

# Lawrence Berkeley National Laboratory

## Lawrence Berkeley National Laboratory

### **Title**

PROCESS DEVELOPMENT STUDIES OF THE BIOCONVERSION OF CELLULOSE AND PRODUCTION OF ETHANOL

### **Permalink**

<https://escholarship.org/uc/item/4ng6c9sd>

### **Author**

Wilke, Charles R.

### **Publication Date**

1981-04-01



# Lawrence Berkeley Laboratory

UNIVERSITY OF CALIFORNIA

## ENERGY & ENVIRONMENT DIVISION

PROCESS DEVELOPMENT STUDIES OF THE BIOCONVERSION  
OF CELLULOSE AND PRODUCTION OF ETHANOL

Charles R. Wilke and Harvey W. Blanch

April 1981

RECEIVED  
LAWRENCE  
BERKELEY LABORATORY  
JUL 14 1981  
LIBRARY  
DOCUMENTS



### TWO-WEEK LOAN COPY

This is a Library Circulating Copy  
which may be borrowed for two weeks.  
For a personal retention copy, call  
Tech. Info. Division, Ext. 6782

LBL-12603  
c.2

## DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

PROCESS DEVELOPMENT STUDIES OF THE BIOCONVERSION  
OF CELLULOSE AND PRODUCTION OF ETHANOL

Charles R. Wilke and Harvey W. Blanch

Lawrence Berkeley Laboratory  
and  
Department of Chemical Engineering  
University of California  
Berkeley, California 94720

SEMI-ANNUAL REPORT

to

SOLAR ENERGY RESEARCH INSTITUTE

April 6, 1981

This work was supported by the Assistant Secretary  
for Conservation and Solar Energy of the U.S.  
Department of Energy under Contract W-7405-ENG-48,  
and by the Solar Energy Research Institute under  
Contract DR-0-9058-1.



## TABLE OF CONTENT

I.	PROCESS DEVELOPMENT STUDIES	
A.	Economic Evaluation of Hydrolysis and Ethanol Fermentation Schemes-----	1
B.	Economic Evaluation of Alternative Fermentation Processes -----	14
C.	Raw Materials Evaluation-----	26
D.	Evaluation of Pretreatment Process-----	44
II.	MICROBIOLOGICAL AND ENZYMATIC STUDIES	
A.	Production of Cellulase Enzyme from High Yielding Mutants-----	46
B.	Hydrolysis Reactor Development-----	48
	(1) Enzyme Recovery in Cellulose Hydrolysis-----	48
	(2) Countercurrent Hydrolysis Reactor Development-----	51
C.	Xylose Fermentation-----	54
	(1) Xylose Utilization by <u>Bacillus macerans</u> -----	54
	(2) Xylose Fermentation by <u>Clostridium thermohydro-</u> <u>sulfuricum</u> -----	61
D.	Xylanase Production-----	64
III.	FERMENTATION AND SEPARATION PROCESSES-----	69
A.	Process Development Studies on Vacuum Fermentation and Distillation-----	69
B.	Evaluation of Low Energy Separations Processes-----	82
C.	Large Scale Hallow Fiber Reactor Development-----	94
IV.	SUPPORT OF GULF-PDU-----	96
A.	General Support Services and Analytic Methods-----	96



## I. PROCESS DEVELOPMENT STUDIES

### A. Economic Evaluation of Hydrolysis and Ethanol Fermentation Schemes

During the past six months, as an evaluation of the hydrolysis of corn stover with cellulase from Trichoderma reesei Rut C-30 was completed.

Corn stover from the University of California at Davis, fall 1980 crop, was hammermilled to 2 mm particle size. The material was acid treated by contacting with .09 M H<sub>2</sub>SO<sub>4</sub> for 5.5 hours at 100°C, washed, the pH was adjusted to pH = 5, and the solids were air dried to approximately 93% moisture.

Samples of the treated and untreated materials were assayed for carbohydrates, lignin, and extractives (1). The acid treated material contained 56% glucan and 11% xylan. Tables IA-1 and IA-2 present a summary of the assays.

Batch hydrolyses were conducted on acid treated material using cellulase grown from batch cultures of T. reesei Rut C-30. The cellulase was produced following the procedures of Tangnu, et al. (2). Its assay was FPA = 7., β1-4 Glucan glucanohydrolase = 193, cellobiohydrolase = .44, β-glucosidase = 7.9 units/ml. The experiments were conducted with a total solution weight of 150 ml, buffered to pH = 5, agitated to approximately 200 rpm and in a bath at constant 45°C temperature.

Hydrolyses were conducted for substrate concentrations of 5 to 25% by weight and enzyme concentrations ranging from 0.5 to 7 IU/ml, and reaction times of 48 hours. Well mixed samples



Table IA-1

U.C.D. Corn Stover  
Fall 1980 Crop

Assay (100% Dry)

<u>% Carbohydrate</u>	<u>% Sugar Equivalent</u>
36.7 Glucan	40.7 Glucose
17.0 Xylan	19.3 Xylose
2.2 Arabinan	<u>2.5 Arabinose</u>
<hr/>	
$\Sigma$ 55.9 Carbohydrate	$\Sigma$ = 62.5 Sugar Equivalent
11.4 Lignin	
8.5 Ash ( $\approx$ 11.5 as actual silicates)	
6.5 BZ/EtOH Extractives	
0.4 Acid insolubles	
<u>N.D. Protein</u>	
<hr/>	
$\Sigma$ = 85.7*	

\*Balance comprised of unknown value for (dirt/oxides) because of the obvious soil present, and probable protein content, not determined.

Table IA-2

Acid Treated U.C.D. Corn Stover  
(1980 crop)

Assay (100% Dry)

<u>% Carbohydrate</u>		<u>% Sugar Equivalent</u>	
55.8	Glucan	62.0	Glucose
11.0	Xylan	12.5	Xylose
$\Sigma =$ 66.8	Carbohydrate	$\Sigma =$ 74.5	Sugar Equivalent
15.1	Lignin		
5.4	Ash ( $\sim$ 7.3 as actual silicates)		
6.0	BZ/EtOH Extractives		
N.D.	Protein		
$\Sigma =$ 95.2*			

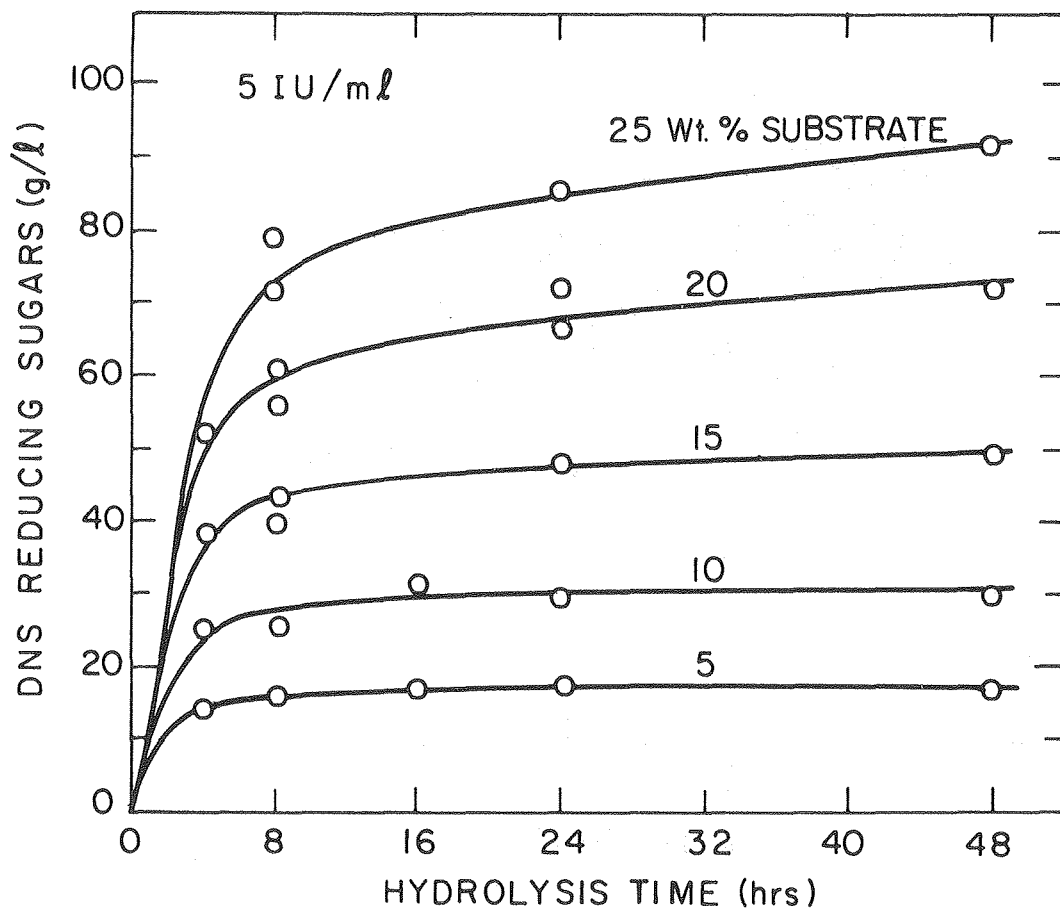
\*Balance comprised of the unknown value for (dirt/oxides) and protein content, not determined.

were withdrawn periodically and measured for reducing sugars using the DNS method. (3). Selected samples were measured for glucose, xylose and cellobiose using a liquid chromatograph. Free enzyme was measured for overall activity (FPA) after precipitation from hydrolysate samples with acetone (4). This method was required because the sugar background readings from hydrolysates overshadowed glucose produced during the test. Selected samples were assayed for component enzyme remaining in solution.

Figure IA-1 presents typical relative production of reducing sugar vs. time profiles. This example is for enzyme activity of 5 IU/ml and substrate concentrations of 5-25%. The reaction experiences a very rapid, initial rate which slows down after about 4-8 hours. Beyond the 8 hour point, the reaction proceeds slowly. In this figure, the reaction rate for the 25% substrate case and for the 5% substrate case appear quite different, the 5% case tapers off rather rapidly for two reasons, the reaction is conducted at higher specific activity (IU/g solids) and the level of sugars in the reaction remains dilute.

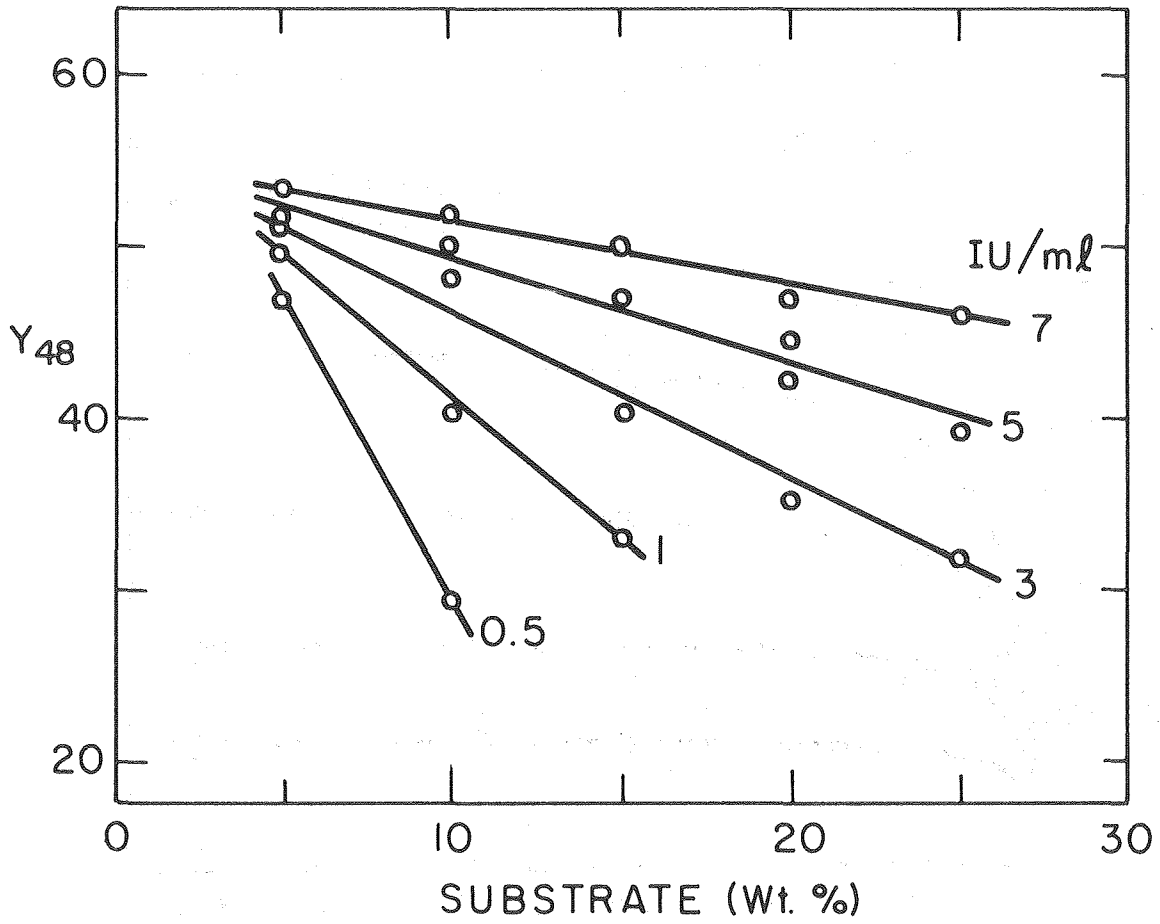
Figure IA-2 presents hydrolysis yields at 48 hours. A maximum conversion of approximately 52% was obtained for the 5% substrate case at enzyme activities beyond 1 IU/ml. Yields decrease with increasing solids concentration.

Figure IA-3 presents hydrolyses yields at 48 hours as a function of specific activity IU/g. For this particular substrate/enzyme mixture the maximum conversion is obtained when using approximately 25 IU/g. Beyond this point and increase in



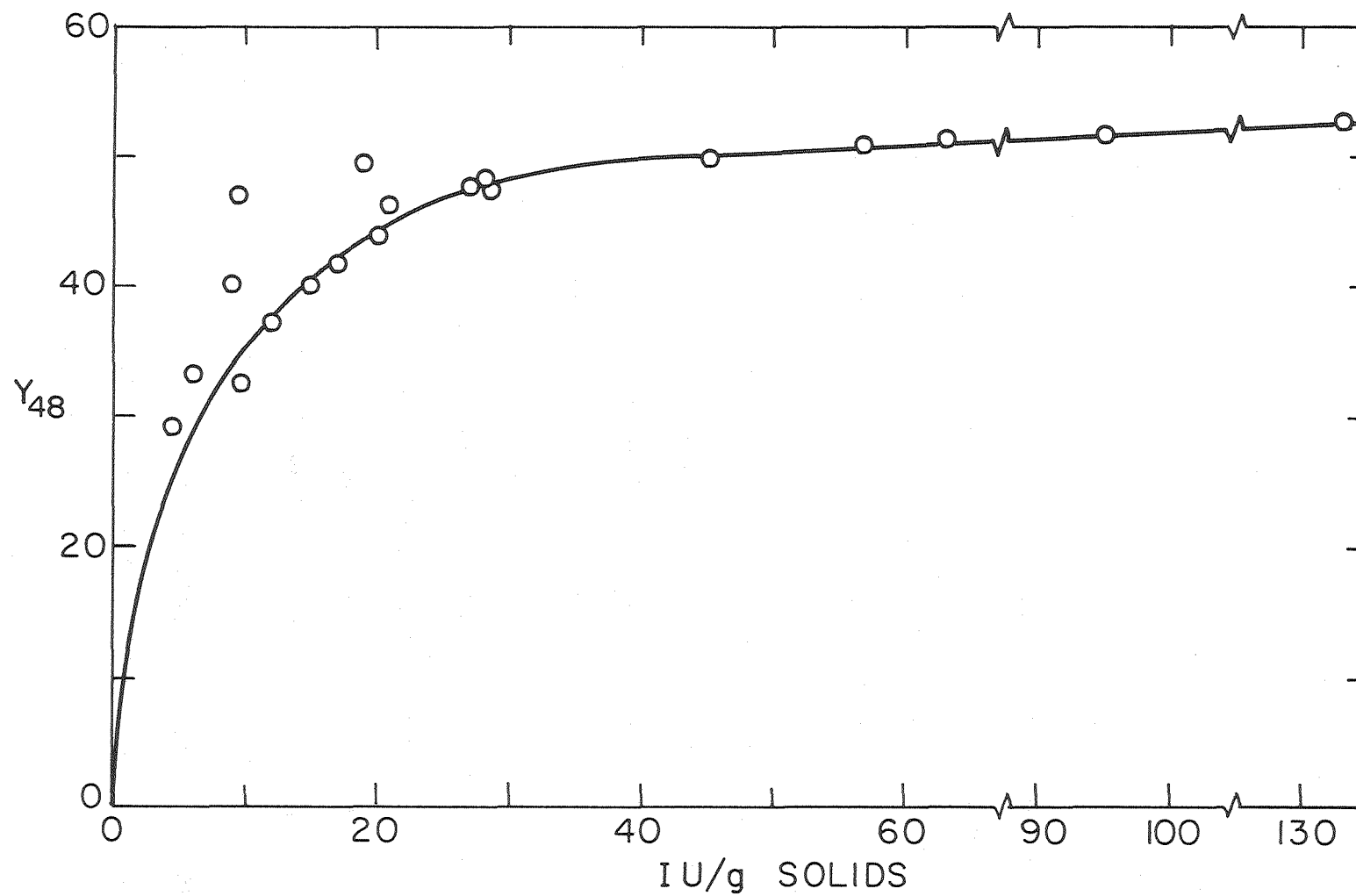
XBL 813-5449

Figure 1A-1. Relative sugar production vs. time profiles at various substrate loadings with Rut-C-30 enzyme.



XBL 813-5447

Figure IA-2. 48 hour glucose conversions (%) vs. substrate loadings at various concentrations of Rut-C-30 enzyme.



XBL 813-5446

Figure IA-3. 48 hour glucose conversion (%) vs. enzyme specific activities.

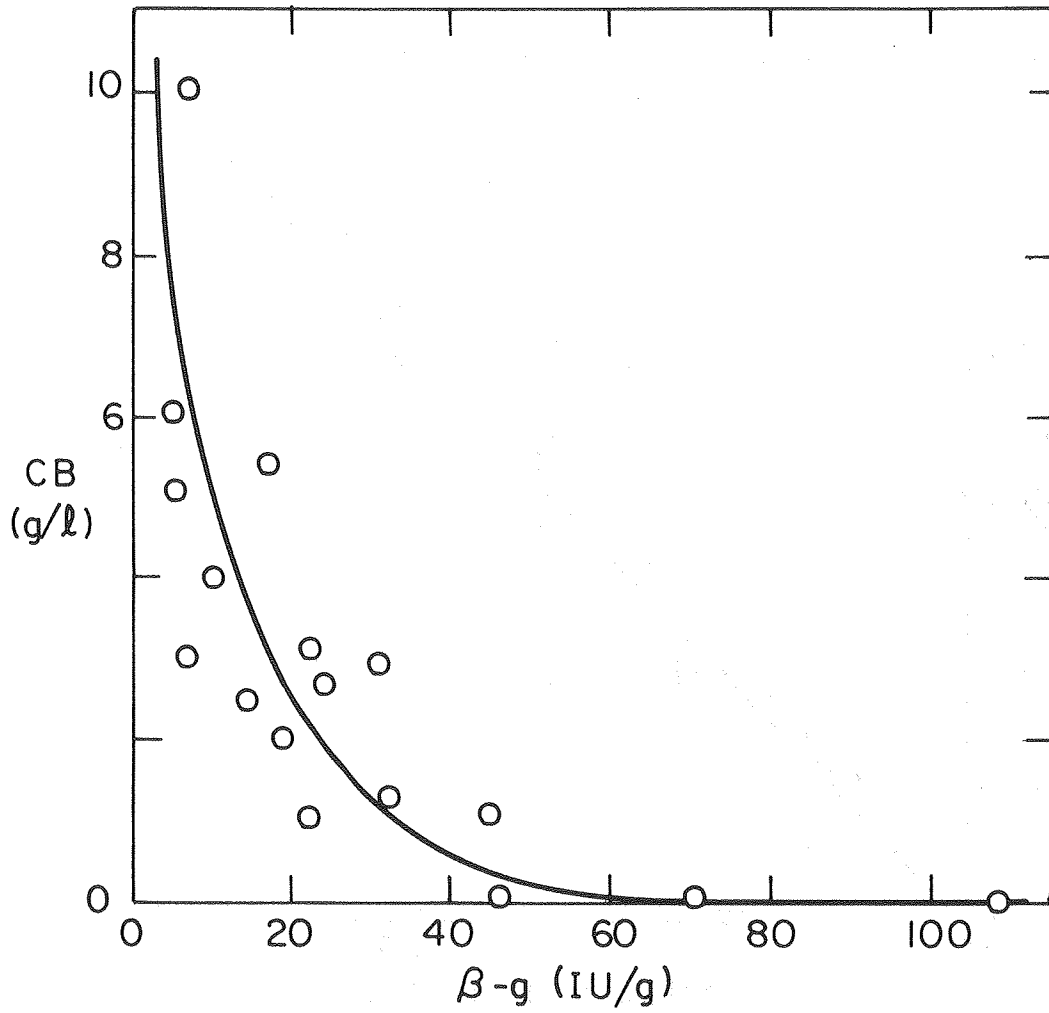
the specific activity brings little or no added conversion.

Figure IA-4 presents cellobiose concentration as a function of specific  $\beta$ -glucosidase activity. Cellobiose concentration decreases to negligible amounts at  $\beta$ -glucosidase levels of 50 IU/g solids. From the specific activity shown, it appears desirable to operate hydrolysis reactions at a FPA to  $\beta$ -glucosidase ratio of 1:2.

Figure IA-5 presents xylose concentration vs. % substrate in hydrolysis. The amount of xylose produced is directly dependent on the substrate concentration and independent of enzyme activity. This seemed to indicate that xylanase in C-30 is not present in limiting amounts..

Figure IA-6 presents enzyme activity as % of original vs. substrate concentration. Activity decreases with increasing substrate concentration and decreasing initial activity. Figure IA-7 shows a plot of enzyme bound (IU/g) vs. free enzyme IU/ml. The data appears to correlate along an adsorption isotherm.

