycerol				0.60	0.020		0.020				1.60	0.052		0.052		#DIV/0!	3.3
Xylitol				0.10	0.003		0.003				3.50	0.115		0.115		#DIV/0!	11.3
Acetic Acid				0.00	0.000		0.000				0.00	0.000		0.000		#DIV/0!	0.0
Lactic Acid				0.00	0.000		0.000				0.00	0.000		0.000		#DIV/0!	0.0
Succinic Acid				0.00	0.000		0.000				0.00	0.000		0.000		#DIV/0!	0.0
Total	0.00	0.000	0.0		1.118	100.0	1.118	0.00	0.000	0.0		1.159	100.0	1.159		#DIV/0!	101.9

C - RECOVERY: 103.7%

CARBON BALANCE: Flask 2: 1% w/v YE, 2% w/v Peptone

Enzyme: N/A
Organism: LNH33C
Feedstock: Pure Sugars
Pretreatment: N/A
Run: Shake Flask
Sample: 45 h

Researcher : Susan Toon
Run Date : 14-Sep-95
CAT Reports : 95-082 PDU

SOLIDS BALANCE	In	Out
Liquor (%):	0.00	0.00
Insoluble Solids (%):	0.00	0.00

PERFORMANCE PARAMETERS

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	100.0%
Overall C5-Sugar Conversion:	97.2%
Ethanol Process Yield (% theor):	80.4%
Ethanol Metabolic Yield (% theor):	81.8%

Carbon Balance: SSF

Component	Carbon In							Carbon Out							Conversion	Product Yield	
	In Solids			In Liquor			Total	In Solids			In Liquor			Total			
	(% dry wt)	(C-mole/Kg Sln)	(% Total In)	(g/L)	(C-mole/Kg Sln)	(% Total In)	(C-mole/Kg Sln)	(% dry wt)	(C-mole/Kg Sln)	(% Total Out)	(g/L)	(C-mole/Kg Sln)	(% Total Out)	(C-mole/Kg Sln)	100 (In-Out)/In (%)	100 g / g C6 cons	100 g / g (C5+C6) cons
Cellobiose				0.00	0.000		0.000				0.00	0.000		0.000			
Glucose	0.00	0.000	0.0	21.00	0.699	100.0	0.699	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	100.0		
Galactose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Mannose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Xylose	0.00	0.000	0.0	32.50	1.082	100.0	1.082	0.00	0.000	0.0	0.90	0.030	100.0	0.030	97.2		
Arabinose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Lignin	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Ethanol				0.40	0.017		0.017				22.40	0.972		0.972		104.8	41.8
Cell Mass				0.45	0.018		0.018				5.52	0.220		0.220		24.2	9.6
Carbon Dioxide												0.478		0.478		100.1	40.0
Glycerol				0.50	0.016		0.016				2.60	0.085		0.085		10.0	4.0
Xylitol				0.10	0.003		0.003				3.40	0.112		0.112		15.7	6.3
Acetic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Lactic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Succinic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Total	0.00	0.000	0.0		1.837	100.0	1.837	0.00	0.000	0.0		1.897	100.0	1.897		254.7	101.7

C - RECOVERY: 103.3%

CARBON BALANCE: Flask 3: 1% w/v YE, 2% w/v Peptone

Enzyme: N/A
Organism: LNH33C
Feedstock: Pure Sugars
Pretreatment: N/A
Run: Shake Flask
Sample: 21 h

Researcher : Susan Toon
Run Date : 14-Sep-95
CAT Reports : 95-082 PDU

SOLIDS BALANCE	In	Out
Liquids (%)	0.00	0.00
Insoluble Solids (%)	0.00	0.00

PERFORMANCE PARAMETERS

Cellulose Conversion:	#DIV/0!
Overall C6 Sugar Conversion:	100.0%
Overall C5-Sugar Conversion:	45.5%
Ethanol Process Yield (% theor):	83.9%
Ethanol Metabolic Yield (% theor):	86.2%