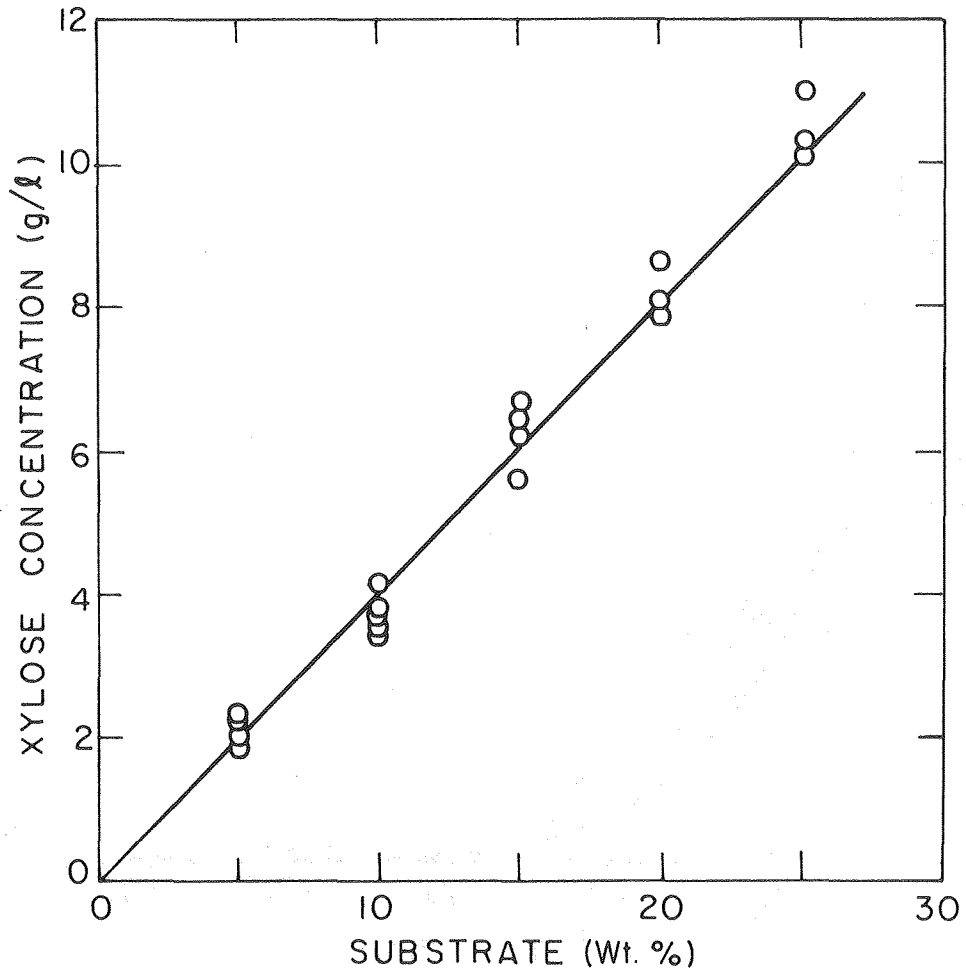
With the information presented, the hydrolysis of acid treated corn stover with T. reesei Rut C-30 cellulase has been sufficiently characterized to enable process evaluation. Qualitatively, under similar operating conditions hydrolysis with cellulase from C-30 vs. cellulase from QM-9414 seem to yield slightly higher yields and react at a faster rate, probably due to higher levels of  $\beta$ -glucosidase activity found in cellulase from C-30. A clear advantage in employing Rut C-30 over QM-9414 appears to be to employ the higher enzyme activities obtained with



XBL 813-5445

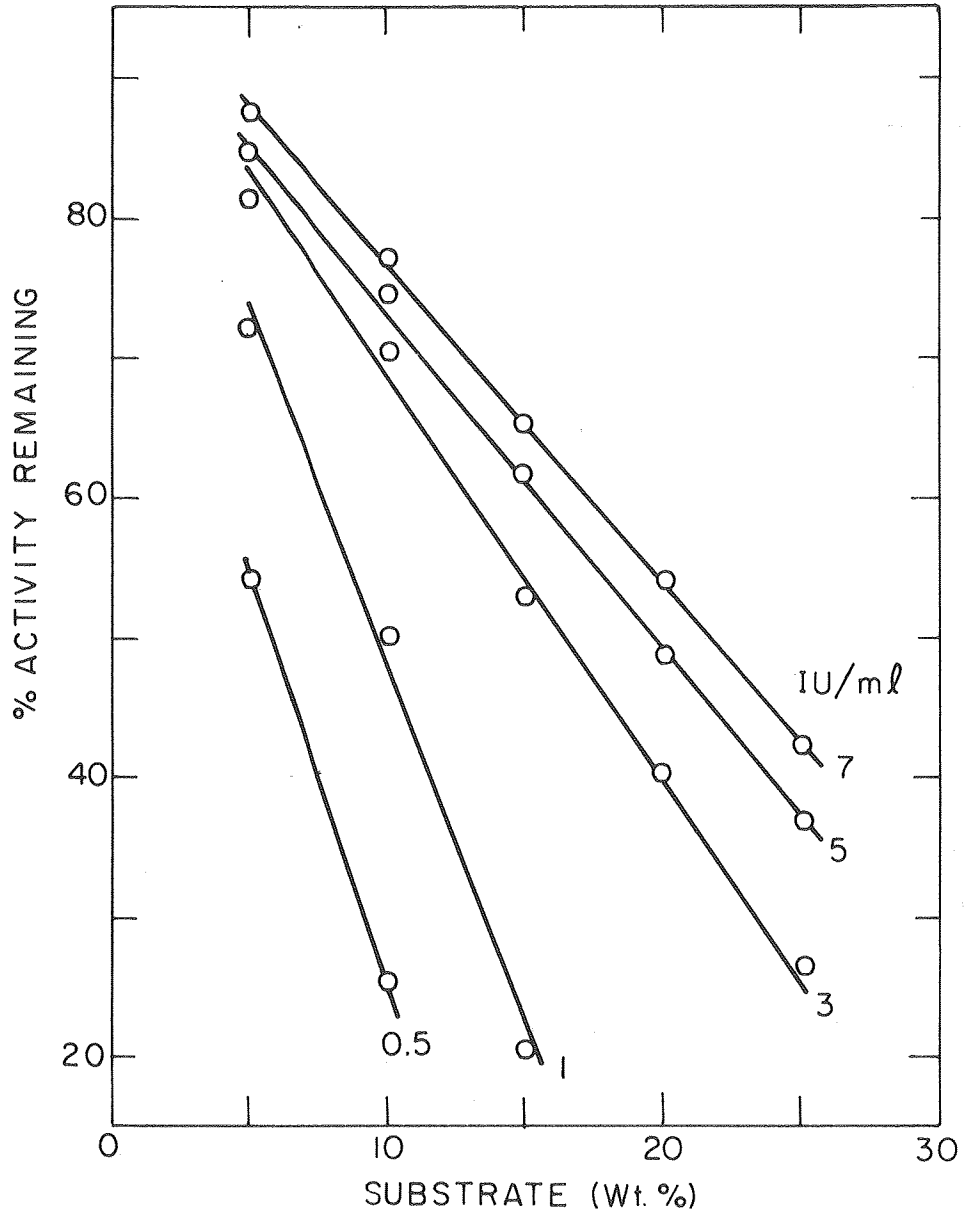
Figure IA-4. The cellobiose concentration produced vs. the specific activity of  $\beta$ -glucosidase in Rut-C-30 enzyme.





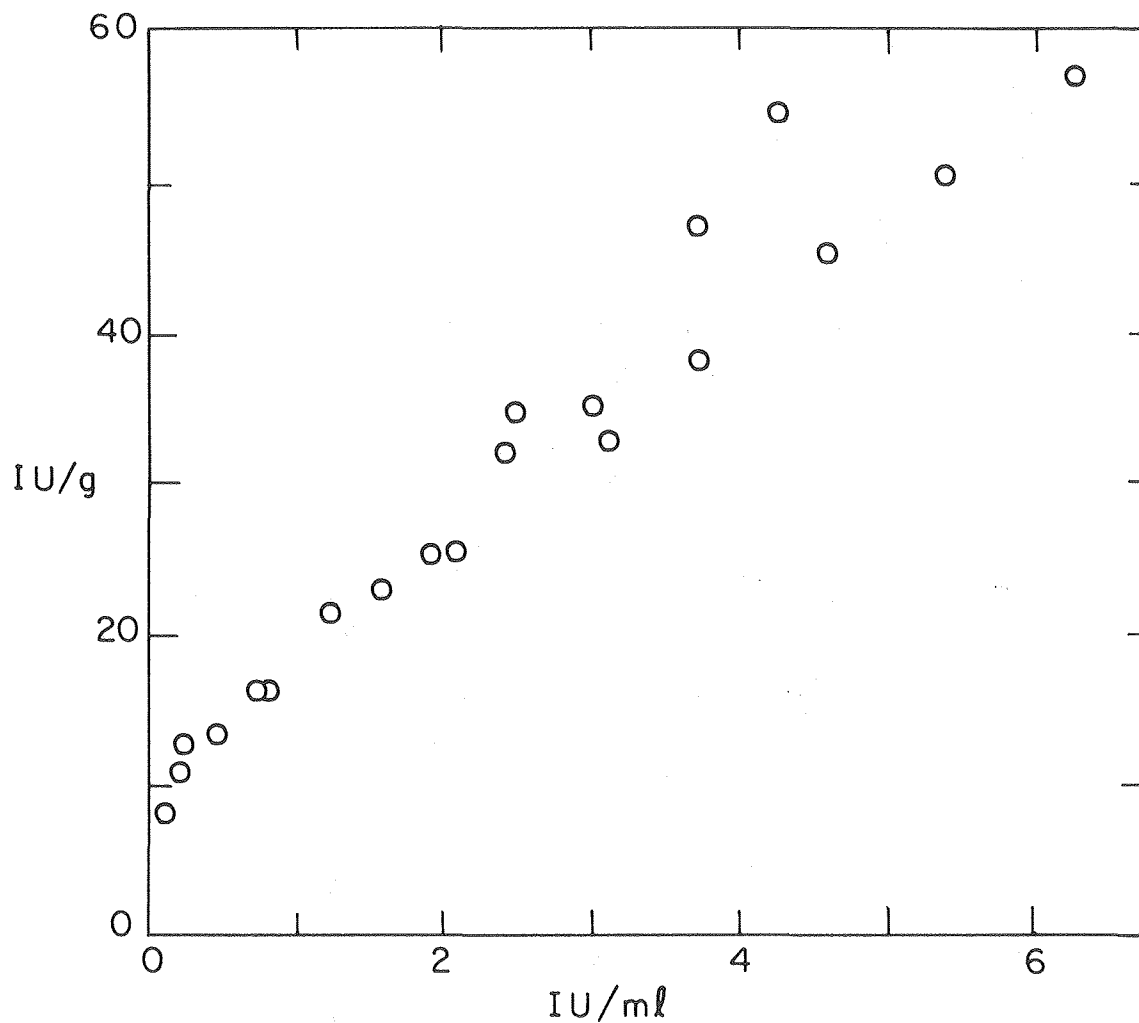
XBL 813-5450

Figure IA-5 Xylose production vs. corn stover loading in Rut-C-30 enzyme.



XBL813-5448

Figure IA-6. The percent of original activity vs. substrate loading at various Rut-C-30 enzyme concentration.



XBL813-5444

Figure IA-7. Apparent adsorption isotherm of bound enzyme (I.U./g) vs. free enzyme (I.U./ml).

C-30 either to operate smaller enzyme production facilities or to obtain more concentrated product streams. However the performances of both enzyme systems under similar specific activity and substrate concentration condition are not different enough to expect radically higher conversion with Rut C-30.

i) Process Design and Evaluation

A computer algorithm was developed for evaluation of alternative glucose manufacturing schemes. Currently, documentation is being prepared for the program and data are being prepared to evaluate the information presented in this report. Process evaluation using cellulase from Rut C-30 will be completed by July 1981.

References

1. R.P. Freitas, B. Long, A. Sciamanna and C.R. Wilke, "Procedures for Analysis of Solids and Liquors from Cellulosic Sources," Lawrence Berkeley Lab, University of California, LBL-5967, December 1977.
2. S.K. Tangnu, H.W. Blanch, and C.R. Wilke, "Enhanced Production of Cellulase, Hemicellulase and B-glucosidase by Trichoderma reesei (Rut-C-30)," Lawrence Berkeley Laboratory, Univ. of California, LBL-11074, June 1980.
3. G.L. Miller, Anal. Chem., 31, 426 (1959).
4. R.T. Ige and C.R. Wilke, "Recovery of the Cellulase Enzyme of Trichoderma viride by Acetone Precipitation." M.S. thesis, Lawrence Berkeley Laboratory, Univ. of California, Berkeley, LBL-3057, June 1974.

## B. Economic Evaluation of Alternative Fermentation Processes

### Introduction

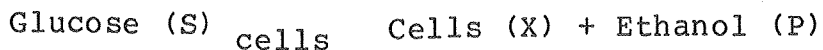
A generalized computer model for evaluation and screening of new fermentation processes is being developed. This new model incorporates a kinetic equation for ethanol production along with design routines for fermentation equipment, centrifuges and selective ethanol removal devices. Design routines may be linked to simulate most new fermentation processes under development. Production and fermentation costs are calculated. The selective ethanol removal routine models devices such as the flashferm flash vessel (1), extractive fermentation extraction train (2), or membrane fermentation membrane module (3). These new selective ethanol removal processes are still in early stages of development and cannot be accurately costed (except for flash fermentation). The new model ascribes no costs to these devices, but does allow evaluation of the potential cost savings in fermentation and distillation equipment, assuming various efficiencies for the separation devices. The required break-even cost for these new separation devices can thus be evaluated in advance of full process development, allowing an early process screening.

### Fermentation Kinetic Model

The rate laws for cell growth and ethanol production are based on the Monod kinetic model applied to the data of Bazua (4) and Cysewski (5) for continuous fermentation of glucose to ethanol

by Saccharomyces cerevisiae var. anamensis (ATCC No. 4126).

The overall fermentation reaction can be represented as



The basic Monod equation expresses the rate for this reaction.

$$v = K'S/(S + C_m)$$

where

$v$  is the specific ethanol production rate [gms ethanol produced /gms cells x hr l];

$K'$  is the reaction rate constant at high substrate concentration ( $\text{hr}^{-1}$ );

$S$  is the glucose substrate concentration [g/L] and  $C_m$  is the Monod constant [g/L].

At high ethanol product concentrations, the fermentation reaction rate is reduced. The basic Monod equation may be expanded (6) to account for this by-product inhibition as:

$$v = k' (1 - P/P_{\max})^{n'} S/(S + C_m)$$

where

$P$  is the ethanol product concentration (g/L), and  $P_{\max}$  is the experimentally determined ethanol concentration above which cells are not viable.

The ethanol production reaction serves to provide energy for cell growth. The cell growth rate  $\mu$  [grams new cells born/grams cells x hr] can thus be related directly to the ethanol production rate as:

$$v = E\mu + M$$

as shown in Figure IB-1.

is proportional to the rate of ATP (energy) production,  $M(\text{hr}^{-1})$  represents a maintenance energy requirement which reduces the ATP available for cell mass production.  $E$  is the substrate utilization efficiency.

The rate of glucose consumption can be calculated from the ethanol yield factor:

$$Y = P / S$$

as the ratio of ethanol produced to glucose consumed.

Using the data of Bazua and Cysewski, the various constants have been evaluated to give the overall model.

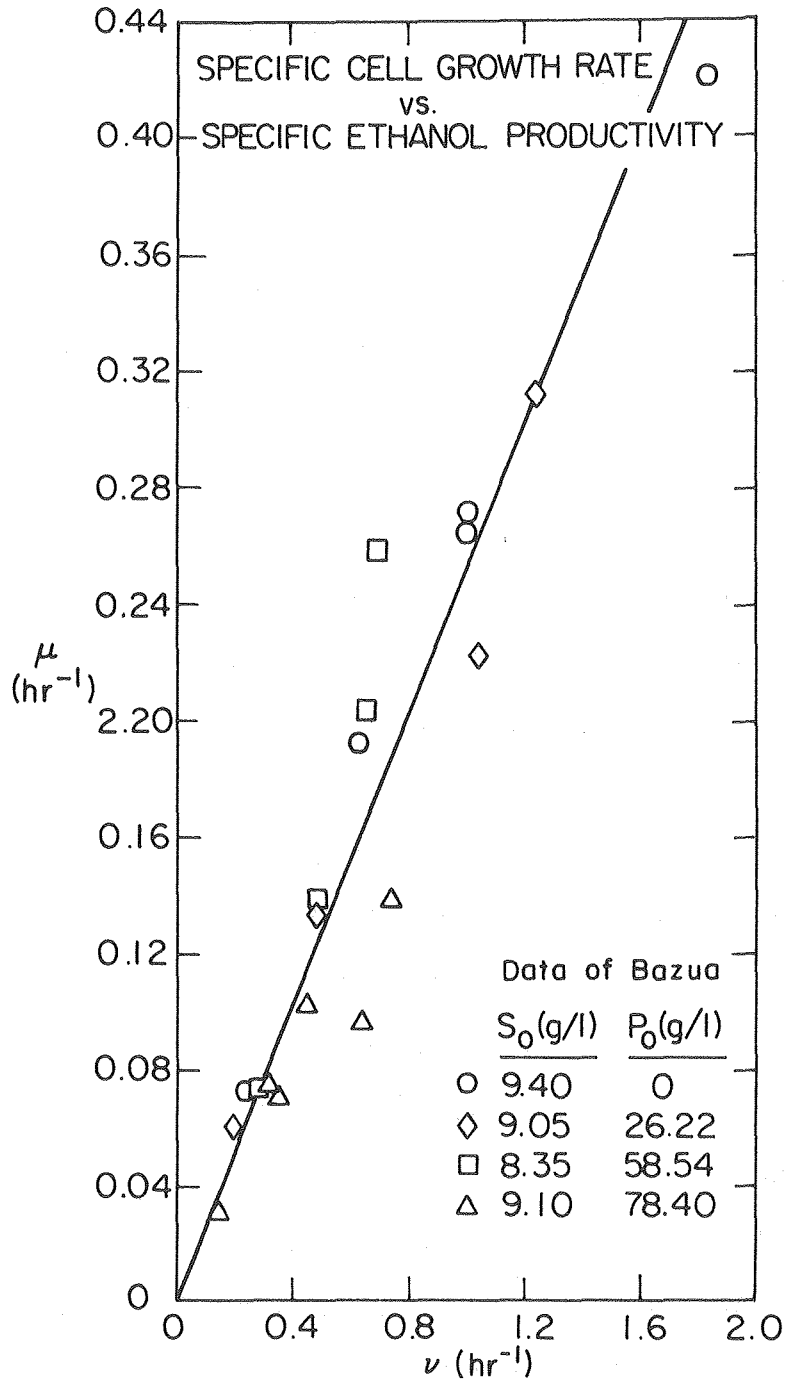
$$v = 1.85 (1 - P/87.5)^{0.36} (S / (S + .315))$$

$$\mu = .249 + 0.0$$

$$Y = .434 = P / S$$

of note,  $P_{\text{max}} = 87.5 \text{ g/L}$ , indicating that a greater than 87.5 g/L ethanol product cannot be produced and hence total utilization of a greater than 201 g/L feed cannot be achieved without an auxiliary ethanol removal system (such as in flash fermentation).  $M = 0.0$ , indicating that for nonstressed cells in the optimized medium of Bazua, the maintenance energy requirement is very small with most ATP utilized in biomass production.

In the computer routines developed from this model, the constants have been maintained as adjustable parameters to allow consideration of the effects of using other microbial strains.



XBL 812-5290

Figure IB-1.



$P_{max}$ , for instance, can be adjusted upward to allow evaluation of the economic advantage possible if a new ethanol tolerant mutant is developed.

Fermentor design routine:

The fermentor design routine utilizes the fermentation kinetic model and mass balance equations to size a fermentor for given inputs and outputs. Consider a CSTR fermentor as shown in Figure IB-2. Given the input substrate, ethanol, and cell mass concentrations we must find the dilution rate required for a specified output product concentration and must find the corresponding output residual substrate and cell mass concentrations. The equations to be used (in order) are:

$$\begin{aligned} S &= S_0 - (P - P_0)/Y \\ X &= E (P - P_0) + X_0 \\ v &= 1.85 (1 - P/87.5)^{0.36} (S/(S + 0.315)) \\ D &= Xv/(P - P_0) \\ V &= F/D \end{aligned}$$

where  $D$ , the dilution rate ( $\text{hr}^{-1}$ ) is the ratio of fermentor volume to feed rate.

This routine may be used to size a single continuous fermentor or (by indexing the outputs from one fermentor to be the inputs to a next) to size combinations of fermentors in series. The plug flow fermentor can be represented as a series of differential reactions and sized by applying the routine over many small reactors in series.

Centrifuge cell recycle routine

SUBROUTINE TO CALCULATE THE FERMENTOR VOLUME AND EXIT  
CONDITIONS OF A CFSTR

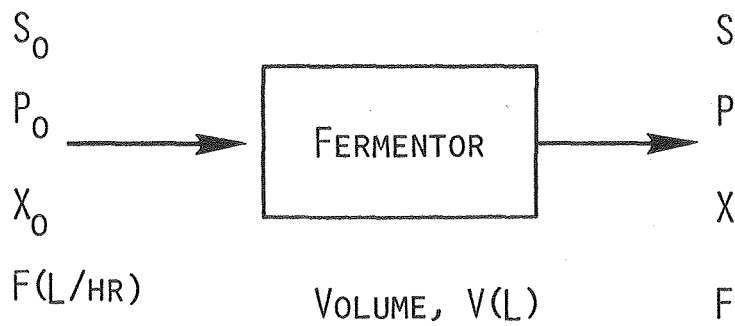


FIGURE IB-2. FLOW DIAGRAM OF A SINGLE-STAGE CONTINUOUS-FLOW,  
WELL-MIXED FERMENTOR SYSTEM

The subroutine for mass balance calculations involving cell recycle streams allows evaluation of processes in which centrifuges (or cell settlers) are used to increase the fermentor cell density. Consider the arrangement shown in Figure IB-3. Given  $P_{out}$ ,  $F$ ,  $S_{feed}$ ,  $X_{feed}$ , (the centrifuge recycle ratio expressed as the ratio of the feed (F) to feed plus recycle (F+R) flow rates), and the concentrated recycle stream cell density  $X_R$ , we must calculate  $S_0$ ,  $P_0$ ,  $X_0$  and  $P$ . The required mass balance relations are:

$$\begin{aligned}P_R &= P_{out} \\P_0 &= (1-\gamma)P_{feed} + P_R \\X_0 &= (1-\gamma)X_{feed} + X_R \\P &= P_{out} \\S_0 &= S_{feed} - [\gamma/(1-\gamma)](P-P_0)/Y\end{aligned}$$

This routine may be incorporated with the previous general fermentor design routine to allow sizing of continuous processes with cell recycle.

#### Selective ethanol removal device routine

This routine simulates selective ethanol removal devices such as the flashferm flash vessel. Consider the arrangement shown in Figure IB-4. Given the input  $F_1$ ,  $P_{feed}$ ,  $X_{feed}$ ,  $S_{feed}$  and specifications for the separator operation, the product flows and compositions must be found.

As in the centrifuge routine, a recycle ratio  $\gamma = F_7/(F_1+F_7)$  and recycle density  $X_R$  are specified. In addition, the separation factor  $K = P_4/P_5$  and fraction of the feed delivered in the concentrated flow  $\Omega = F_4/F_1$  are given to specify the separator

SUBROUTINE FOR MASS-BALANCE CALCULATION INVOLVING CELL RECYCLE SYSTEMS

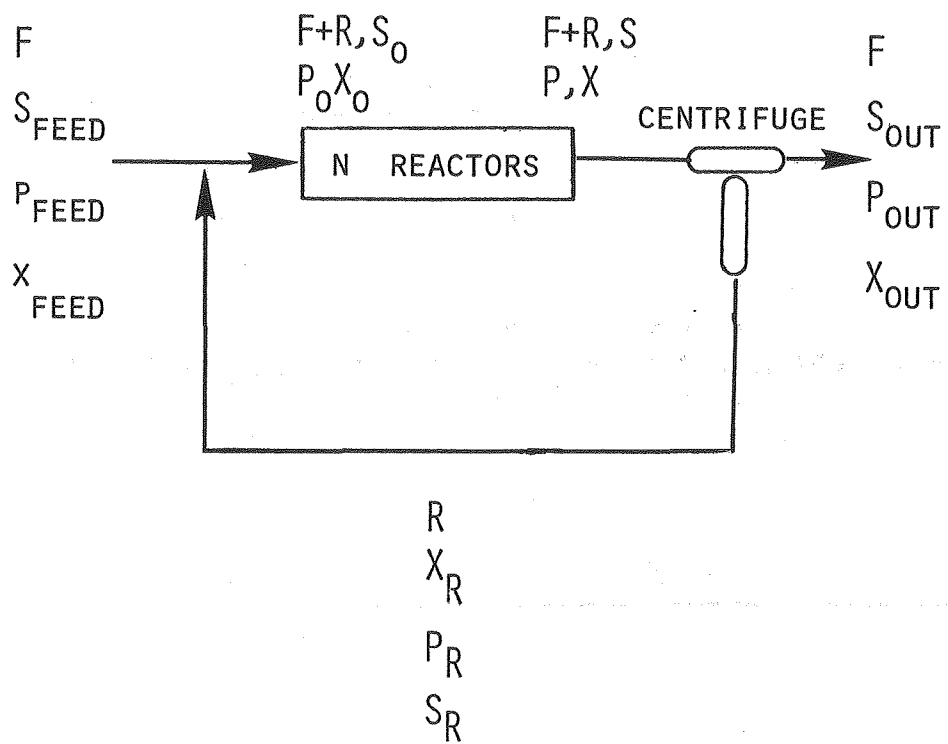


FIGURE IB-3. PROCESS FLOWS FOR RECYCLE MASS-BALANCE CALCULATION.

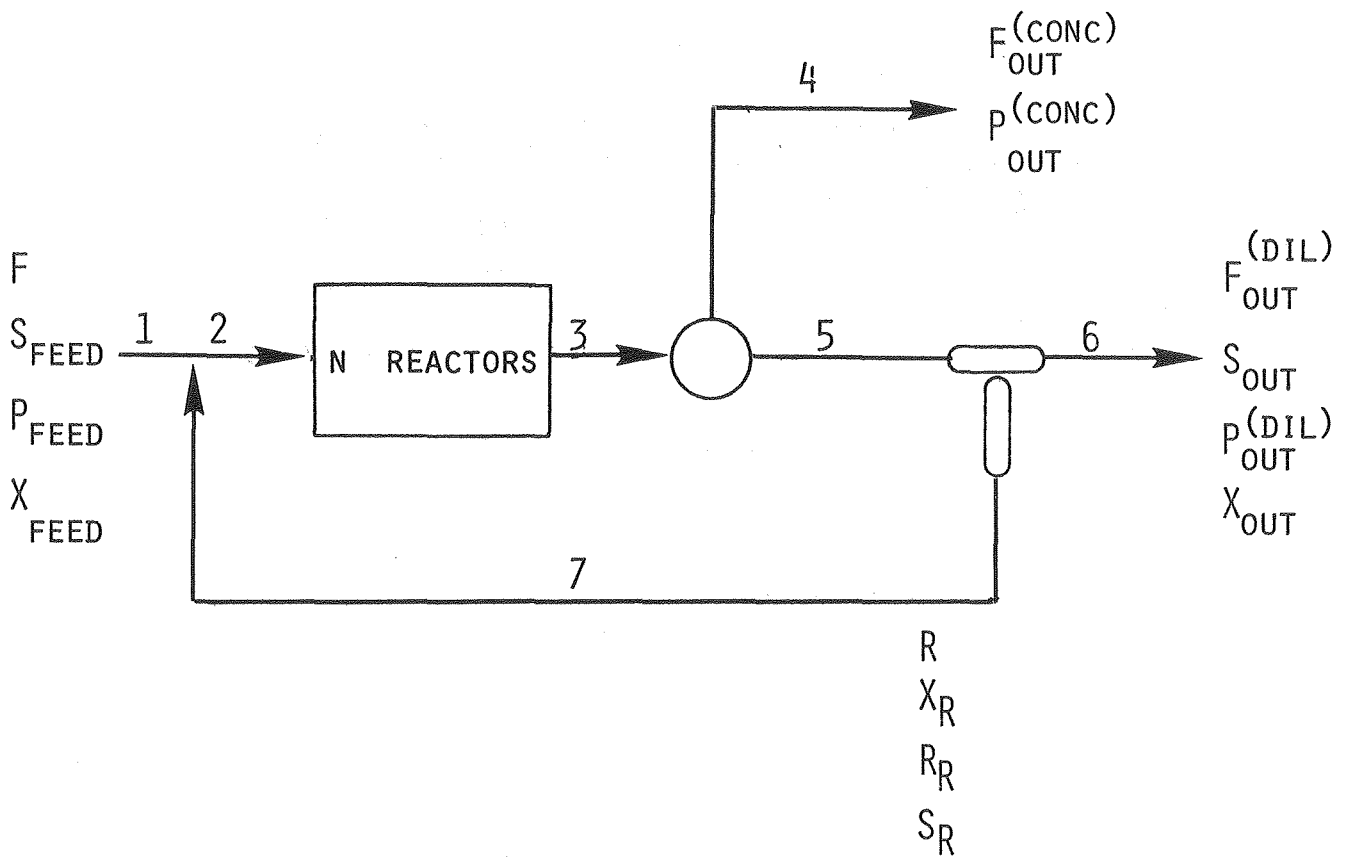


FIGURE IB-4 MASS BALANCE CALCULATION ON SELECTIVE ETHANOL REMOVAL SYSTEM

efficiency. The overall sugar utilization (ratio of sugar input to sugar consumed)  $C = (F_1 S_1 - F_6 S_6) / F_1 S_1$  is then chosen to fully specify the system.

The flows and compositions of all streams can then be specified as follows.

Flows

$$F_1 = \text{Given}$$

$$F_2 = F_1 [1/(1-\gamma)]$$

$$F_3 = F_2 = F_1 [1/(1-\gamma)]$$

$$F_4 = \Omega F_1$$

$$F_5 = F_3 - F_4 = F_1 [1/(1-\gamma) - \Omega]$$

$$F_6 = F_1 - F_4 = F_1 (1-\Omega)$$

$$F_7 = F_1 [\gamma/(1-\gamma)]$$

Sugar Balance

$$S_1 = \text{Given}$$

$$S_6 = [(1-C)F_1 S_1] / F_6 = [(1-C)/(1-\Omega)] S_1$$

$$S_5 = S_6 = [(1-C)/(1-\Omega)] S_1$$

$$S_7 = S_6 = [(1-C)/(1-\Omega)] S_1$$

$$S_4 = 0$$

$$S_3 = (F_5/F_3) S_5 = [1-\Omega(1-\gamma)] [(1-C)/(1-\Omega)] S_1$$

$$S_2 = [F_1 S_1 + F_7 S_4] / F_2 = [(1-\gamma) + \{[(1-C)\gamma]/(1-\Omega)\}] S_1$$

Ethanol Balances

$$P_1 = \text{Given}$$

$$P_7 = [\gamma(F_2 S_2 - F_3 S_3) + F_1 P_1] / (K F_4 + F_5 - F_7)$$

$$P_6 = P_7$$

$$P_5 = P_7$$

$$P_4 = KP_5$$

$$P_3 = [P_4F_4 + P_5F_5]/F_3$$

$$P_2 = [F_1P_1 + F_7P_7]/F_2$$

#### Cell Balance

$$X_1 = \text{Given}$$

$$X_7 = \text{Given}$$

$$X_2 = [F_1X_1 + F_7X_7]/F_2$$

$$X_3 = (1/F_3) [(Y/E)(F_2S_2 - F_3S_3) + F_2X_2]$$

$$X_4 = 0$$

$$X_5 = (F_3/F_5)X_3$$

$$X_6 = [F_5X_5 - F_7X_7]/F_6$$

#### Conclusion

The fermentation design program described, allows the sizing of alternative ethanol fermentation processes. Costing routines are to be added. The generalized computer model will then be used to assess alternative fermentation processes now under development. It will be possible to estimate the potential value of the several new processes under consideration on an equal basis.

#### References

1. B. Maiorella, H. Blanch, and C.R. Wilke, "Rapid Ethanol Production via Fermentation," Presented at AIChE National Meeting, San Francisco, CA, November 29, 1979.
2. E. Pye, and A. Humphrey, "Production of Liquid Fuels from Cellulosic Biomass," Proc., 3rd Annual Biomass Energy Systems Conf., p.69 USDOE, SERI, Golden, Colo., June 5, 1980.

3. H. Gregor, " Membrane Processes of Separation and Concentration in Biomass Harvesting, Production and Refining," Proc. 3rd Annual Biomass Energy Systems Conf. p. 39.

4. C.D. Bazua, Ethanol Effects on the Kinetics of a Continuous Fermentation with Saccharomyces cerevisiae, M.S. thesis. LBL-4423, November, 1976.

5. G. Cysewski, Fermentation Kinetics and Process Economics for the Production of Ethanol, Ph.D. thesis, LBL-4480, March, 1976.

6. O. Levenspiel, Biotech and Bioeng., 8, p. 2672 (1980).



### C. Raw Materials Evaluation

The SERI program at the Lawrence Berkeley Laboratory has undertaken support functions for the Gulf Jayhawk Pilot Plant, Pittsburg, Kansas. Studies to date have focused primarily on enzymatic hydrolysis of corn stover and the subsequent fermentation of the resulting sugars to ethanol. Additional work performed in conjunction with Gulf has been the analysis of corn stover from Nebraska, cotton gin trash from Mississippi and aspen from Canada that was steam exploded by Iotech. These substrates were also enzymatically hydrolyzed on bench scale to establish the benchmark for the Process Demonstration Unit (PDU).

The corn stover, fall of 1979 crop grown in Nebraska, after milling to approximate 0.5 to 2 mm size particles was enzymatically hydrolyzed with cellulase enzyme derived from the Rutgers C-30 strain of Trichoderma reesei. There was obtained an approximately 40% conversion of the available carbohydrate to sugars. This result is much lower than observed for corn stover grown in Indiana and California for the fall 1976, 1977, and 1978, respectively. With an acid pretreatment (1) followed by enzymatic hydrolysis, there was obtained only a 55% carbohydrate conversion. A summary of the results are shown in Figure IC-1. It is unknown why the expected conversion of at least 80% was not obtained. The analysis (2) of this substrate shown in Table IC-1 did not have an unusual composition. In fact, it is a little higher in carbohydrate content compared to those mentioned previously. Essentially, the

SUMMARY

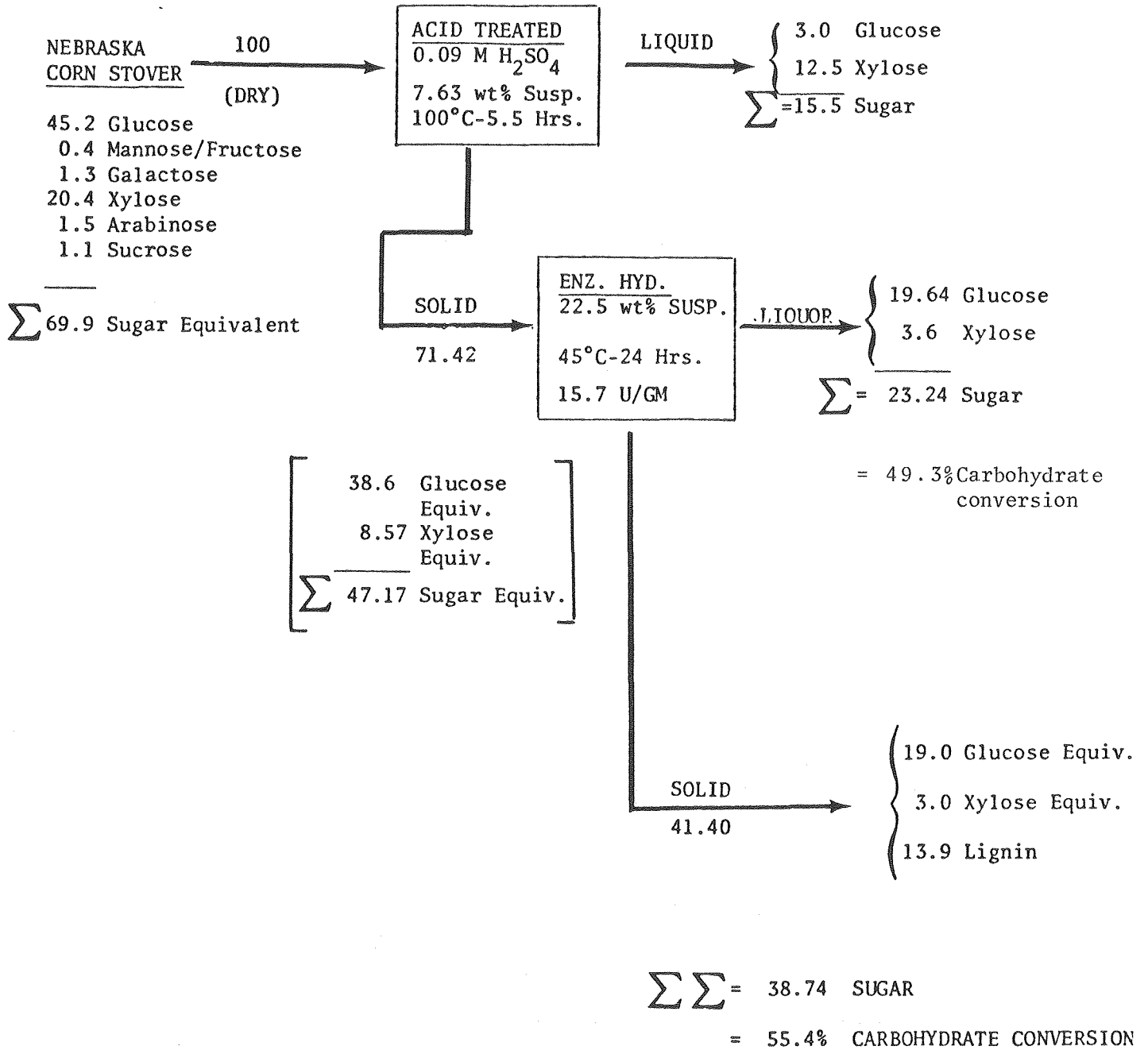


Figure I C-1 Summary of Enzymatic Hydrolysis of Acid Treated Nebraska Corn Stover. Based on 100 lbs of Dry Material.

Table IC-1  
 CORN STOVER<sup>(a)</sup>  
Zeamays-L. (Prairie Valley-76S)  
 From Polk County, Nebraska, Fall Crop 1979  
 ASSAY<sup>(b)</sup>

(%) <u>Carbohydrate</u>	<u>% Sugar and/or Equivalent</u>
40.7      Glucan	45.2      Glucose
0.36     Mannan	0.4       Mannose and/or Fructose
1.2       Galactan	1.3       Galactose
18.0      Xylan	20.4      Xylose
1.36      Arabinan	1.5       Arabinose
	1.1       Sucrose
$\Sigma$ 61.6      Carbohydrate	$\Sigma$ 69.9      Sugar and/or Equivalent
14.8      Lignin	
5.1       Ash { 0.457 Fraction is Acid Insoluble }	
	{ 0.543 Fraction is Acid Soluble }
6.1       Azeotropic Benzene/Alcohol Extractives	
4.2       Other Acid Insolubles (organic)	
4.4       Protein	

(a) 1 inch tub milled particles, courtesy of Charles Wosel and Mark Holscher, Gulf Oil Chemicals Co., Pittsburgh, Kansas 66762.

(b) 2 mm Wiley milled, 0.25 - 0.35 mm fraction, 100% dry.

same results were obtained at the SERI/Gulf PDU on enzymatic hydrolysis of much larger batches (approximately 100 lbs) of this residue.

While awaiting delivery of steam explosive decompressed wood to arrive from Iotech Co., Canada, work was started on cotton gin trash that was on hand at the Gulf Jayhawk plant. About 5 KG. sample of Gulf cotton gin trash, grown in Indianola, Mississippi, 1975 harvest was supplied to us. This was analyzed and the analysis is shown in Table IC-2. This cotton gin trash is considerably higher in carbohydrate content compared to what was generated in California (1). Upon enzymatic hydrolysis there was obtained a carbohydrate conversion of only 19%, nearly equal to what was obtained with the residue from California. A summary of results are shown in Figure IC-2. Even though the residue from Mississippi contains about 2.5 times more carbohydrate, apparently the cellular carbohydrate is tightly bound or completely sheathed with lignin thus preventing enzyme accessibility. Since this residue appears to be an unlikely candidate for economic conversion of the carbohydrate to fuel alcohol, no further studies were performed here. A 100 pound batch hydrolysis done at Gulf resulted in essentially the same results.

Results from preliminary enzymatic hydrolysis on one of the samples of steam exploded wood showed better than 90% carbohydrate conversion. This wood, treated by Iotech, was shipped and stored at  $-37^{\circ}\text{C}$  to decrease the rate of crystallization of the

Table IC-2  
COTTON GIN TRASH

GOSSYPIUM HIRSUTUM-L.

GROWN IN INDIANOLA, MISS, 1975 HARVEST (a)

ASSAY<sup>(b)</sup>

<u>(%) CARBOHYDRATE</u>		<u>% SUGAR EQUIVALENT</u>	
48.9	Glucan	54.4	Glucose
2.0	Other Hexosan (c)	2.2	Other Hexose
16.9	Xylan	19.3	Xylose
0.5	Arabinan	0.6	Arabinose
$\Sigma$ 68.3	Carbohydrate	$\Sigma$ 76.5	Sugar Equivalent
20.7	Lignin		
4.8	Ash		
2.8	Other Acid Insolubles		
4.2	Azeotropic Benzene/alcohol Ext.		

(a) 0.032 inch Hammer Milled Particles, Courtesy of Charles Wosel, Gulf Oil Gulf Oil Chemicals Co., Pittsburgh, Kansas

(b) 2 MM Wiley Milled, Unsieved fraction, 100% dry.

(c) Includes Mannan (Mannose) and Galactan (Glactose)

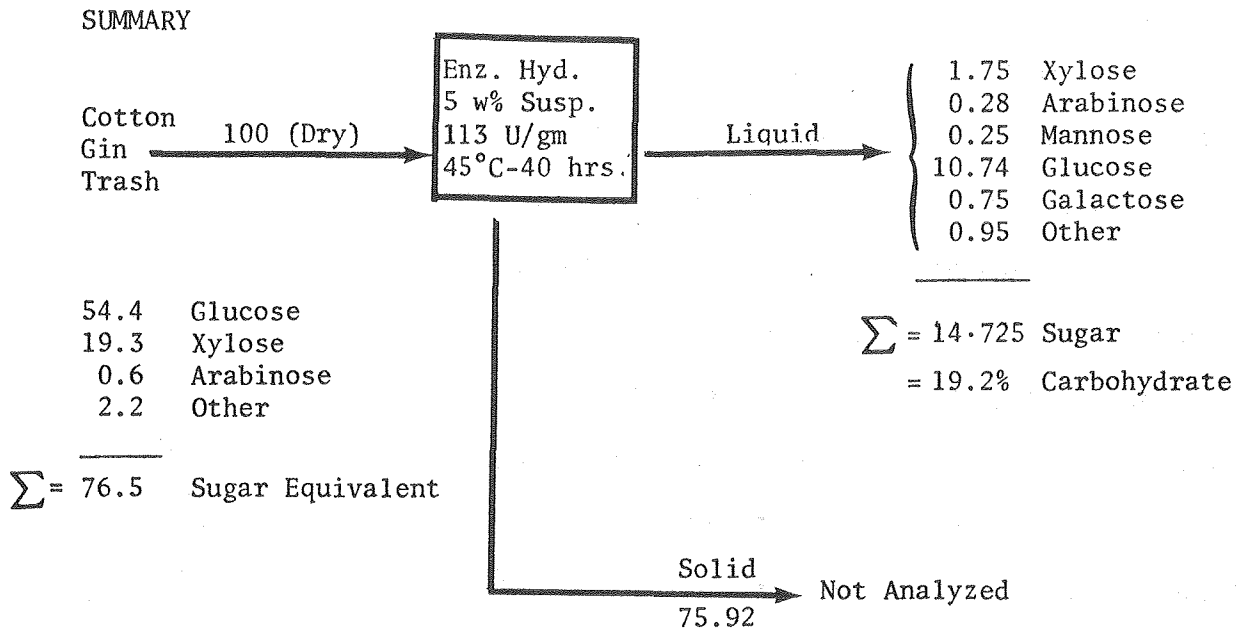


Figure I C-2. Summary of Enzymatic Hydrolysis of Mississippi Cotton Gin Trash. Based on 100 lbs of dry material.

cellulose. A moisture determination on an inner core sample showed about 75% moisture (25.25% dry). The freeze dried brown powder appeared to be very fine, comparable to -400 mesh (approximately 35 $\mu$ M) ball milled populus.