Carbon Balance: SSF

Component	Carbon In							Carbon Out							Conversion	Product Yield	
	In Solids			In Liquor			Total	In Solids			In Liquor			Total			
	(% dry wt)	(C-mole/Kg Sln)	(% Total In)	(g/L)	(C-mole/Kg Sln)	(% Total In)	(C-mole/Kg Sln)	(% dry wt)	(C-mole/Kg Sln)	(% Total Out)	(g/L)	(C-mole/Kg Sln)	(% Total Out)	(C-mole/Kg Sln)	100 (In-Out)/In (%)	100 g / g C6 cons	100 g / g (C5+C6) cons
Cellobiose				0.00	0.000		0.000				0.00	0.000		0.000			
Glucose	0.00	0.000	0.0	21.50	0.716	100.0	0.716	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	100.0		
Galactose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Mannose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Xylose	0.00	0.000	0.0	1.10	0.037	100.0	0.037	0.00	0.000	0.0	0.60	0.020	100.0	0.020	45.5		
Arabinose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Lignin	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Ethanol				0.40	0.017		0.017				10.10	0.438		0.438		45.1	44.1
Cell Mass				0.46	0.018		0.018				3.09	0.123		0.123		12.2	11.9
Carbon Dioxide												0.211		0.211		43.1	42.1
Glycerol				0.60	0.016		0.016				1.00	0.033		0.033		2.3	2.3
Xylitol				0.10	0.003		0.003				0.10	0.003		0.003		0.0	0.0
Acetic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Lactic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Succinic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Total	0.00	0.000	0.0		0.808	100.0	0.808	0.00	0.000	0.0		0.828	100.0	0.828		102.8	100.4

C - RECOVERY: 102.5%

CARBON BALANCE: Flask 4: 3% w/v CSL

Enzyme: N/A
Organism: LNH33C
Feedstock: Pure Sugars
Pretreatment: N/A
Run: Shake Flask
Sample: 45 h

Researcher : Susan Toon
Run Date : 14-Sep-95
CAT Reports : 95-082 PDU

SOLIDS BALANCE	In	Out
Lignin (%):	0.00	0.00
Insoluble Solids (%)	0.00	0.00

PERFORMANCE PARAMETERS

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	#DIV/0!
Overall C5-Sugar Conversion:	95.1%
Ethanol Process Yield (% theor):	73.1%
Ethanol Metabolic Yield (% theor):	76.9%

Carbon Balance: SSF

Component	Carbon In: In Solids (% dry wt)	In Solids (C-mole/Kg Sln)	In Solids (% total In)	In Liquor (g/L)	In Liquor (C-mole/Kg Sln)	In Liquor (% total In)	Total (C-mole/Kg Sln)	Carbon Out: In Solids (% dry wt)	In Solids (C-mole/Kg Sln)	In Solids (% total Out)	In Liquor (g/L)	In Liquor (C-mole/Kg Sln)	In Liquor (% total Out)	Total (C-mole/Kg Sln)	Conversion 100 (In-Out)/In (%)	Product Yield 100 g / g C6 cons	Product Yield 100 g / g (C5+C6) cons
Cellobiose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Glucose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Galactose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Mannose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Xylose	0.00	0.000	0.0	30.50	1.016	100.0	1.016	0.00	0.000	0.0	1.50	0.050	100.0	0.050	95.1		
Arabinose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Lignin	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Ethanol				0.40	0.017		0.017				11.80	0.512		0.512		#DIV/0!	39.3
Cell Mass				0.51	0.020		0.020				3.99	0.159		0.159		#DIV/0!	12.0
Carbon Dioxide												0.247		0.247		#DIV/0!	37.6
Glycerol				0.50	0.016		0.016				1.70	0.055		0.055		#DIV/0!	4.1
Xylitol				0.10	0.003		0.003				3.80	0.125		0.125		#DIV/0!	12.8
Acetic Acid				0.00	0.000		0.000				0.00	0.000		0.000		#DIV/0!	0.0
Lactic Acid				0.00	0.000		0.000				0.00	0.000		0.000		#DIV/0!	0.0
Succinic Acid				0.00	0.000		0.000				0.00	0.000		0.000		#DIV/0!	0.0
Total	0.00	0.000	0.0		1.073	100.0	1.073	0.00	0.000	0.0		1.149	100.0	1.149		#DIV/0!	105.8

C - RECOVERY: 107.1%

CARBON BALANCE: Flask 5: 3% w/v CSL

Enzyme: N/A
Organism: LNH33C
Feedstock: Pure Sugars
Pretreatment: N/A
Run: Shake Flask
Sample: 45 h

Researcher : Susan Toon
Run Date : 14-Sep-95
CAT Reports : 95-082 PDU

SOLIDS BALANCE	In	Out
Lignin (%)	0.00	0.00
Insoluble Solids (%)	0.00	0.00

PERFORMANCE PARAMETERS

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	100.0%
Overall C5-Sugar Conversion:	95.9%
Ethanol Process Yield (% theor):	76.3%
Ethanol Metabolic Yield (% theor):	78.3%