For enzymatic hydrolysis, and to maintain comparable enzyme activity as used in the past for 5 w/w% suspensions, the 7.1 I.U./ml (F.P.A.) Rutgers C-30 enzyme solution was freeze concentrated by a factor of 1.18. The enzymatic solution was concentrated rather than freeze drying the exploded wood substrate because of probable glucan recrystallization and consequent longer hydrolysis time for a comparable glucan conversion to glucose.

5 w/w% suspensions were hydrolyzed at 45°C for forty hours and sampled at 20 and 40 hours. The relative sugar, as shown by DNS, was 42 and 43.0 g/L, respectively. A plot of relative sugar production vs. time shows the hydrolyses were essentially 98% complete in 20 hours and about 93% complete in 10 hours HPLC analysis of the enzyme hydrolyzate showed an average composition of:

22. % xylose

1.9% mannose at 39.56 $\pm$ 0.45 grams of sugar/liter

76. % glucose

The 100% dried hydrolyses residues averaged 24.7% of the starting amount of (dry based) exploded wood. It should be noted that the residue was 95.1% lignin as determined by colorimetric analysis by a modified method of Moore, Johnson and Zank (3). A summary of the results of enzymatic hydrolysis are shown in Figure IC-3.

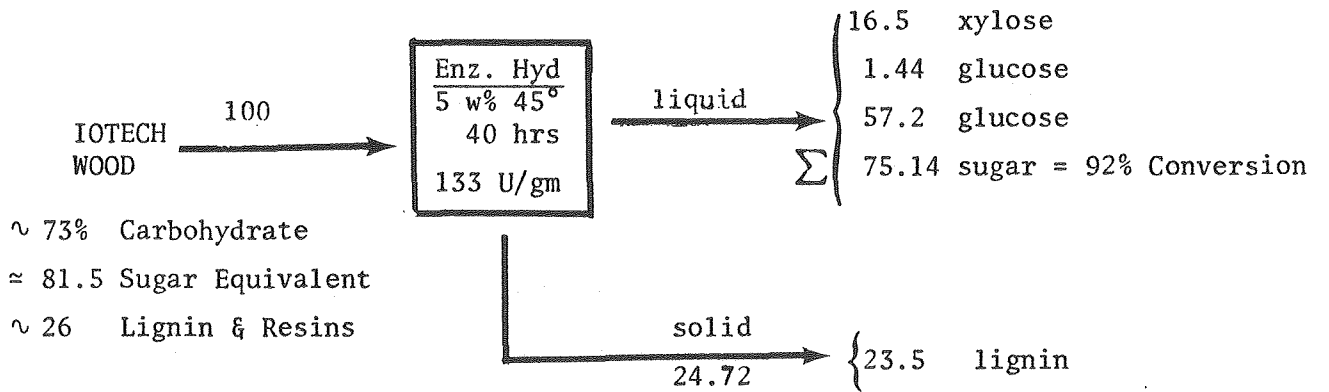


Figure I C-3 Summary of Enzymatic Hydrolysis of Aspen, Iotech Steam Exploded #W-800421-2. Based on 100 lbs of (dry) substrate.



Since the enzymatic hydrolysis of this steam exploded wood was considered exceptional, enzyme recovery experiments were performed on the hydrolyzates. The sugars were removed by ultrafiltration. The results of enzyme recovery are shown in Table IC-3. As can be seen that with only the enzyme solution present and stirred for 40 hours at 45°C, about 14% of the cellobiohydrolase ( $C_1$ ) activity, and about 20% of the beta glucosidase (B-g) activity was lost. The Beta 1-4 glucanase ( $C_x$ ) activity remained the same as original starting amount as did the soluble protein (S.P.). It would appear that a certain amount of activity was lost due to thermal denaturation.

With 5 w/w% suspensions of Iotech wood in Rut-C-30 enzyme solution, 10 to 40% of the various activities are not recovered. Since it was unknown what effect lignin has on the enzyme components including the apparent thermal denaturation mentioned above, a mixture of 1.24 w/w% lignin (recovered from hydrolyzed Iotech wood) in enzyme was stirred for 40 hours and 45°. This suspension is comparable to what was obtained as a residue from 40 hours hydrolysis of the wood carbohydrate. As shown in Table IC-3, 14 to 25% of the various activities are not recovered. The approximate 11% of the soluble protein not recovered from enzymatic hydrolysis of the treated wood is apparently not due to adsorption on predominantly lignin residue. The S.P. was nearly all recovered from the mixture when only the ligneous residue was present and the other activities still decreased including that due to thermal

Table IC-3

Enzyme Recovery of Hydrolysis of Aspen, Iotech Steam Exploded #W800421-2.

Rut-C-30 original composition per ml of solution:

$C_1$	=	0.407 IU
$C_x$	=	254 IU
$\beta$ -g	=	2.45 IU
S.P.	=	16.6 mg

	% of original component remaining in solution after ultra filtration			
	$C_x$	$C_1$	$\beta$ -g	S.P.
Original enzyme @ 45°C. & 40 hours	100	86	82	100
Above + 1.24 w% lignin	87	86	76	97
Above + 5 w% Iotech wood	82	60	76	89

denaturation. Therefore, it was concluded that the 97% recovered was not due to the possible effect that the ligneous residue was already saturated with protein from the previous hydrolyses.

A tentative conclusion indicated by these experiments is that about 15 to 20% of the original enzyme activity is not recovered due to thermal denaturation. The other losses are not necessarily due to adsorption of Rut-C-30 derived enzyme solutions on steam exploded wood where most of the carbohydrate is dissolved and hydrolyzed to sugars.

Samples of aspen, steam exploded by Iotech, #V-56 and H-60, for the P.D.U. at Gulf Oil Chemicals Jayhawk Plant were received. The several pounds of #V-56 and H-60 were refrigerated to help maintain uniform moisture content and slow down apparent bacteria or mold growth in the case of #V-56.

An inner core sample of #V-56 showed 62.22% moisture. Duplicate 5 w/w% suspensions of #V-56 were hydrolyzed with our standard (7.0 I.U./ml) Rut-C-30 enzyme. Since the activity of this stock enzyme was relatively high, it was not freeze concentrated nor was the wood substrate freeze dried to maintain the constant 133 U/GM of substrate, as was done previously for the hydrolyses with Iotech aspen #W800421-1. The hydrolyses were sampled at 16 hours and 40 hours and showed by DNS a relative sugar production of 34 and 39 grams of sugar per liter, respectively. Analyses for the sugar content of the 40 hour hydrolyzates by HPLC gave an average composition of:

15.4% xylose

82.5% glucose at 29.82  $\pm$ 0.24 grams of sugar/liter

0.9% galactose

1.2 mannose

A summary of the results are shown in Figure IC-4 considering the age of this treated substrate, the carbohydrate conversion of about 86% is quite good though not surprising when it is noted that nearly 10% of the treated wood is already sugar.

The assay of the Iotech aspen #V-56 is shown in Table IC-4. The assay procedure (2) was modified to accommodate the relatively high sugar and high water soluble lignin, resins and acids encountered with this substrate. This procedure will be published in the future when further variations are performed with this procedure on other substrates.

As shown in Table IC-4, nearly 27% of the dry wood was hot water (80°C soluble). This result was obtained by a dozen single pass (not reboiled) rather than the usual multipass reboil Soxhlet extraction. The combined single pass extracts were then freeze dried to concentrate the water soluble fraction. The sugar content was determined on known portions of the redissolved solute by HPLC. Another portion was subjected to secondary acid hydrolysis for total sugars, as the monomers and determined by HPLC and GLC. The soluble lignin was determined spectrophotometrically (3) after a portion of the dried solute was extracted with benzene. The organic acids were estimated by titration to pH 8 and the result was

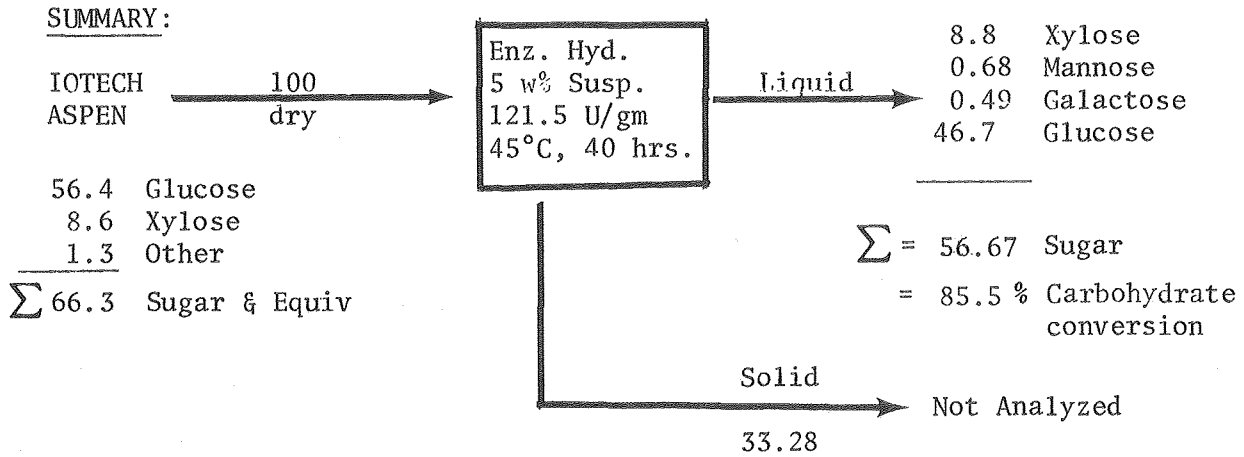


Figure I C-4. Summary of Enzymatic Hydrolysis of Iotech Aspen #56-V. Based 100 lbs. of Dry Material.

Table IC-4

Aspen

Populus Tremuloides

Hardwood, Steam Exploded, Iotech #V-56<sup>(a)</sup>

ASSAY<sup>(b)</sup>

<u>% Carbohydrate and Sugars<sup>(c)</sup></u>		<u>% Sugar Equivalent</u>
49.4	Glucan	56.4 Glucose
1.4	Polyglucose(d)	8.6 Xylose
1.5	Xylan	
6.9	Polyxylose (d)	1.3 Other Sugars
1.3	Other Sugars	
$\Sigma$ 60.6	Carbohydrate and Sugars	$\Sigma$ 66.3 Sugar Equivalent
13.5	Lignin with a distribution: 0.403 (80°C) water soluble and 0.597 water insoluble	
22.5	Benzene/Alcohol Extractive(e)	
0.6	Ash	
0.3	Acid Insolubles	

(a) 37.8% dry wood, Iotech Steam Exploded. Courtesy of Charles Wosel, Gulf Oil Chemical Co., Pittsburg, Kansas 66762, and John Leigh, Iotech Corp., 220 Laurier Ave.-West, Ottawa, Ontario, Canada.

(b) Freeze Dried to 100% dry, 2 mm Wiley milled to repulverized the aggregated powder, 0.25 to 0.35 mm fraction.

(c) 26.75% of the dry wood was (80°C) water soluble with a distribution after secondary acid hydrolysis:

0.053 Glucose  
 0.006 Mannose  
 0.257 Xylose  
 0.010 Arabinose  
 0.020 Ribose  
 0.012 Unknown hexose  
 0.204 Lignin  
 ~ 0.44 Acids & Resins.

(d) Polyglucose is the monomer and oligomers including hot water soluble glucans and substituted glucans. The polyxylose is the monomer and oligomers including hot water soluble xylans.

(e) Total azeotropic benzene/alcohol extractive was 35.85% with a distribution: 0.373 hot water soluble acids and resins, and 0.627 water insoluble.

combined with resins, since the "water soluble resin" apparently is variable.

The 22.5% azeotropic benzene/alcohol extractive is counted only as the water insoluble fraction, since about 0.37 of the total extractive (35.85%) is also water soluble. Water soluble lignin was also observed in the fraction of the extractive.

The Iotech aspen #V-56 was washed at the Jayhawk PDU with warm water in a 3 w% suspension. It was then vacuum filtered resulting in 23.2% dry solids and labelled by us as #V-56W. Iotech aspen #V-56 was washed to reduce the lignin content for its hopeful use as substrate for enzyme production. A certain measure of success on the effort was thus obtained at the Jayhawk PDU. Unfortunately, as can be seen in Table IC-5, most of the readily available sugar was also removed. In Iotech #V-56, about 16% of carbohydrate is already sugar and almost all of this was removed including about 20% of the lignin (or about 40% of the water soluble lignin). About 94% of the water soluble acids and resins component of the azeotropic benzene/alcohol extractive was also removed.

Iotech aspen #H-60 was also used as a substrate at the Jayhawk PDU. The assay is shown in Table IC-6. The striking difference is the much larger available glucose content of 10% of the total carbohydrate compared to about 2% in Iotech aspen #V-56. Because the water soluble lignin content in #H-60 is 6.1% vs. 5.4 in #V-56, and coupled with the overall decrease in carbohydrate content in the former one could conclude the former was subjected to higher

Table IC-5

ASPEN

Populus Tremuloides

Hardwood, Steam Exploded, Iotech #V-56-W<sup>(a)</sup>

ASSAY<sup>(b)</sup>

<u>% Carbohydrate and Sugar</u> <sup>(c)</sup>	<u>% Sugar Equivalent</u>
62.7 Glucan	69.7 Glucose
4.7 Xylan	
0.50 Polyxylose (d)	5.8 Xylose
$\Sigma$ 67.9 Carbohydrate and Sugars	$\Sigma$ 75.5 Sugar Equivalent
11.0 Lignin with a distribution: 0.293 (80°C) water soluble and 0.707 water insoluble.	
19.4 Benzene/alcohol extractives (e)	
0.5 Ash	
1.0 Acid insolubles	

- (a) 23.2% dry wood resulting from vacuum filtering of water washed (in a 3 wt% suspension) Iotech #V-56 steam exploded aspen. Courtesy of Charles Wosel and Dan Stoops, Gulf Oil Chemical Co., Pittsburgh, Kansas 66762, and John Leigh, Iotech Corp., 220 Laurier Avenue-West, Ottawa, Ontario, Canada.
- (b) Freeze Dried to 100% dry, 2 mm Wiley milled to repulverize the aggregated powder, 0.25 to 0.35 mm fraction.
- (c) 7.87% of the dry wood was (80°C) water soluble with a distribution after secondary acid hydrolysis:
- |        |                |
|--------|----------------|
| 0.063  | Xylose         |
| 0.412  | Lignin         |
| ~ 0.53 | Acids & Resins |
- (d) Polyxylose is the monomer and oligomers including hot water soluble xylans.
- (e) Total azeotropic benzene/alcohol extractive was 20.12% with a distribution: 0.038 hot water soluble acids and resins and 0.962 water insoluble.



Table IC-6

ASPEN  
Populus Tremuloides  
 Hardwood, Steam Exploded, Iotech #H-60<sup>(a)</sup>  
 ASSAY<sup>(b)</sup>

% Carbohydrate & Sugar <sup>(c)</sup>	% Sugar Equivalent
42.0 Glucan	52.4 Glucose
5.8 Polyglucose (d)	
5.3 Xylan	9.3 Xylose
3.4 Polyxylose (d)	
1.0 Other Sugars	<u>1.1 Other Sugars</u>
$\Sigma$ 57.6 Carbohydrate & Sugars	$\Sigma$ 62.8 Sugar Equivalent
13.6 Lignin with a distribution: 0.449 (80°C) water soluble and 0.551 water insoluble.	
26.8 Benzene/Alcohol Extractives (e)	
0.5 Ash	
0.2 Acid Insolubles	

- (a) 22.5% dry wood, Iotech Steam Exploded. Courtesy of Charles Wosel, Gulf Oil Chemicals Co., Pittsburg, KS 66762, and John Leigh, Iotech Corp., 220 Laurier Avenue-West, Ottawa, Ontario, Canada.
- (b) Freeze Dried to 100% dry, 2mm Wiley milled to repulverize the aggregated powder, 0.25 to 0.35 mm fraction.
- (c) 31.93% of the dry wood is (80°C) Water soluble with a distribution after secondary acid hydrolysis:
- |        |                 |
|--------|-----------------|
| 0.180  | Glucose         |
| 0.007  | Mannose         |
| 0.105  | Xylose          |
| 0.011  | Arabinose       |
| 0.012  | Ribose          |
| 0.006  | Unknown Hexose  |
| 0.191  | Lignin          |
| ~ 0.49 | Acid and Resins |
- (d) The polyglucose is the monomer and oligomers including hot water soluble glucans and substituted glucans. The polyxylose is the monomer and oligomers including water soluble xylans.
- (e) Total azeotropic benzene/alcohol extractives was 42.11% with a distribution: 0.366 hot water soluble acid and resins, and 0.636 water insoluble.

temperature and/or residence time in the treatment. The assignment of the presence of ribose in both cases is based solely on emergence times from HPLC and GLC and therefore is not yet totally conclusive.

#### References

1. A.F. Sciamanna, R.P. Freitas, C.R. Wilke, "Composition and Utilization of Cellulose for Chemicals from Agricultural Residues," Lawrence Berkeley Laboratory, University of California, LBL-5966, December 1977.
2. R. Freitas, B. Long, A. Sciamanna, and C.R. Wilke, "Process for Analysis of Solids and Liquors from Cellulosic Sources," Lawrence Berkeley Laboratory, University of California, LBL-5967, December 1977.
3. W.E. Moore, and D.B. Johnson, "Procedures for Chemical Analysis of Wood and Wood Products," U.S. Dept. of Agriculture, Forest Service, Forest Products Laboratory, Madison, Wisconsin, December 1967. Also D.B. Johnson, W.E. Moore, and L.C. Zank, Tappi, 44, 793 (1961).

#### D. Evaluation of Pretreatment Process

A comparison of the acid pretreatment process used here on a hardwood and a steam explosive/decompressionn treatment on a similar hardwood it would appear that the latter is considerably more effective in enhancing rate as well as overall yield of fermentable sugars by enzymatic hydrolysis. With the hardwood, Populus tristis, a carbohydrate conversion of 9% was obtained (1). When the same wood was pretreated with boiling 0.09 M (approximately 1 w%) sulfuric acid, followed by enzymatic hydrolysis, the carbohydrate conversion doubled to about 19% including the sugars in acid liquor. The experiments described in the previous section with steam exploded/decompressed wood, 85 to 92% conversions were obtained from enzymatic hydrolysis. As mentioned previously the steam treated wood has about 17% available sugar present which provides a portion of the considerable increase in carbohydrate conversion. Removing the available sugar by washing the substrate, the conversions are still a respectable 70 to 75 %, and comparable to those obtained with more easily hydrolyzable agricultural residues, such as acid, and/or steam treated corn stover.

With the steam treated wood, the 5 to 6% water soluble lignin and the 13 to 15% water soluble resins will have to be taken into consideration. If as proposed in those designs that utilize lignin and resins as fuel for plant steam, it would appear this form of lignin is not readily available as a solid fuel. Additionally, these soluble materials represent an

additional load on the system up to and including an anaerobic digester for utilization of fermentation and distillation slops.

Washing the steam exploded wood with hot water to remove the water soluble components, especially the water soluble lignin and resins for enzyme production, it would appear that a wash ratio of about 150 parts of hot water per weight part of wood is necessary for effective extraction. This ratio might not be reasonable on a single pass basis. It is unknown at this time whether a multiple pass or countercurrent extraction is economically feasible including disposal of the liquor and extracts. It is assumed that the extracts, if fermented for pentose removal and dried somewhat would at least have plant fuel value if not by-product credit.

If all of the treated wood that is used for hydrolysis as well as enzyme production is extracted to obtain a pentose stream then further studies are needed to evaluate feasibility of fermentation of these pentoses in the presence of considerable amounts of other substances. Also it might be advantageous to use a low boiling recoverable solvent for extraction rather than hot water.

#### References

1. C.R. Wilke, "Process Development Studies on the Bioconversion of Cellulose and Production of Ethanol," Lawrence Berkeley Laboratory, University of California, Berkeley, LBL-7880, Sept. 1978, pp. 7-9.

## II. MICROBIOLOGICAL AND ENZYMATIC STUDIES

### A. Production of Cellulase Enzyme from High Yielding Mutants

In the process of converting biomass to ethanol, via a glucose syrup, there are two major economic obstacles; the high cost of substrate pretreatment, and the production of cellulolytic enzymes. It is therefore essential to use optimum conditions for cellulase production, in order to gain improved activity and so lower the costs involved.

Past work with QM-9414 has shown that cellulase productivity could be increased two-fold by raising the substrate concentration from 2% to 5%, along with increased nitrogen content and pH control. More recent work has aimed to optimize environmental parameters for cellulase production by the C-30 strain, predominantly using Solka Floc at a concentration of 1%. Although, the presence of Tween 80 has been shown to increase the level of extracellular cellulase, this is most effective at low concentrations (0.01%-0.02%) for the C-30 strain. Increasing the concentration of Tween 80 to 0.1% resulted in a 40% decrease in filter paper activity.

The addition of urea (0.3 g/l) to the medium produced higher initial levels of  $C_1$  and  $C_x$  activity, but final maximum levels were similar whether or not urea was present. Filter paper activity was higher in the absence of urea.

Over a range of pH values investigated, pH 5.0 resulted in the highest production of filter paper activity,

whereas pH 4.0 produced higher levels of  $C_1$  and  $C_x$  activities. Similarly, a temperature of 25°C resulted in maximum filter paper activity, while maximum  $\beta$ -glucosidase,  $C_1$ , and  $C_x$  activities were achieved at 28°C.

Although extensive work has been done with QM-9414 in 2-stage continuous fermentation, limited information is so far available for the C-30 strain. Indications to date suggest a temperature of 28°C and pH 5.0. Work is proceeding in this area to obtain further information about control variables, and to increase the productivity of the organism.

## B. Hydrolysis Reactor Development

### (1) Enzyme Recovery in Cellulose Hydrolysis

Urea is introduced into the cellulose hydrolysis to enhance cellulase enzyme recovery. The effects of urea on the sugar fermentation of Saccharomyces cerevisiae are being studied. Through three months of continuous operation, a urea concentration of 0.500 M was achieved before contamination became severe. It is not yet apparent if any adaptation by the yeast occurred.

At 0.500 M urea, the exponential specific growth rate is  $0.272 \text{ hr}^{-1}$ . At a dilution rate of  $0.146 \text{ hr}^{-1}$  the outlet ethanol concentration was 25. g/L with productivity of 3.7 g/(L-hr). This compares favorably with the base case (4.0 g/(L-hr): no urea). The cell density at a dilution rate of  $0.146 \text{ hr}^{-1}$  is 5.6 g/L compared to 9.1 g/L: no urea, giving a very high specific ethanol productivity of 0.67 g EtOH/(g cells-hr) compared to 0.44 g/(g-hr): no urea. This high conversion is supplemented with an ethanol yield factor (g ethanol produced/g glucose consumed) of 0.42 compared to 0.36: no urea.

Experiments on complete sugar utilization indicate that over the range of 0.0-0.5 M urea, glucose is consumed to less than 1.0 g/L, albeit at dilution rates less than  $0.10 \text{ hr}^{-1}$ .

Effects of urea on S. cerevisiae are given in Table IIB(1)-1, with ethanol productivities for two dilution rates.

At 1.0 M urea, the behavior of the yeast becomes erratic. A large initial inoculum results in more vigorous growth. At an initial glucose concentration of 100 g/L, the

Table IIB(1)-1

Effect of Urea on Cell Growth Rate  
and Ethanol Productivity

Urea Concentrations (M)	Exponential Growth Rate (hr <sup>-1</sup> )	Ethanol Productivity (g/L-hr)	
		<u>D = 0.25</u>	<u>D = 0.15</u>
0.000	0.450	4.7	4.0
0.125	0.375	4.6	4.2
0.250	0.341	4.7	3.9
0.500	0.272	-	3.7
0.750	0.153	-	-
1.000	0.070	-	-



highest batch cell growth achieved levels off at 2 g/L. The exponential growth rate is  $0.07 \text{ hr}^{-1}$ . However, the glucose concentration drops to 1 g/L. Attempts to run continuously at a dilution rate of  $0.04\text{-}0.06 \text{ hr}^{-1}$  resulted in wash-out. Ethanol production rates in batch growth are being explored.

It is apparent that urea concentrations higher than 0.75 M cause severe physiological anomalies in yeast metabolism.

(2) Countercurrent Hydrolysis Reactor Development

In the proposed scheme of enzymatic hydrolysis of cellulose a continuous hydrolysis stage is coupled with an enzyme recovery stage. The purpose of such a scheme is to recycle the enzyme as much as possible, thereby reducing the amount of make-up enzyme required.

The enzyme recovery stage is a countercurrent adsorption process where pretreated cellulose solids are contacted countercurrently with the hydrolysis produce stream, which consists of sugar and enzyme in solution. A considerable amount of the enzyme in solution should adsorb onto the cellulose solids, resulting in a sugar solution containing much less enzyme. The cellulose solids, after adsorbing as much of the enzyme as possible, are then sent to the enzymatic hydrolysis stage. The enzymatic hydrolysis stage is supplemented with additional enzyme to make up for incomplete recovery and deactivation of enzyme.

Present studies will focus on the development of the enzyme recovery stage. Countercurrent contacting of a solid phase with a liquid phase presents a difficult handling problem. Rather than attempting to design an elaborate continuous countercurrent system it was decided to achieve countercurrency by means of a "batch countercurrent multiple-contact system." In such a system the cellulose solids remain stationary in each tank and are contacted, by means of vigorous stirring, with the enzyme sugar solution. The wetted solids are then filtered and contacted with fresh enzyme

sugar solution, while the filtrate is contacted with fresh solids. The overall effect is that as the solution becomes weaker in enzyme concentration it is contacted with additional cellulose solids, so the final solution contains as little enzyme as possible. The batch countercurrent multiple-contact system offers simplicity in design and has been used industrially for the extraction of coffee from coffee beans.

Previous studies on the adsorption of the cellulase enzyme onto cellulose indicate that various problems need to be investigated, the most important of which concerns the fact that the cellulase enzyme produced by Trichoderma viride actually consists of three separate enzymes. It has been observed that the three cellulase components,  $C_1$ ,  $C_x$  and  $\beta$ -glucosidase, exhibit selective adsorption onto cellulose.  $\beta$ -glucosidase seems to adsorb very little in comparison with the  $C_1$  and  $C_x$  enzymes.

Adsorption of the cellulase components onto cellulose is dependent upon the available surface area. Various methods of pretreatment need to be investigated since they result in different amounts of available surface area. The possibility of using a steam explosion process as a means of pretreatment will be investigated. The present design calls for acid pretreatment. The use of various types of cellulose substrates may be investigated as well, since this also determines the degree of adsorption.

In addition to using various pretreatments and cellulose substrates, the temperature dependence of the degree of adsorption will be investigated. Previous studies have shown that the

temperature dependance is strong.

In summary, adsorption of the three cellulase components onto various types of pretreated cellulose will be studied. Initial studies will be concerned with adsorption in a single batch reactor. Information from the initial single batch data will be used to develop an adsorption isotherm. The results will be used to design a multiple-contact system which will be operated in parallel with an enzymatic hydrolysis stage.

C. Xylose Fermentation

(1). Xylose Utilization by Bacillus macerans

Bacillus macerans has been grown anaerobically on xylose to study its growth characteristics. In these studies it was grown in a continuous flow stirred tank fermenter. One notable feature of this kind of operation was its instability. More specifically, whenever it was growing at steady-state an increase in available xylose would result in the culture partially washing-out, but then recovering.

Experiments to study this phenomenon showed that when the xylose concentration in the fermenter was low--as it would be during steady state operation at dilution rates well below  $\mu_{max}$ --a step-change to a higher dilution rate--still well below  $\mu_{max}$ --would result in a halt in cell growth (see Figures IIC(1)1-2). Use of a continuous CO<sub>2</sub> analyzer showed that this halt in growth occurred within 5 to 20 minutes after a change (See Figure IIC(1)-3). Further, it was found by simply injecting a few tenths of a gram of xylose into the one liter fermenter, the same results could be obtained. Recovery from these upsets would take from 20 to 100 hours to begin, and were quite rapid once started. Upsets were not observed when a step-change went to a lower dilution rate or when the xylose concentration in the fermenter was high before a step-change up.

At no time during the whole upset would the xylose concentration be near inhibitory levels; nor were the fermentation products near inhibitory levels either. The phenomenon appears to

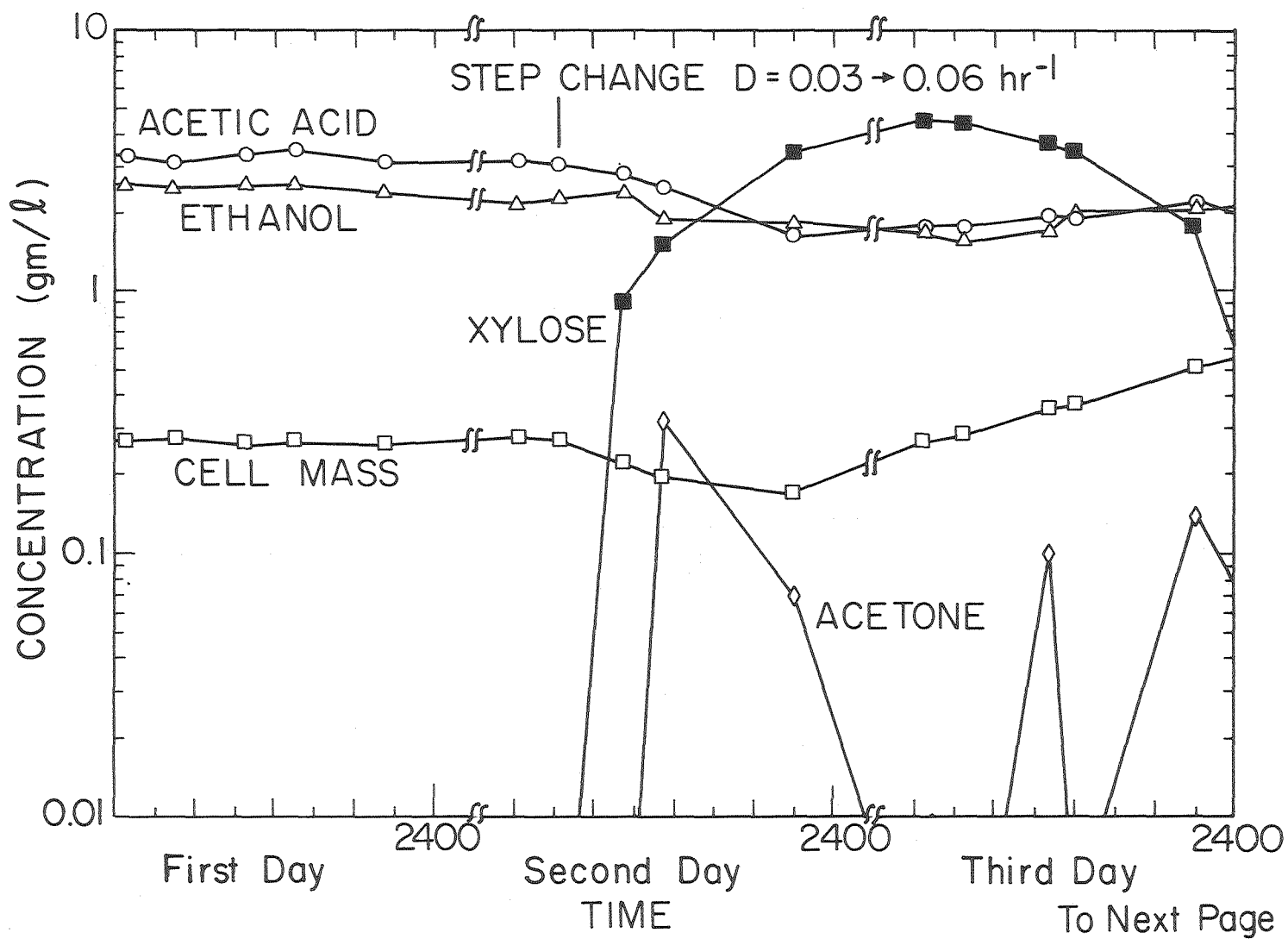


Figure IIC(1)-1. Reaction of *B. macerans* to a step change in dilution rate (D): The slope of the cell mass line following the change is approximately  $-0.06 \text{ hr}^{-1}$  indicating the bacteria have stopped multiplying.

XBL 813-5439

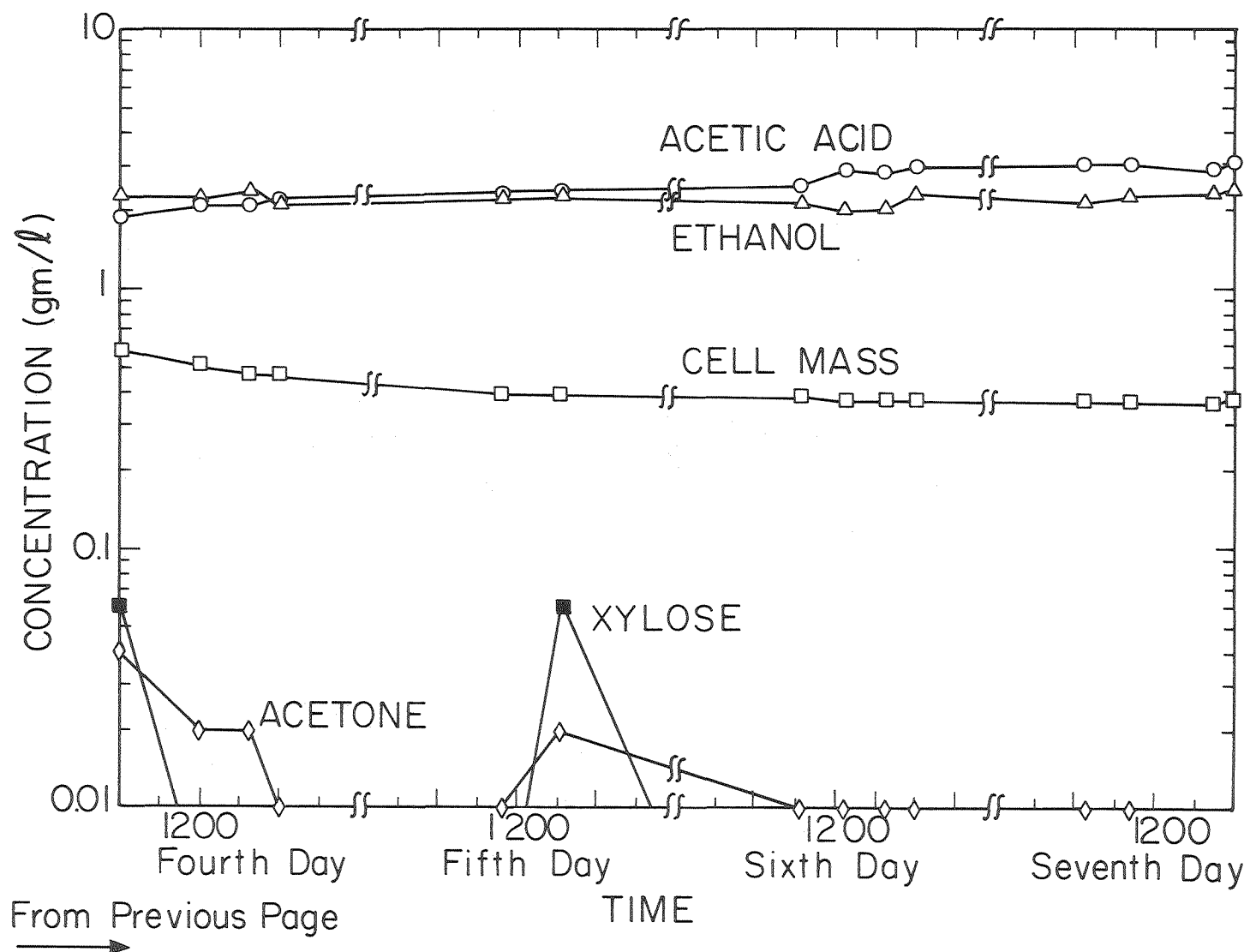
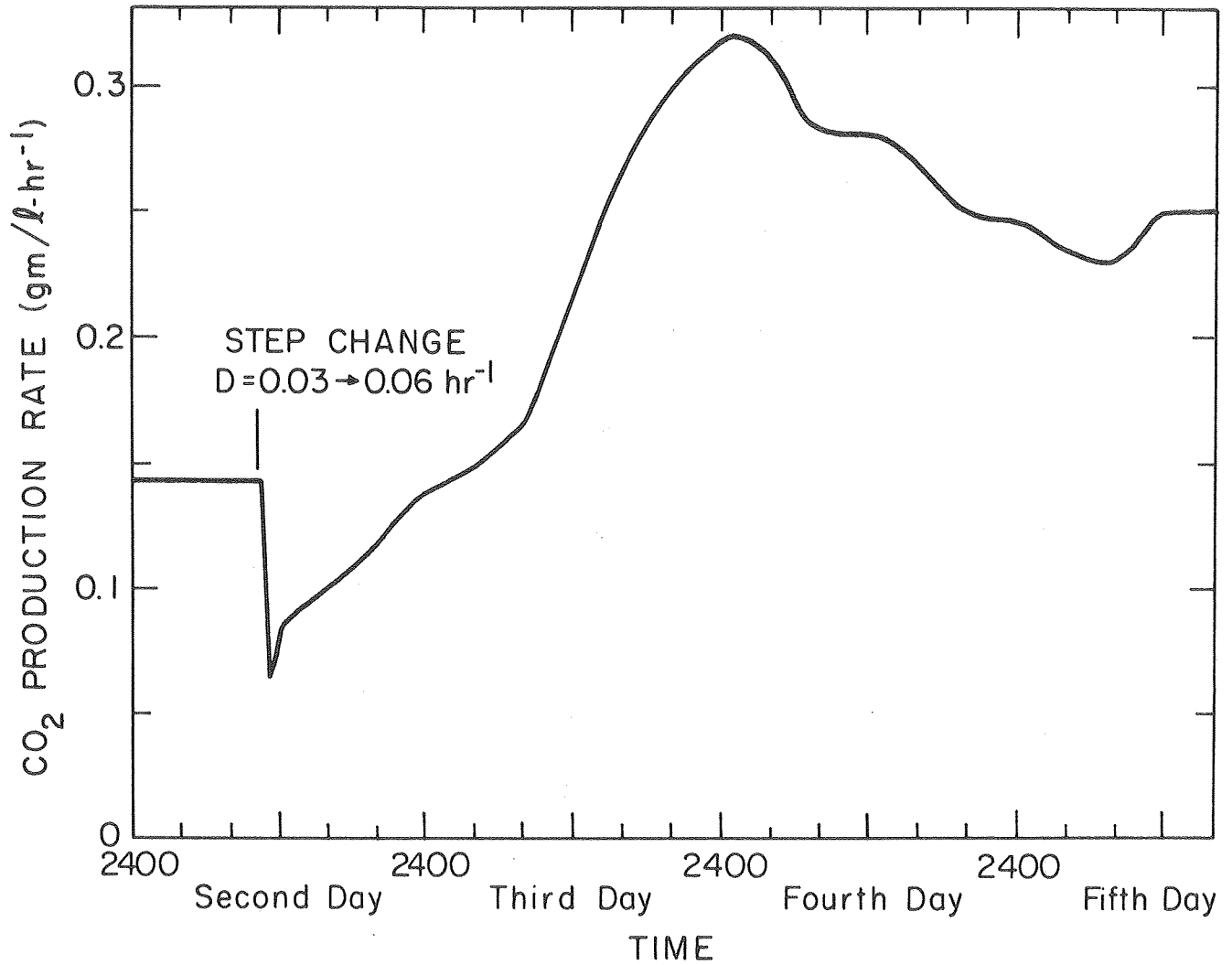


Figure IIC(1)-1 continued.

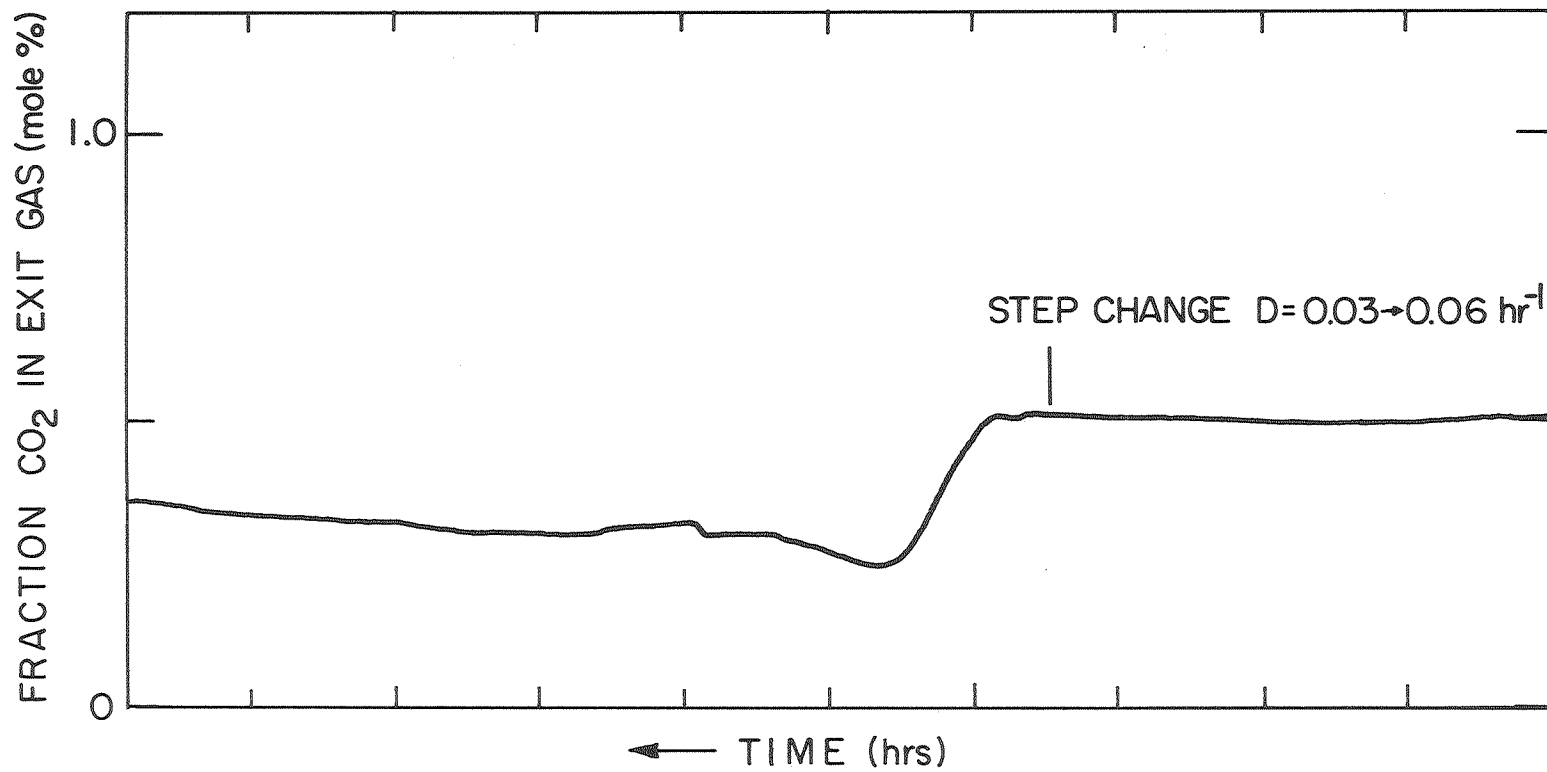
XBL 813-5440



XBL 813-5438

Figure IIC(1)-2. The profile of CO<sub>2</sub> production following the step change shown in Figure IIC(1)-1.





XBL 813-5437

Figure IIC(1)-3. The trace of the continuous CO<sub>2</sub> analyzer before and after the step change shown in Figure IIC(1)-1. Some of the roughness of the curve is due to periodic adding of concentrated NaOH by the pH controller.

be a problem of transport control. Xylose only crosses cell membranes by active transport (1). The transport mechanism for glucose has been shown to have feed-back control (2). It can be assumed that xylose transport mechanisms have a similar feature. In the case of B. macerans apparently control is poor.

With glucose, regulation of the transport mechanism is obtained by the level of an intermediate downstream in the metabolic chain (2). If this is also the case with xylose, a possible explanation for the observed behavior is a lag in response to higher xylose concentrations by the metabolic system. That is the metabolic system doesn't speed up quickly enough to build-up the level of the controlling intermediate before too much xylose or its derivatives cause growth to cease. This conclusion is supported by the CO<sub>2</sub> analyzer trace which shows no rise in CO<sub>2</sub> output just before the fall-off.