Carbon Balance: SSF

Component	Carbon In							Carbon Out							Conversion	Product Yield	
	In Solids			In Liquor			Total	In Solids			In Liquor			Total			
	(% dry wt)	(C-mole/Kg Sln)	(% Total In)	(g/L)	(C-mole/Kg Sln)	(% Total In)	(C-mole/Kg Sln)	(% dry wt)	(C-mole/Kg Sln)	(% Total Out)	(g/L)	(C-mole/Kg Sln)	(% Total Out)	(C-mole/Kg Sln)	100 (In-Out)/In (%)	100 g / g C6 cons	100 g / g (C5+C6) cons
Cellobiose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Glucose	0.00	0.000	0.0	21.10	0.703	100.0	0.703	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	100.0		
Galactose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Mannose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Xylose	0.00	0.000	0.0	31.40	1.046	100.0	1.046	0.00	0.000	0.0	1.30	0.043	100.0	0.043	95.9		
Arabinose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Lignin	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Ethanol				0.40	0.017		0.017				20.90	0.907		0.907		97.2	40.0
Cell Mass				0.46	0.018		0.018				4.77	0.190		0.190		20.4	8.4
Carbon Dioxide												0.445		0.445		92.8	38.2
Glycerol				0.50	0.016		0.016				3.40	0.111		0.111		13.7	5.7
Xylitol				0.10	0.003		0.003				3.70	0.122		0.122		17.1	7.0
Acetic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Lactic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Succinic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Total	0.00	0.000	0.0		1.804	100.0	1.804	0.00	0.000	0.0		1.818	100.0	1.818		241.2	99.4

C - RECOVERY: 100.8%

12

CARBON BALANCE: Flask 6: 3% w/v CSL

Enzyme: N/A
Organism: LNH33C
Feedstock: Pure Sugars
Pretreatment: N/A
Run: Shake Flask
Sample: 21 h

Researcher : Susan Toon
Run Date : 14-Sep-95
CAT Reports : 95-082 PDU

SOLIDS BALANCE

	In	Out
Lignin (%):	0.00	0.00
Insoluble Solids (%):	0.00	0.00

PERFORMANCE PARAMETERS

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	100.0%
Overall C5-Sugar Conversion:	63.6%
Ethanol Process Yield (% theor):	79.9%
Ethanol Metabolic Yield (% theor):	81.3%

Carbon Balance: SSF

Component	Carbon In: In Solids (% dry wt)	In Solids (C-mole/Kg Sln)	In Solids (% Total In)	In Liquor (g/L)	In Liquor (C-mole/Kg Sln)	In Liquor (% Total In)	Total (C-mole/Kg Sln)	Carbon Out: In Solids (% dry wt)	In Solids (C-mole/Kg Sln)	In Solids (% Total Out)	In Liquor (g/L)	In Liquor (C-mole/Kg Sln)	In Liquor (% Total Out)	Total (C-mole/Kg Sln)	Conversion 100 (In-Out)/In (%)	Product Yield 100 g / g C6 cons	Product Yield 100 g / g (C5+C6) cons
Cellobiose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Glucose	0.00	0.000	0.0	21.90	0.729	100.0	0.729	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	100.0		
Galactose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Mannose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Xylose	0.00	0.000	0.0	1.10	0.037	100.0	0.037	0.00	0.000	0.0	0.40	0.013	100.0	0.013	63.6		
Arabinose	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Lignin	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Ethanol				0.40	0.017		0.017				9.80	0.425		0.425		42.9	41.6
Cell Mass				0.49	0.020		0.020				2.84	0.114		0.114		10.7	10.4
Carbon Dioxide												0.204		0.204		41.0	39.7
Glycerol				0.50	0.016		0.016				1.30	0.042		0.042		3.7	3.5
Xylitol				0.10	0.003		0.003				0.10	0.003		0.003		0.0	0.0
Acetic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Lactic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Succinic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.0	0.0
Total	0.00	0.000	0.0		0.823	100.0	0.823	0.00	0.000	0.0		0.802	100.0	0.802		93.3	95.3