An explanation of the high potential for xylose transport is the possible existence of two xylose transport mechanisms. From the handful of organisms for which xylose transport kinetics are known, two have been shown to have two transport mechanisms. (3,4). In one of these the faster mechanism has been shown to be inducible by starvation (4). If B. macerans has this feature, a low external xylose concentration may be sufficient to induce the faster mechanism, thereby giving it a high potential for transport.

A high rate of xylose transport combined with a lag in response by the metabolic system leads to a build up of xylose

inside the cell. The xylose builds past an inhibitory level causing metabolism to slow and growth to stop. Recovery cannot occur until the transport system is controlled and the inhibition relieved. Since metabolism is running so slowly, it takes a long time for the level of controlling intermediate to build and so the transport system remains uncontrolled for a long time also.

#### References

1. M. Höfer, P. Dahle, European Journal of Biochemistry, 29(2), 326-32 (1972).
2. A. Kepes, In "The Cellular Functions of Membrane Transport," (J.Hoffman, ed.)p. 155. Englewood Cliff, N.J., Prentice Hall, 1974.
3. D. Shamanna, and K. Sanderson, Journal of Bacteriology, 139(1), 64-70 (1979).
4. M. Alcorn, and C. Griffin, Biochimica et Biophysica Acta, 510 361-71 (1978).

(2) Xylose Fermentation by Clostridium  
thermohydrosulfuricum

Background

Due to the problems encountered in fermenting xylose with Bacillus macerans, other bacteria are being examined as useful xylose fermenters. Presently, the most promising organism for producing high yields of ethanol from xylose is Clostridium thermohydrosulfuricum.

C. thermohydrosulfuricum is an extreme thermophile which grows only under strictly anaerobic conditions. These extreme growth requirements are beneficial since most commonly encountered contaminants do not grow in such a severe environment.

Strain 39E, which has the highest ethanol yield of any strain in this species, produces 0.47 gm of ethanol per gram of glucose fermented. This result was obtained using yeast extract as a nitrogen source and in the absence of ethanol inhibition (i.e. less than 1% ethanol by weight in the medium). The yeast extract may also provide necessary vitamins and amino acids for growth. By-products of the fermentation include acetic acid: 0.035 gm/gm glucose and lactic acid 0.084 gm/gm glucose.

The specific productivity of ethanol for strain 39E has not been reported, but the high specific growth rate ( $0.78 \text{ hr}^{-1}$  under optimal conditions) suggests that the specific productivity is also fairly high.

An undesirable property of this organism is that it produces hydrogen sulfide gas from sulfur containing amino acids

(present in yeast extract) and sulfite ion. The source of sulfite in the medium is oxidized sulfide. Sulfide is a necessary component of the medium since strictly anaerobic organisms grow only in a reducing environment. Most of the results cited above from the literature are for growth on glucose. Results for xylose fermentation are not yet available but are forthcoming.

### Objectives

Most of the  $H_2S$  production can be eliminated by removing sulfide ion from the medium. Experiments will be done using non-sulfur reducing agents, such as ascorbic acid. The affect of ascorbic acid on ethanol production will also be examined.

Since the cost of separating the ethanol product from the medium is directly related to the concentration of ethanol in the medium, experiments will be performed to condition or select a strain able to tolerate more than 1% alcohol. Strain 39E has been shown to grow in 3% ethanol.

Due to the high cost of yeast extract, alternative sources of organic nitrogen and growth factors will be tested. One possibility is corn steep liquor, which contains many amino-acids and vitamins and is less expensive than yeast extract.

Parameters, such as optimal pH and temperature for ethanol Production will be determined. The specific ethanol productivity during growth on xylose will also be measured.

Finally, experiments in vacuum fermentation may be attempted. Due to the high growth temperature (approximately

65°C) only moderately low pressure (3.6 psia) is needed to continuously boil the medium. Vacuum fermentation is attractive because it continuously removes ethanol from the medium, which allows higher rates of ethanol production.

#### D. Xylanase Production

As most agricultural wastes and woods contain 15 to 30% hemicellulose, it is clear that the utilization of these pentose polymers is desirable to achieve economic conversion of biomass. Enzymatic hydrolysis would appear to be the most favored process. The xylose produced in this manner finds application in foods or confectionary, and may be converted to alcohol, 1,4 butanediol or acetic acid. Various organisms have been found to produce xylanase, including Aspergillus foetidus, A. oryzae, Fusarium oxysporum, Trichoderma viride and Chaetomium trilaterale. A xylanase-producing strain of Streptomyces xylophagus has been studied. The medium used to cultivate this bacterium is shown in Table IID-1.

Xylanase activities were measured for a range of growth substrates including wood gum xylan, larchwood xylan and wheat bran. Activity was determined as the release of reducing sugars (mg, via DNS) from larchwood xylan in 15 minutes at 55°C. It was found that increasing the concentration of wheat bran from 1.75% to 3.0% gave only approximately 25% increase in xylanase activity (up to 9.35 X.U.), and increasing the substrate concentration to 7% resulted in no further increase. This affect may be attributed to oxygen transfer limitations, or enzyme de-activation by adsorption to the surface of the wheat bran.

During growth in a fermentor where pH control was possible, xylanase activities were improved compared to those obtained during growth in shake flasks. The conditions maintained for xylanase production are shown in Table IID-2. During

Table IID-1  
Xylanase Production Medium

<u>Constituents</u>	<u>Concentration (g/L)</u>
Xylan	10.0
$K_2HPO_4$	1.0
$MgSO_4 \cdot 7H_2O$	0.5
KCl	0.1
$FeSO_4$	0.01
Bacto-peptone	0.2 - 0.18
Tap water	to 1 liter



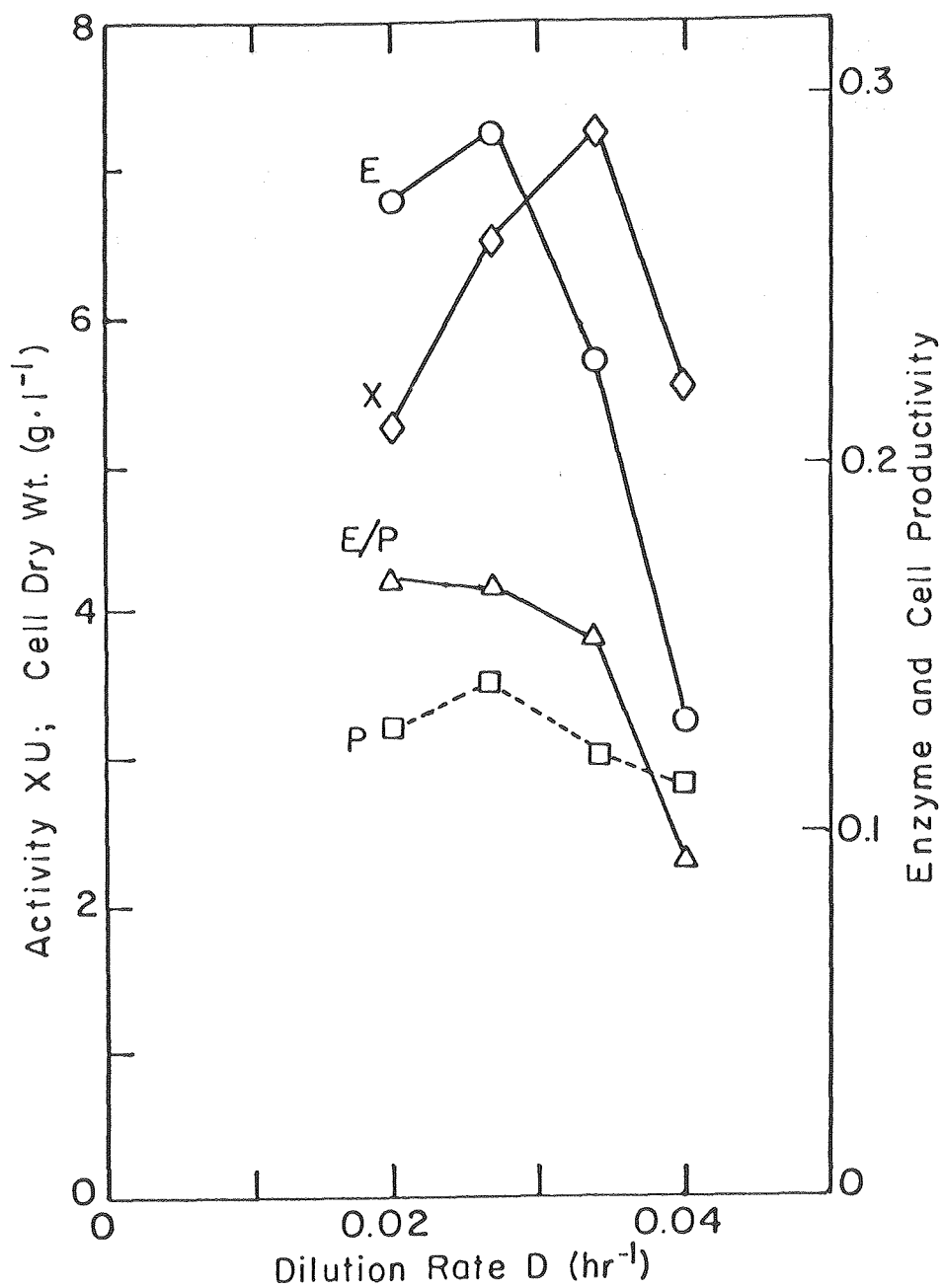
Table IID-2

Optimum Conditions for Xylanase Production

<u>Control Variables</u>	<u>Values</u>
pH	7.4
Temperature (°C)	30
Substrate concentration (g/L)	
commercial xylan	—
wheat bran	10
Bacto-peptone (g/L)	3

single-stage, continuous fermentation, a dilution rate of  $0.027 \text{ hr}^{-1}$  appears optimal.

Chaetomium trilaterale was also studied for production of xylanase under continuous cultivation at pH 7.4, a temperature of  $30^{\circ}\text{C}$  over a range of dilution rates from 0.02 to  $0.04 \text{ hr}^{-1}$ . The results for xylanase activity, cell productivity and cell mass are shown in Figure IID-1.



XBL 814-5533

Figure IID-1. Steady State Activities of *C. trilaterale* in Continuous Culture.

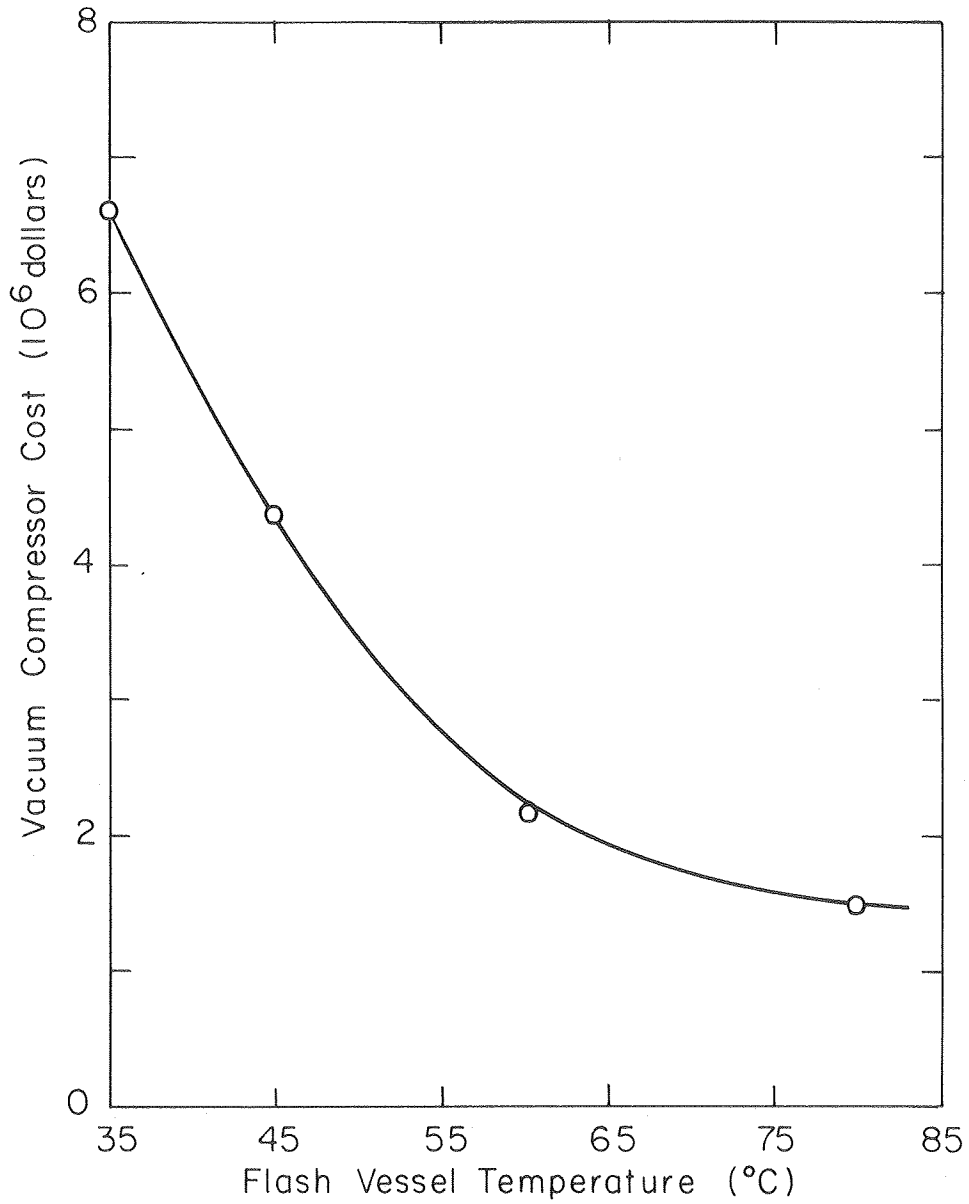
### III. FERMENTATION AND SEPARATION PROCESSES

#### A. Process Development Studies on Vacuum Fermentation and Distillation

##### Introduction

Extremely high capital costs for low pressure vacuum compressors is the major drawback to flash fermentation implementation. The energy requirements for flashferm compressors are kept low by utilizing a vapor recompression heating system so that product must be compressed only a small fraction of the way from flash vessel to atmospheric pressure (1). At flash vessel pressure, however, the specific volume of product vapors is very high and the required compressors are large. Operation of the flash vessel at higher temperature would allow the flashing operation to be conducted at higher pressure. Process energy requirements would not be affected, but the compressors could be much smaller and less costly. If the flash vessel temperature could be raised from 35 to 45°C a 35% compressor capital cost savings would result. At 60°C a 69% savings and at 80°C a 77% savings would result (see Fig. IIIA-1).

Work by Walsh and Bungay (2) indicates that a short duration temperature shock (20°C temperature increase for 3 minutes) temporarily halts fermentation activity, but cell activity is rapidly resumed on return to a hospitable fermentation environment. Work in our flashferm apparatus has shown that the pressure shock in cycling from an atmospheric pressure fermentor into the vacuum flash vessel has no adverse effects on the yeast



XBL 813-5442

Figure IIIA-1. Compressor purchased equipment cost for 25 million gallon/year capacity fermentation plant.

productivity (3). Now to be considered is a process where yeast are cycled from an optimum fermentation temperature atmospheric pressure fermentor into an elevated temperature low pressure flash vessel. If fermentation activity is retained upon return of the yeast to the fermentor, flashferm process economics would be greatly improved.

Experimental Program:

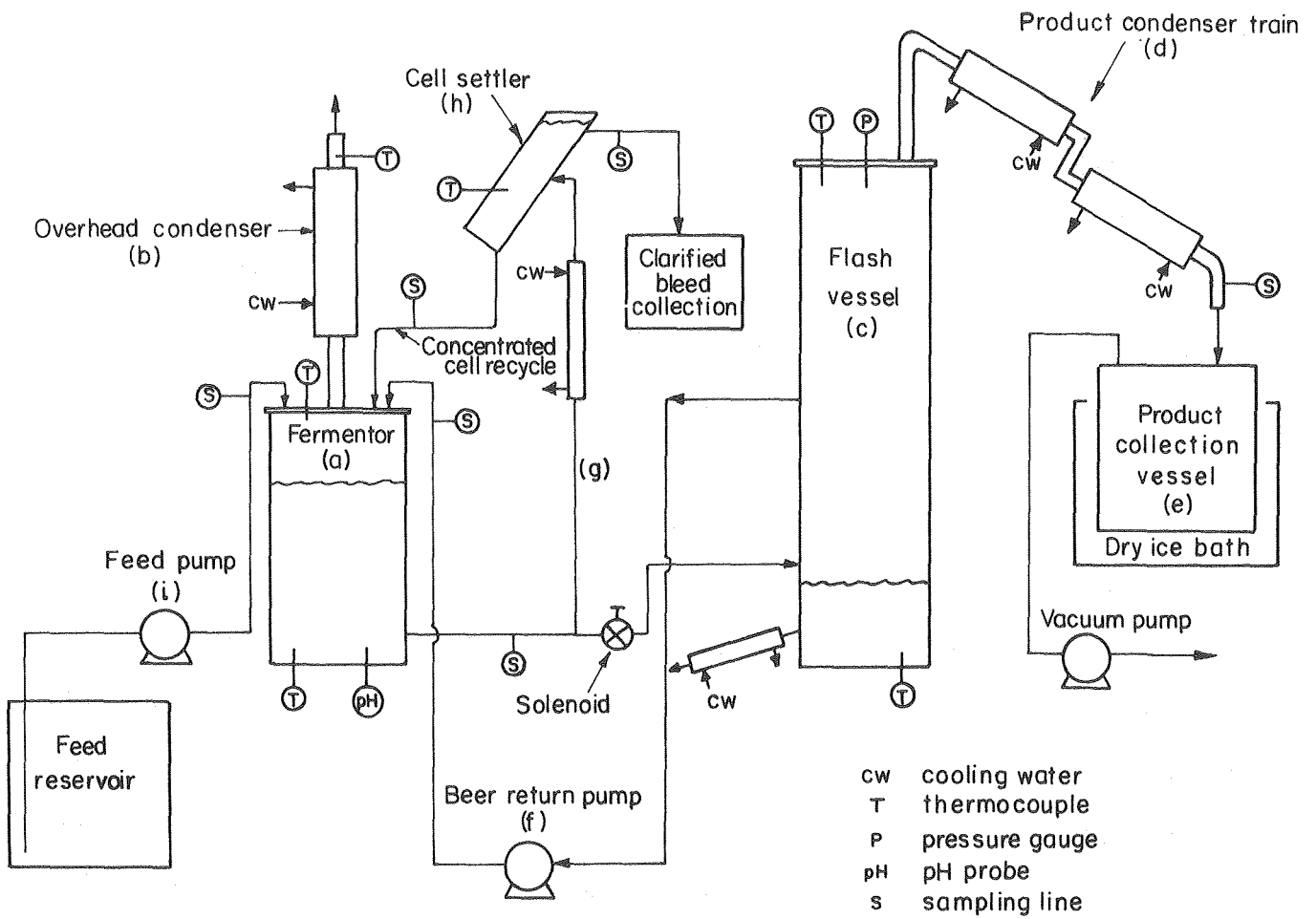
The following experimental program is underway to test the possibility of high temperature flashferm operation:

- 1) Pressure shift experiments
- 2) Elevated temperature experiments
- 3) Temperature shift experiments
- 4) Combined pressure and temperature shift experiments
- 5) Flashferm experiments with ethanol vapor removal.

Experiments are designed to show both the independent and synergistic effects of pressure and temperature shifts on fermentation activity. The pressure shift experiments reported last Quarter (3) resulted in retained high productivity. Elevated temperature experiments conducted this quarter, set a benchmark fermentation activity as a function of temperature. The effect of combined temperature and pressure shifts will be studied next. Finally, flashferm experiments (with ethanol vapor removal) will be conducted at the optimum temperature and pressure conditions.

Fermentation Activity Temperature Dependence

The flashferm apparatus (shown schematically in Figure IIIA-2, and described in detail in an earlier report (4)) was used



FLASH - FERM APPARATUS OVERVIEW

XBL 7912-5497

Figure IIIA-2.

to find the continuous fermentation temperature dependence. The full apparatus was used with cycling of the beer through the flash vessel. The cell settler was not used and a dilution rate of  $0.15 \text{ hr}^{-1}$  was maintained by taking a continuous bleed from the fermentor without settling and recycle of cells. So that fermentor beer could be cycled from the fermentor to the flash vessel, the flash vessel was kept at a slight vacuum (0.95 atm.). Both the fermentor and flash vessel overhead condensers were operated to return any ethanol vapor to the fermentor. A 125 g/L glucose feed was used (Table IIIA-1).

The experiment was conducted by operating fermentor and flash vessel at the same temperature. Samples of the fermentor beer were taken daily. In addition, the collection bottle was changed and the dilution rate was measured each day. When the cell density was constant for two days in a row, the temperature in the flash vessel and fermentor was changed.

The ethanol concentration in the samples was determined by gas chromatography. The cell densities were determined optically using a spectrophotometer; the yeast viability by the methylene blue stain test; and the glucose concentration by the dinitrosalicylic acid (DNS) method. A more detailed discussion of these procedures can be found elsewhere (5). The dilution rate was calculated by measuring the time needed to collect a 10 ml sample. In addition, the collection bottles were calibrated to provide a check on the first method.



Table IIIA-1

Medium Preparation (160, 120 g/L Glucose)

Yeast Extract Solution

<u>Substance</u>	<u>Conc.</u>	<u>Total Added</u>
Yeast Extract	8.5 g/L	1700 g
NH <sub>4</sub> Cl	1.32 g/L	269 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.11 g/L	22 g
CaCl <sub>2</sub>	0.06 g/L	12 g
Antifoam	0.5 ml/L	100 ml

Tap water added to make 30 L volume.

Glucose Solution

<u>Substance</u>	<u>Conc.</u>	<u>Total Added</u>
Glucose	125 g/L	20,000 g
Ampicillin	.051 g/L	10.15 g
Streptomycin	.010 g/L	2.03 g
Penicillin G	.320 g/L	63.96 g

Tap water added to make 130 L volume.

Total volume = (130 + 30) = 160 L

### Results and Discussion

Table IIIA-2 gives the primary results for each of the eight temperatures investigated. These same results are shown graphically in Figure IIIA-3. Both the cell concentration and ethanol productivity are a maximum at 33°C. In addition, the cell concentration and ethanol productivity fall off much faster above the optimum temperature than below it.

The specific productivity of the cells is lowest at the optimum temperature, increasing slightly at both elevated and depressed temperatures. This is reasonable in that cells at other than the optimum temperature will be stressed. Maintenance energy requirements will be increased, depressing cell mass production while ethanol production must be maintained or increased.

No loss of cell viability was found in the experiment, even at the highest temperature. The ethanol and cell yields ( $Y_{P/S}$  and  $Y_{X/S}$ ) also were not affected by temperature changes.

The steep drop in cell concentration and ethanol productivity in the superoptimal region can be explained by looking at the proposed reaction kinetics for cell growth. An Arrhenius dependence on temperature has been proposed (6) for many enzyme catalyzed reactions. An Arrhenius plot ( $\log(DX)$  vs.  $1/T$ ) for the full temperature range investigated is shown in Figure IIIA-4. Up to 33°C the plot is straight, indicating the Arrhenius dependence is correct. Above 33°C the plot falls off with increasing sharpness. Enzyme denaturation takes place at higher temperature (6) and is the most likely explanation for this non-Arrhenius

Table IIIA-2

Temperature (°C)	Dilution Rate (hr <sup>-1</sup> )	Cell Mass (g/L)	Ethanol Productiv. (G/L-hr)	Specific Producti. (hr <sup>-1</sup> )	Glucose in Sample (g/L)	Glucose Consumed	Y <sub>P/S</sub>	Y <sub>X/S</sub>
T	D	X	Q	V	g			
24.8	.149	3.69	4.03	1.09	58.6	66.4	.407	.056
27.4	.154	3.96	3.90	.985	43.3	81.7	.310	.048
30.2	.141	5.21	4.79	.92	33.8	91.2	.372	.057
32.8	.151	5.88	5.50	.94	28.6	96.4	.377	.061
35.2	.159	5.05	5.00	.99	32.1	92.9	.338	.054
37.9	.156	4.13	4.21	1.02	57.0	68	.397	.061
41.3	.153	.763	.776	1.02	120.6	4.4	-	-
44.4	.153	.148	.144	.973	130.5	- 5.5	-	-

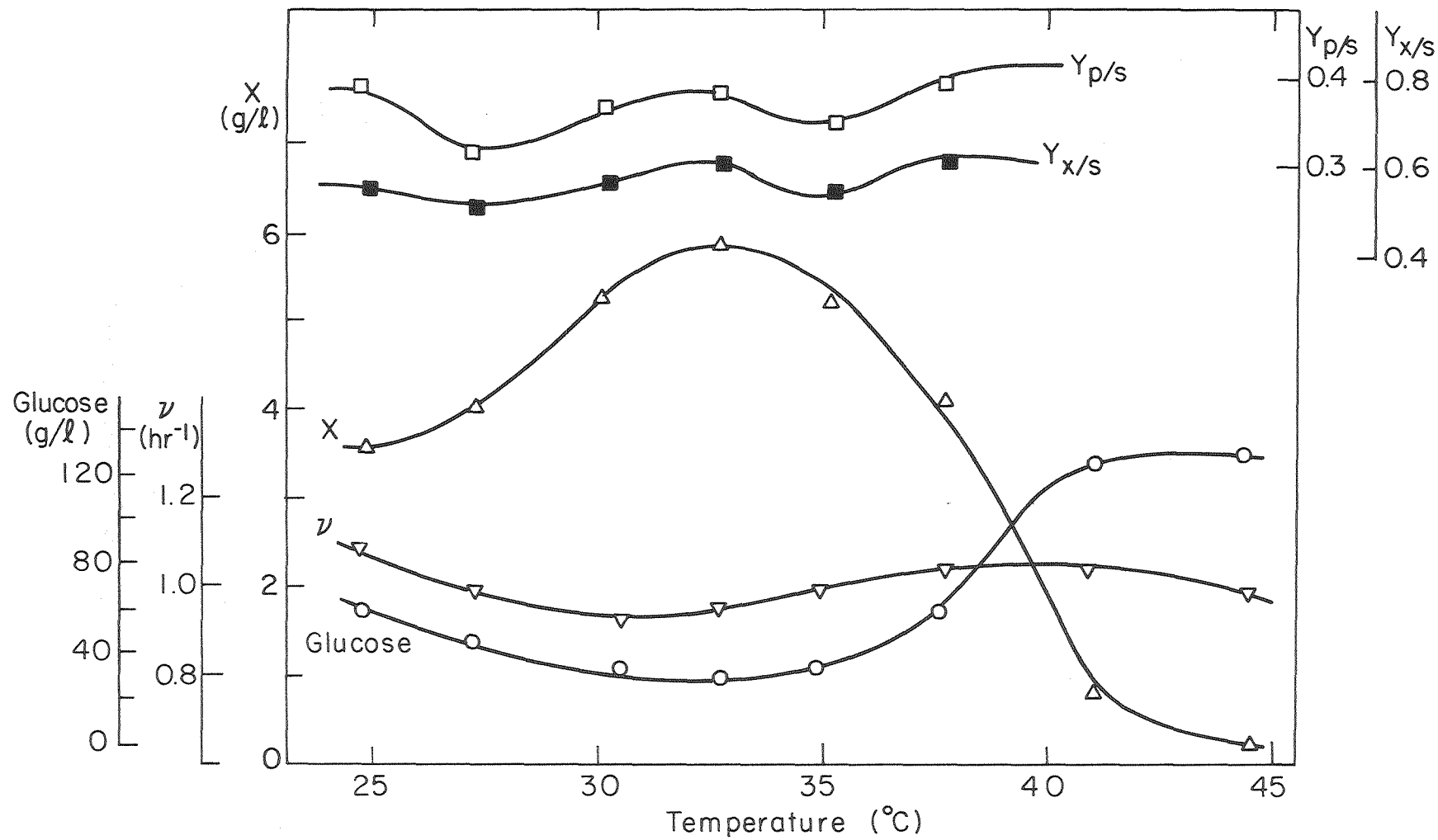
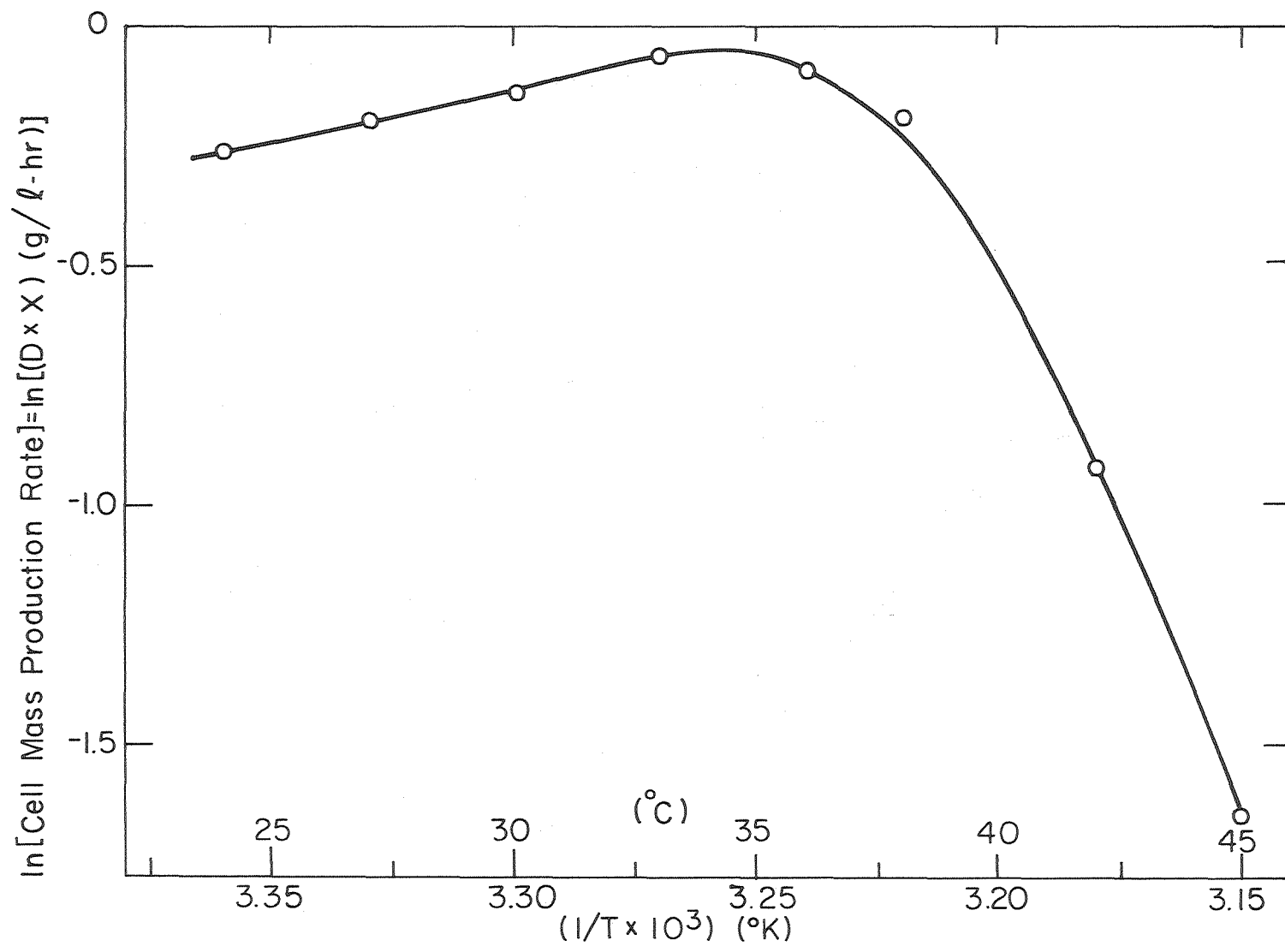


Figure IIIA-3. Continuous fermentation at dilution rate =  $0.15 \text{ hr}^{-1}$ .

XBL 813-5443



XBL 813-5441

Figure IIIA-4. Arrhenius plot of cell mass production for temperature range investigated.

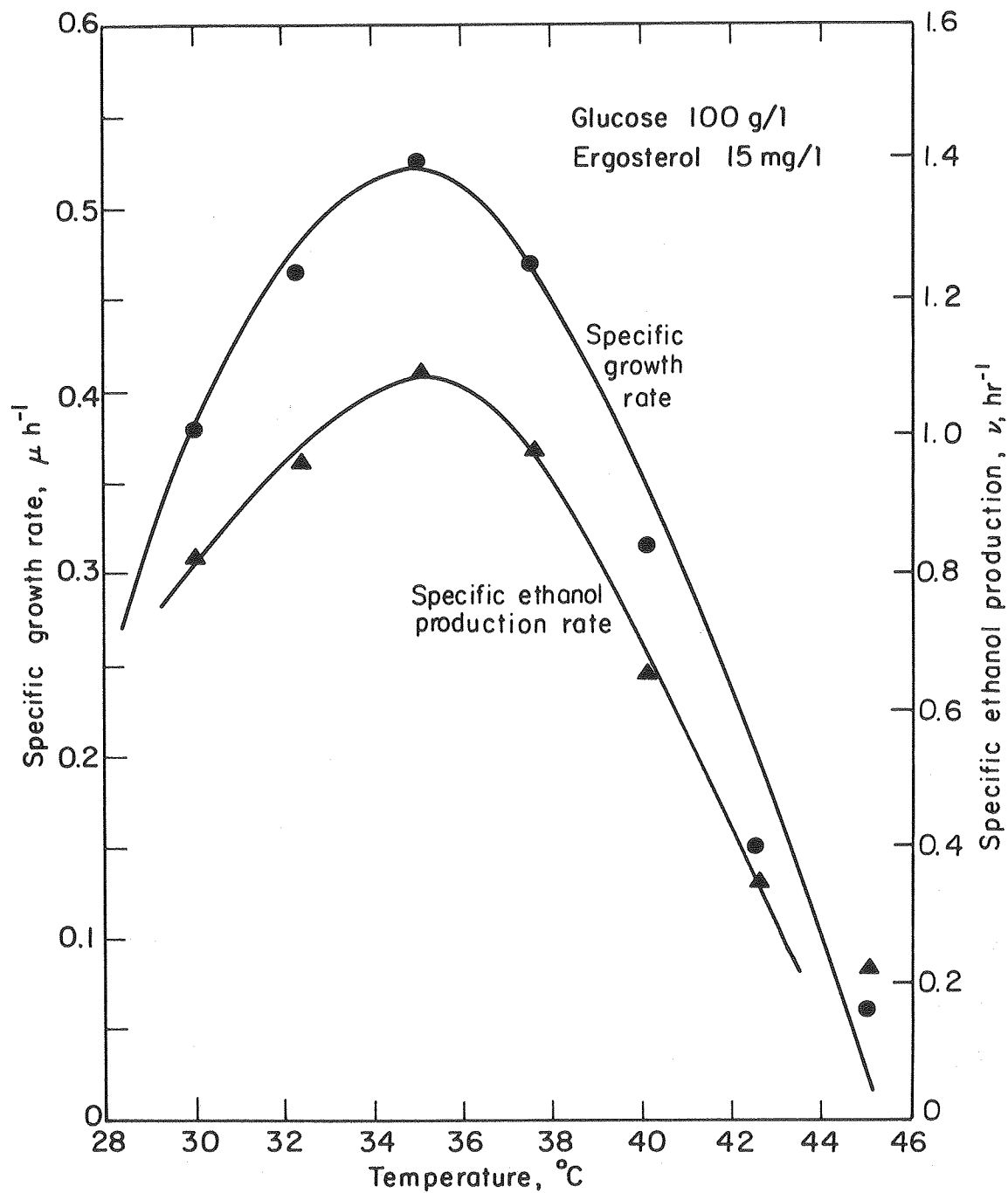
behavior. Indeed, there is much evidence that the reversible denaturation of key enzymes is the dominant factor in the kinetics of growth in the superoptimal temperature region (6). It is interesting to compare the temperature effects found here for continuous fermentation with the results from an earlier batch study (Fig. IIIA-5). The batch temperature optimum was 3° higher than the continuous process optimum. There are two possible explanations for the difference in optimum fermentation temperatures for batch and continuous growth. If we assume Michaelis-Menton kinetics, the equation for cell growth with limiting substrate of concentration "S" is of the form:

$$\text{rate} = K_1 e^{(-E_1/RT)} [S/(K_2+S)] \quad (1)$$

Let us assume that this particular step is limiting for batch growth. For batch growth all nutrients are available in excess, but in continuous growth there is a limiting nutrient. If some substrate other than S is limiting in the continuous process, then a different rate step (with a different E) may become rate limiting and so the optimum fermentation temperature changes. A second possibility is that there is ethanol inhibition in some of the continuous process steps and so, as before, the rate limiting step changes.

#### Project Goals

Temperature shift, combined temperature and pressure shift, and high temperature flash fermentation experiments will be



XBL 76I-6170

Figure IIIA-5. Effect of temperature on specific growth and specific ethanol production in batch fermentation, from Cysewski and Wilke (5).

completed next quarter.

Walsh and Bungay (2) investigated heat shocking of yeasts over a large temperature range. They found that the yeast could be heated at 50°C for 20 minutes with no loss of viability. At 55°C, however, shocking times as short as 5 minutes resulted in poor recovery of viability after return to fermentation temperature. Since the residence time in our flash vessel is on the order of two minutes, we should be able to operate it at at least 50°C and possibly in the 55 to 60°C range, corresponding to a possible 68% compressor capital cost saving.

#### References

1. B. Maiorella, H. Blanch and C.R. Wilke, "Rapid Ethanol Production Via Fermentation," Presented at the AIChE Meeting, San Francisco, November 29, 1979.
2. T.J. Walsh and H.R. Bungay, Biotechnology and Bioengineering, 21, 1085 (1979).
3. Annual Report (1981), Energy and Environment Division, Lawrence Berkeley Laboratory.
4. Solar Energy Research Report, Fall 1979.
5. G.R. Cysewski and C.R. Wilke, "Process Design and Economic Studies of Alternative Fermentation Methods for the Production of Ethanol, Ph.D. Thesis, University of California, Lawrence Berkeley Laboratory Report 5963 (1977).
6. R.Y. Stanier, M. Doudoroff and E.A. Adelberg, The Microbial World, Prentice Hall, Inc., N.J., 1970).



B. Evaluation of Low Energy Separation Processes

Introduction

The objectives of the project on novel ethanol-water separations are to investigate and develop the potential of alternatives to distillation for ethanol recovery. Earlier work has included assessment of fractional crystallation (freeze separation) and a type of field-flow fractionation. These approaches were determined to be technically or economically unfeasible, however, and all subsequent and continuing work has focused on various aspects of solvent extraction.

The main rationale for seeking alternatives to distillation as a product recovery method lies in the hope of reducing the considerable energy cost of distillation in the case of ethanol-water. As shown in Table IIIB-1, the energy required to produce 95 wt. % ethanol by distillation is over forty times the reversible thermodynamic work required to completely separate a 5 wt% ethanol solution into pure ethanol and water. This is due to the nature of the ethanol-water vapor-liquid equilibrium, particularly to the existence of the ethanol-water azeotrope. Operation at reduced pressure can reduce the energy requirement by altering the vapor-liquid equilibrium favorably, but the savings in energy is offset somewhat by considerable capital expense for compressors and other equipment.

Table IIIB-1

Energy Requirements for Ethanol Recovery

	<u>Energy Required (kilojoules/liter)</u>
Theoretical Minimum (complete separation of $X_{\epsilon} = 0.02$ )	147
Distillation, Conventional (to azeotrope only)	6216
Distillation, Vacuum (to azeotrope, calculated)	3568
Extraction (potential, calculated)	~ 1700

Current Research  
Extraction Solvent Selection

The key to a solvent extraction process for fermentation product recovery lies in the discovery of a solvent which meets several important criteria. The most obvious of these is that the solvent have a high distribution coefficient,  $D_E$ , defined by  $D_E = ([EtOH] \text{ solvent phase}) / ([EtOH] \text{ aqueous phase})$  where  $[EtOH]$  is ethanol concentration in g-moles/liter (or other appropriate units). An analogous coefficient,  $D_W$ , can be defined for water distribution. A further desirable property of an extraction solvent is that it have a high separation factor,  $S$ , defined by  $S = D_E / D_W$ . In addition, a solvent should be easily regenerable, inexpensive, and should have low water solubility.

All of these factors are interrelated and the quantitative requirements in each case are affected by the specific details of the process. For example, in the process schemes currently being considered, regeneration of solvent would be accomplished by vaporization of the ethanol at elevated temperature. This affects the requirement of high distribution coefficient because of the need to supply heat to the whole solvent stream. It also favors the selection of high-boiling solvents which separate easily from the volatile ethanol. The requirement of low water solubility is to prevent solvent loss and is thus associated with the solvent cost requirement. Furthermore, in processes under consideration which involve recycle of the fermentation broth after product extraction, this requirement is altered to one involving toxicity of the solvent to

the fermentation. Finally, a variation on the conventional solvent extraction process scheme is being considered which changes the low water solubility requirement to a low volatility one. This involves replacing conventional liquid-liquid contacting with a stream of inert gas alternately stripping the fermentation broth of ethanol and is in turn depleted of ethanol by absorption of the product into the solvent. Such a process also has the advantage of avoiding the formation of emulsions which can accompany attempts to apply liquid-liquid contacting to fermentation broths.

It is clear, then, that an extraction solvent and the process in which it is employed are mutually dependent. So it is appropriate to examine a range of solvent to determine the feasibility of ethanol extracton.

#### Measured Properties of Solvents

Distribution coefficient data and some separation factor data for a number of solvents are presented in IIIB-2. Distribution coefficients for ethanol were measured in this work by gas chromatographic analysis of samples from a batch equilibration experiments using pure ethanol in water and reagent grade solvents. The work has been hampered to date by lack of a sensitive technique for determination of water in the solvent phase. Shown in Table IIIB-2 also are some results of Roddy (1981) who used  $^{14}\text{C}$  labelled ethanol and tritiated water to determine  $D_W$  as well as  $D_E$ , and of Korenman and Chernorukova who used a spectrophotometric technique based on reaction of alcohol

Table IIIB-2  
 Properties of Solvents, 20°-25°C

solvent	D <sub>E</sub>	S
alkanes		
n-alkanes (C <sub>5</sub> -C10)	.008 <sup>c</sup>	-
isooctane	.006 <sup>d</sup>	-
cyclohexane	.007 <sup>b</sup>	310
aromatics		
benzene	.046 <sup>b</sup> , .024 <sup>c</sup>	110
toluene	.034 <sup>b</sup> , .020 <sup>c</sup>	100
ethyl benzene	.029 <sup>b</sup> , .007 <sup>c</sup>	97
diethyl benzene	.022 <sup>b</sup>	120
triethyl benzene	.097 <sup>a</sup>	-
oxygenated		
2,6-dimethyl-4-heptanone	.143 <sup>a</sup> , .088 <sup>b</sup>	19
acetophenone	.257 <sup>a</sup>	-
dibutyl phthalate	.123 <sup>a</sup>	-
tributyl phosphate	.430 <sup>a</sup> , .430 <sup>c</sup> , .540 <sup>b</sup>	8.3
chlorinated		
methylene chloride	.145	-
chloroform	.120 <sup>b</sup> , .140 <sup>c</sup>	170
carbon tetrachloride	.056 <sup>a</sup> , .040 <sup>c</sup>	-

Sources: a-this work  
 b-Roddy, 1981  
 c-Korenman & Chernorukova, 1974  
 (all S values from Roddy, 1981)

with nitrous acid to determine alcohol concentrations. Most of the values in Table IIIB-2 are for aqueous alcohol concentrations at or near 1.0 M, but all investigators find that distribution coefficients vary little over a range of dilute ethanol concentration.

Several important points can be made about the data in Table IIIB-2. First, even the best value of  $S$  is rather low considering the fact that to produce an azeotropic (95 wt%) ethanol from 1 M (5 wt%) aqueous solution in one step would require  $S \geq 390$ . Second, the only solvent with selectivities approaching this value (the alkanes are presumably all similar to cyclohexane) have quite low distribution coefficients. Also of interest is the difference between the oxygen-containing and chlorine-containing compounds. Both types of compounds are capable of participating in hydrogen-bonding with either water or ethanol (excepting carbon tetrachloride). However, the oxygen-containing compounds can act only as nucleophilic hydrogen bonding partners while the partially chlorinated hydrocarbons act only as electrophilic hydrogen-bonding partners. The distribution coefficients for ethanol are of the same order of magnitude for both types of compounds, suggesting that ethanol interacts to roughly the same degree with either type of hydrogen-bonding. However, there is an order of magnitude difference in the separation factors (i.e., in  $D_W$ ) for the two kinds of compounds, implying that water is much less able to interact with the electrophilic H-bonding sites than with the nucleophilic ones.