C - RECOVERY: 97.5%

Flask 1: 1% Yeast extract, 2% peptone, 3% xylose								
Time (h)	OD (600 nm)	Cell mass (g/L)	pH	Glucose (g/L)	Xylose (g/L)	Ethanol (g/L)	Glycerol (g/L)	xylitol (g/L)
0	0.992	0.496	5.05	0	31.9	0.3	0.6	0.1
2.5	1.39	0.695	5.05	0	32.1	0.5	0.5	0.1
5	1.905	0.9525	5.09	0	31.1	0.8	0.6	0.1
7	2.25	1.125	5.1	0	30.4	1.2	0.6	0.3
21	5.325	2.6625	4.93	0	18.8	5.2	1.1	1.7
28.5	6.95	3.475	4.85	0	10.2	8.9	1.4	2.5
45	8.19	4.095	4.79	0	1.8	11.9	1.6	3.5

Flask 2: 1% Yeast extract, 2% peptone, 3% xylose, 2% glucose								
Time (h)	OD (600 nm)	Cell mass (g/L)	pH	Glucose (g/L)	Xylose (g/L)	Ethanol (g/L)	Glycerol (g/L)	xylitol (g/L)
0	0.894	0.447	5.04	21	32.5	0.4	0.5	0.1
2.5	1.79	0.895	4.94	18.4	32.5	1.5	0.6	0.1
5	4.58	2.29	4.71	7.1	32.4	7	1.1	0.1
7	6.44	3.22	4.72	0	30.3	10.6	1.5	0.1
21	10.05	5.025	4.58	0.1	12.2	17.4	2.3	2.1
28.5	11	5.5	4.54	0	5.1	20.7	2.5	2.9
45	11.03	5.515	4.57	0	0.9	22.4	2.6	3.4

Flask 3: 1% Yeast extract, 2% peptone, 2% glucose								
Time (h)	OD (600 nm)	Cell mass (g/L)	pH	Glucose (g/L)	Xylose (g/L)	Ethanol (g/L)	Glycerol (g/L)	xylitol (g/L)
0	0.924	0.462	5.06	21.5	1.1	0.4	0.5	0.1
2.5	1.88	0.94	4.95	18.6	1.1	1.5	0.6	0.1
5	4.99	2.495	4.71	5.8	1.1	7.4	1	0.1
7	6.12	3.06	4.77	0	1	9.8	1	0.1
21	6.175	3.0875	4.71	0.4	0.6	10.1	1	0.1

Flask 4: 3% CSL, 2% xylose								
Time (h)	OD (600 nm)	Cell mass (g/L)	pH	Glucose (g/L)	Xylose (g/L)	Ethanol (g/L)	Glycerol (g/L)	xylitol (g/L)
0	1.012	0.506	5.06	0	30.5	0.4	0.5	0.1
2.5	1.46	0.73	5.05	0	30.7	0.8	0.6	0.1
5	2.06	1.03	5.07	0	29.6	1.1	0.6	0.1
7	2.46	1.23	5.1	0	28.9	1.5	0.6	0.2
21	4.975	2.4875	4.97	0	16.9	5.7	1.1	1.6
28.5	6.71	3.355	4.91	0	9.9	8.6	1.4	2.4
45	7.97	3.985	4.89	0	1.5	11.8	1.7	3.8

Flask 5: 3% CSL, 2% xylose, 2% glucose								
Time (h)	OD (600 nm)	Cell mass (g/L)	pH	Glucose (g/L)	Xylose (g/L)	Ethanol (g/L)	Glycerol (g/L)	xylitol (g/L)
0	0.922	0.461	5.04	21.1	31.4	0.4	0.5	0.1
2.5	1.73	0.865	4.92	18.2	30.8	1.6	0.6	0.1
5	4.12	2.06	4.61	7.3	30.3	6.6	1.3	0.1
7	5.66	2.83	4.59	0	29.3	10.4	1.9	0.1
21	8.038	4.019	4.53	0	13.6	16.3	2.8	2
28.5	9.14	4.57	4.54	0	7.2	18.7	3.1	3
45	9.53	4.765	4.68	0	1.3	20.9	3.4	3.7

Flask 6: 3% CSL, 2% glucose								
Time (h)	OD (600 nm)	Cell mass (g/L)	pH	Glucose (g/L)	Xylose (g/L)	Ethanol (g/L)	Glycerol (g/L)	xylitol (g/L)
0	0.982	0.491	5.05	21.9	1.1	0.4	0.5	0.1
2.5	1.8	0.9	4.93	18.9	1.1	1.5	0.6	0.1
5	4.6	2.3	4.59	6.6	1	6.8	1	0.1
7	5.6	2.8	4.63	0	0.8	9.8	1.3	0.1
21	5.688	2.844	4.67	0	0.4	9.8	1.3	0.1