Admittedly, this observation is based on little data as yet, but more evidence for it from the literature will be presented later.

A preliminary assessment of the toxicity of some solvents has also been done. This involved batch culture experiments with yeast growing in medium saturated with the solvent. The alkanes tested, cyclohexane and a mixture of high-boiling alkanes, had virtually no effect on growth or substrate utilization, possibly because of their low solubility. Aromatic compounds tested showed varying levels of toxicity, though all were more toxic than alkanes. Tributyl phosphate was toxic. Dibutyl phthalate, while showing no toxicity, formed a stable emulsion with the medium.

#### Improvement of Extraction Systems

It is apparent from the results in the previous section that anything which can be done to improve distribution coefficients, separation factors, or preferably both, will be very helpful, if not essential, to the realization of a solvent extraction recovery process for ethanol production.

Increasing the temperature can have a dramatic effect on distribution coefficients. Thus Roddy (1981) notes that increasing the temperature from 20°C to 40°C can roughly double distribution coefficients. It seems likely that this is due to a decrease in hydrogen bonding in the aqueous phase since the hydrogen bond, like other dipole-dipole interactions, requires specific orientation and thus have a strong inverse temperature dependence. This is just as true for water as it is for ethanol,

though, with the result that temperature has very little effect on separation factors.

An approach which does improve both distribution coefficients and separation factors is the addition of an appropriate salt to the aqueous phase. This salting out effect has been studied for a great number of salts in ethanol-water solutions. Its potential application in distillation has been reviewed by Furter (1977) among others. It is equally applicable to extraction processes, however. The improvement of distribution coefficient is related directly to the Sechenow parameter (or salt effect parameter),  $K_A$ , defined

$$\ln (C_{AS}/C_A) = K_A Z_S$$

where  $C_A$  and  $C_{AS}$  are the concentrations of solute (ethanol in this case) in the solvent in equilibrium with aqueous solution without and with salt, respectively.  $Z_S$  is a measure of salt concentration, usually molality mole fraction.

Some experiments have been done to confirm the salting out of ethanol from fermentation broths. Particularly effective salts include potassium carbonate, potassium phosphates and ammonium sulfate. An increase in the ethanol distribution coefficient by orders of magnitude is possible with sufficiently high salt concentrations. At saturation, for example, potassium carbonate salts out a separate phase of over 90 weight % ethanol from dilute solution without any solvent phase present. However,



no salt is inexpensive enough to consider using it in this way without either recovery or recycle.

A third method for improving distribution coefficients is to employ a mixed solvent system which has desirable extraction properties. An example of this is the use of trioctyl phosphine oxide (TOPO) in 2,6-dimethyl 4-heptanone. Results are shown in IIIB-3. It is seen that  $D_E$  is improved at low concentration of ethanol with the improvement reduced as concentration is increased. When the results are expressed in terms of the additional ethanol brought into the organic phase by the TOPO, it is seen that a saturation appears to be reached at 0.47 moles of ethanol per mole of TOPO. The reason for this is not known, but appears to be related to the relative weakness of ethanol in competition with water for nucleophilic H-bonding sites mentioned previously. It is expected that separation factors in this case, which are poor for the pure ketone, are not improved by TOPO addition.

One further way of improving distribution coefficients is being investigated. In reviewing the literature on salting out, it was observed that certain salts have the opposite effect. That is they salt ethanol into the aqueous phase, reducing its volatility. Notable among these salts are those involving the large tetraalkyl ammonium ions. It was reasoned that, by using salts of these ions which would have very little water solubility but be soluble instead in an organic phase, an improved extraction solvent for ethanol might be devised. Table IIIB-4 shows some

Table IIIB-3

Mixed Solvent Extraction Using TOPO  
(0.521 M TOPO in 2,6-dimethyl-4 heptanone)

Aqueous Ethanol Concentration, M	D <sub>E</sub>	Extra Moles EtOH Per Mole TOPO
0.39	0.41	0.21
1.49	0.31	0.47
3.18	0.22	0.47

NOTE: D<sub>E</sub> for range of aqueous ethanol concentrations 0.5 - 3.5 M  
with no TOPO added is constant at 0.14.

Table IIIB-4  
Salting Ethanol Into Organic Solvents

Solvent	Salt	[EtOH] <sub>aq</sub> , <u>M</u>	D <sub>E</sub>
dibutyl phthalate	none	1.0	.123
dibutyl phthalate	TBA benzoate, .04 <u>M</u>	1.0	.116
triethyl citrate	none	1.5	.072
triethyl citrate	TBA benzoate, .03 <u>M</u>	1.5	.091
triethyl benzene	none	1.0	.097
triethyl benzene	TBA iodide, sat.	1.0	.088
triethyl benzene	TBA benzoate, .18 <u>M</u>	1.0	.171

TBA ≡ tetrabutyl ammonium

results of initial tests of this idea. While the effects in the case of the ester solvents are small due to the small concentrations employed, at higher concentration in triethyl benzene, tetrabutyl ammonium benzoate clearly salts ethanol into the organic phase. The extra ethanol brought in is slightly more than 0.5 moles of ethanol per mole of salt. In the case of tetrabutyl ammonium iodide, however, salting out of ethanol is indicated, pointing to the importance of an anion in this interaction. This is confirmed by the work of Arnett, et al. (1977) who found, in a study of the hydration of ions in non-aqueous media, that the influence of the tetraalkyl ammonium cation is small compared to the anion effect. The salting out by iodide is in keeping with other evidence that water is better able to compete with ethanol for nucleophilic sites in an organic medium than electrophilic. In the case of the larger benzoate ion, the hydration forces are not as strong and presumably the cation dominates the overall effect.

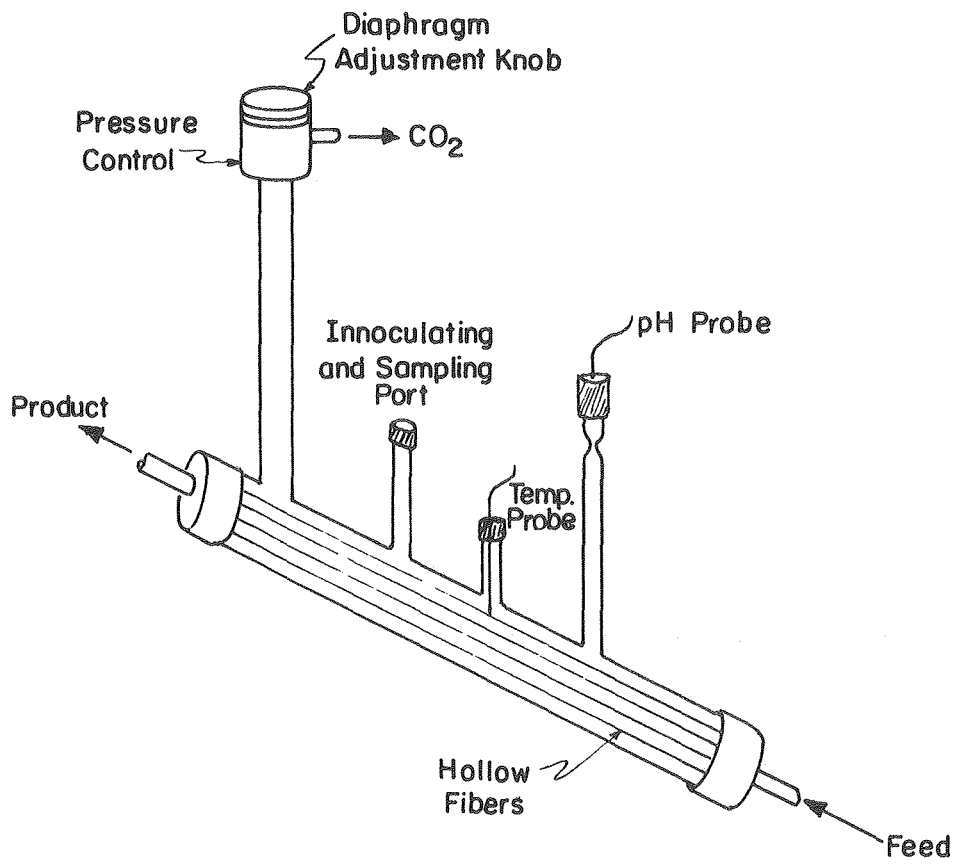
#### References

1. E.M. Arnett, B. Chawla and N.J. Hornung J Solution Chem 6-12. 781 (1977).
2. W.F. Furter, Can. J. Chem. Eng. 55229 (1977).
- 3/ I.M. Korenman and Z.G. chernorukova, J. Appl. Chem., USSR, 47,11 pt.2, 2595 (1974).
4. J.W. Roddy, Ind. Eng. Chem. Proc. Dev. 20, 104 (1981).

C. Large Scale Hollow Fiber Reactor Development

A fermentation unit with one set of fibers traversing the length of the cartridge was successfully constructed. Using Saccharomyces cerevisiae anamensis (ATCC 4126), an ethanol producing fermentation was carried out. The run was terminated after four days when it was noticed that about half of the fibers had elongated. Some growth of yeast through the tubes was noticed, and upon disassembly a few pin hole leaks were found in the fibers which were not present before the fermentation. The unit's productivity was only about 5% of the theoretical maximum. Thus, although the fermentation gases vented easily this was not a rigorous test of the unit. The cause of the low productivity is being investigated. At the moment it is believed to be partially due to CO<sub>2</sub> inhibition.

Another unit with two sets of hollow fiber membranes was constructed, as shown in Figure IIIC-1. In this unit one set of tubes supplies the nutrients to the culture, and the other set removes the products. In principle, this provides for a convective flux of fluid going past the cells. Difficulties arose in the fermentation when fluid backed up in the shell side and did not pass into the tubes. It is believed that membrane compaction and fouling are responsible for the decreased product flux. Some other types of fibers will be looked at to minimize the membrane compaction effect.



HOLLOW FIBER FERMENTOR WITH MONITORING PROBES  
AND PRESSURE CONTROL

XBL 813-5317

Figure IIIC-1.

#### IV. SUPPORT OF GULF-PDU

##### A. General Support Services and Analytic Methods

The process demonstration unit at the Gulf Oil Chemicals Jayhawk Plant has been used with several variations in substrates, enzyme production, and media formulation.

A considerable cost in enzyme production is the use of Solka Floc substrate and the various chemicals used in the growth media. Corn steep liquor has been suggested as a substitute for supplying some of the nutrients, especially for protein nitrogen. The published (1) composition of corn steep liquor shows about 7.5% total nitrogen on a dry basis and 4.05% organic nitrogen predominantly protein, peptides, and free amino acids. Since corn steep liquor appears to cost less than technical grade proteose peptone by about a factor of sixteen on a per ton basis, a schedule was prepared as a preliminary starting point for the substitution of corn steep liquor for the peptone and steam exploded wood for the Solka Floc. This schedule is shown in Table IVA-1.

The last row was added on the expectation that if enzyme production was successful at 73 grams per liter of steam treated wood (equivalent to 50 gms/liter or 4.8 w% Solka Floc) then it might be possible to obtain higher enzyme activity from about 103 grams steam treated wood per liter (equivalent to 70 g/L or approximately 6.5 w% Solka Floc).

The experimental values of proteose peptone requirements

Table IVA-1

Schedule of components for substitution of corn-steep liquor for proteose peptone and steam treated--washed aspen for Solka Floc in enzyme production ( gm/L ).

Solka Floc (94% Glucan)	Cellulose	Dry Iotech Aspen (64% Glucan)	Proteose Peptone (16% N)*	Corn Steep Liquor	
				Solid (7.52% N)	Liquid (54% Solids)
10.0	9.4	14.7	1.0	2.37	4.4
10.6	10.0	15.6	1.03	2.4	4.5
25.0	23.5	36.7	1.6	3.9	7.0
26.6	25.0	39.1	1.7	4.0	7.4
50.0	47.0	73.4	2.9	6.85	12.7
53.2	50.0	78.1	3.1	7.3	13.6
70.0	65.8	102.8	4.1	9.7	17.9
74.5	70.0	109.4	4.4	10.4	19.3

\*Reagent grade proteose peptone is about 16% organic nitrogen and commercial grade is about 10% N.



vs. Solka Floc used in enzyme production (2) is plotted in Figure IV(A)1. From this nearly linear curve the other values of proteose peptone listed in Table IV(A)1 were obtained.

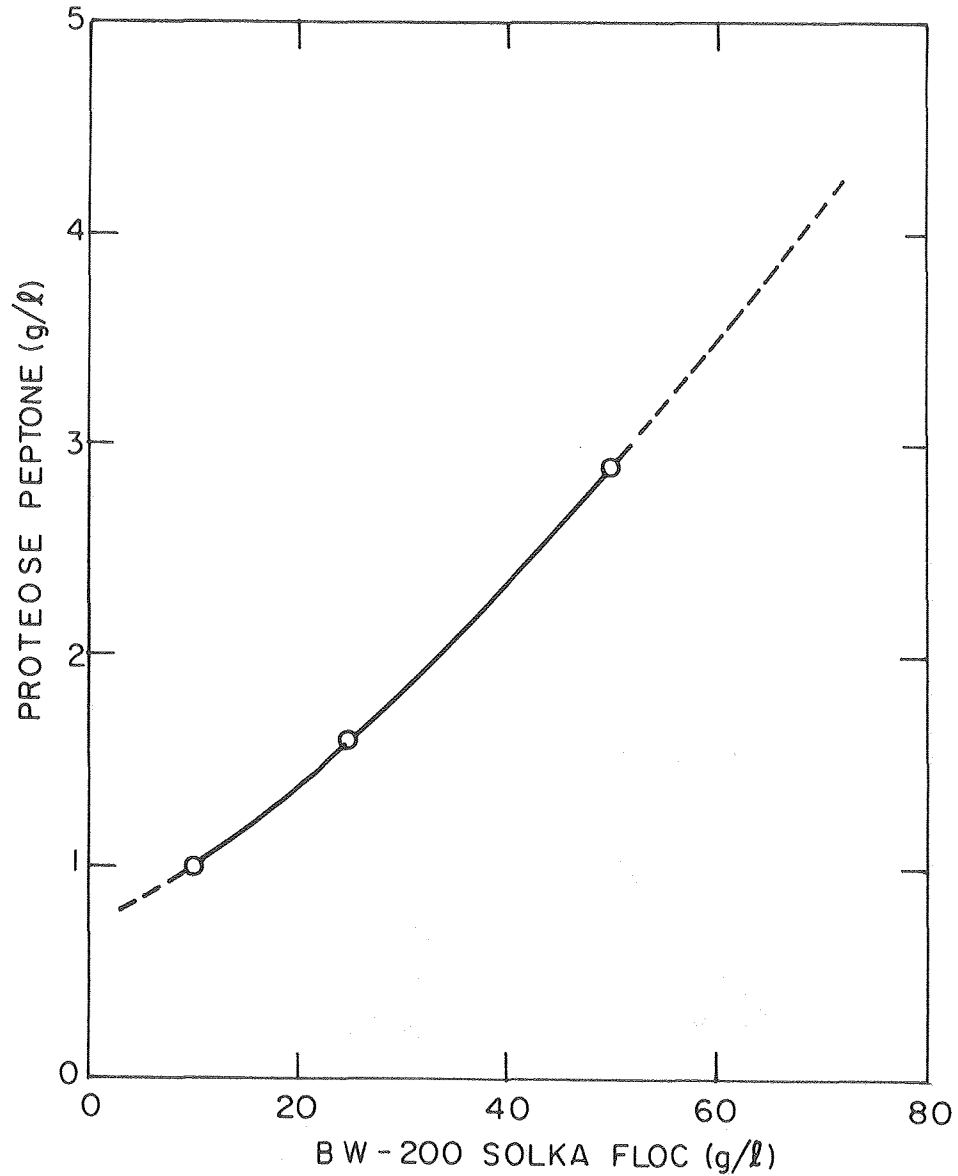
The potassium dihydrogen phosphate used serves a dual purpose. It is a source of the phosphorus requirement and buffering action. A plot of experimental requirement of phosphorus and ammonia is shown in Figure IV(A)2. As can be seen the potassium dihydrogen phosphate is constant at lower suspensions of Solka Floc for its primary maintenance of buffering action. Given the 1.1% phosphorus content on a dry basis in corn steep liquor, then 5.2 to 7.9% of the requirement is provided by the liquor. About one half of the balance would be required for the buffering action. The other half would have to be provided by another source.

Based on the listed composition of a 0.1 distribution of free ammonia in the total of 7.5% nitrogen in corn steep liquor, then from 3.2 to 10.6% of the ammonia is provided by the corn steep liquor. The balance could be provided by using ammonium hydroxide rather than sodium hydroxide for maintenance of pH.

Based solely on protein requirements, a savings of approximately 75% is realized on the substitution of corn steep liquor for proteose peptone.

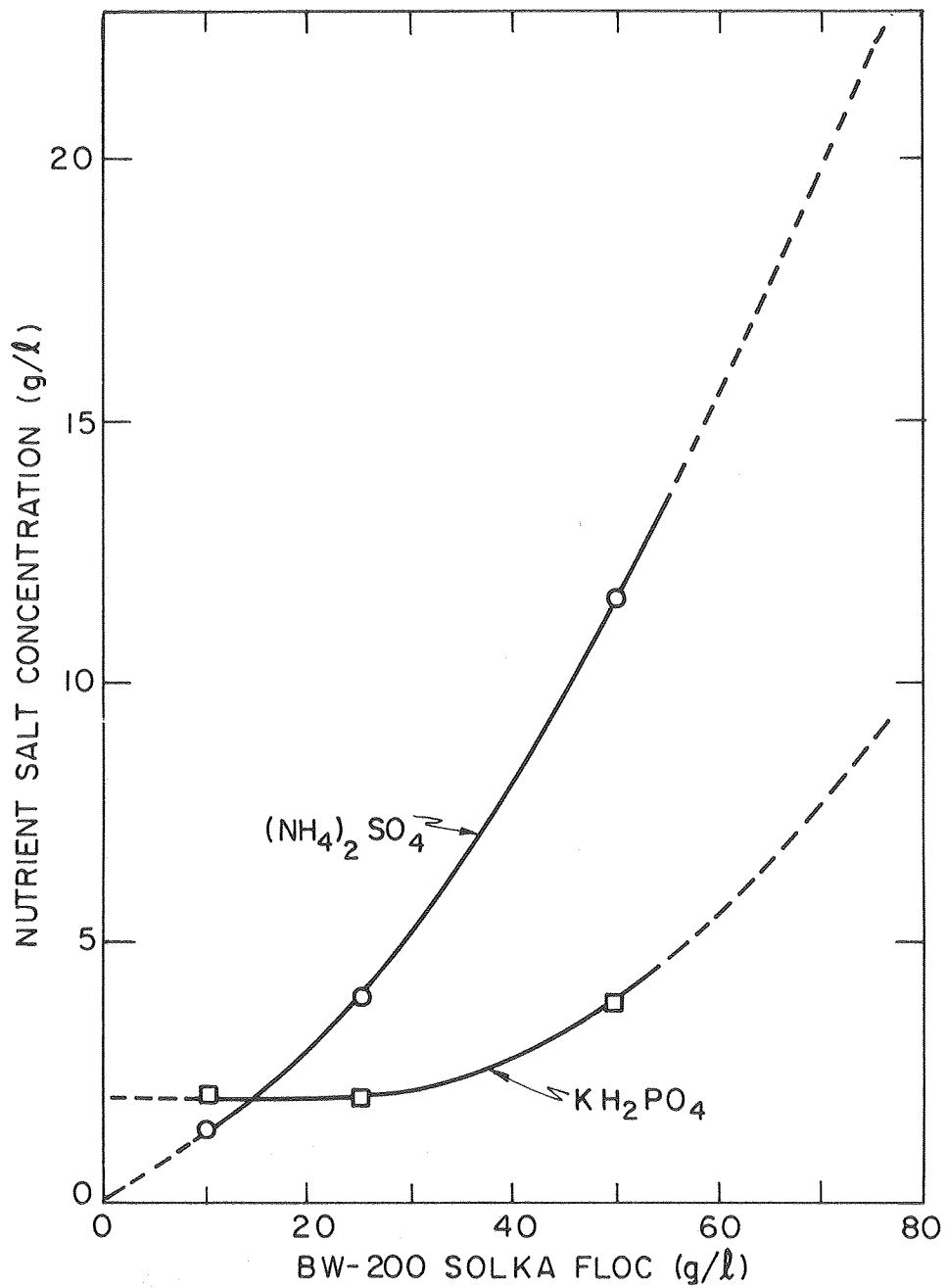
#### References

1. Argo Steep Water #E801, C.P.C. International Inc., Corn Products Div., International Plaza, Englewood Cliffs, New Jersey 07632.



XBL 813-5451

Figure IVA-1. Reagent grade proteose peptone required for corresponding Solka Floc suspensions in Rut-C-30 enzyme production. Commercial grade requirements is about 1.5 times above.



XBL 813-5452

Figure IVA-2. Reagent grade salts used for corresponding Solka Floc suspensions in Rut-C-30 enzyme production.

2. S.K. Tangnu, H.W. Blanch and C.R. Wilke, "Enhanced Production of Cellulase, Hemicellulase and B-glucosidase by Trichoderma reesei (Rut C-30), Lawrence Berkeley Laboratory, University of California, Berkeley, LBL-11074, June 1980.

APPENDIX

BIOCONVERSION OF CELLULOSE AND PRODUCTION OF ALCOHOL

Project Staff

Faculty Investigators

Charles R. Wilke, Professor of Chemical Engineering  
Harvey W. Blanch, Associate Professor of Chemical Engineering

Staff Scientist

Neil Hendy

Graduate Students

1. Ph.D. Candidates

Frances Arnold  
Brian Maiorella  
Bruce Vick Roy  
Dale Wiley  
Harry Wong

2. MS Candidates

Robert Antonoplis  
Thomas Delfino  
George Dove  
Tony Mancusco  
Steven Orichowsky  
Javier Perez  
Stephen Wald

3. Support Staff

Aldo Sciamanna, Senior Chemist  
Bill Long, Technician  
Linda Sindelar, Technician

ACKNOWLEDGEMENT

This work was supported by the Assistant Secretary for Conservation and Solar Energy of the U.S. Department of Energy under Contract W-7405-ENG-48, and by the Solar Energy Research Institute under Contract DR-0-9058-1.