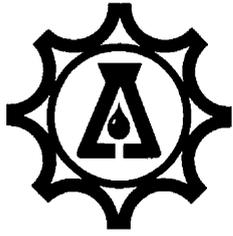


# Alcohol Fuels Program Technical Review

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Produced for the United States Department of Energy by the Solar Energy Research Institute

Spring 1984



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## Introduction

The spring meeting of the alcohol fuels program subcontractors was held on May 3-4, 1984, in Washington, D.C. The purpose of the meeting was to review the research results of the subcontractors for the past six months. This was the first meeting at which both the research and engineering contractors participated. Discussion was lively and the exchange between the two groups informative.

The alcohol fuels program consists of in-house and subcontracted research for the conversion of lignocellulosic biomass into fuel alcohols via thermoconversion and bioconversion technologies. SERI is the field manager of the program for the Biomass Energy Technology Division at DOE. The production of alcohols from lignocellulosic biomass has moved from a long-range technology a few years ago to one with a real mid-term potential for commercial development.

In the thermoconversion area, the SERI gasifier has been operated on a one-ton per day scale and produces a clean, medium-Btu gas that can be used to manufacture methanol with a relatively small gas-water shift reaction requirement. Recent research has produced catalysts that make methanol and a mixture of higher alcohols from the biomass-derived synthetic gas. These alcohol mixtures will significantly enhance the value of methanol as a fuel extender by improving its compatibility with gasoline.

In the last three years we have seen rapid development of the acid and enzymatic hydro-

lysis processes. Three processes have emerged as candidates for more focused research. They are:

- A high-temperature, dilute-acid, plug-flow approach based on the Dartmouth reactor
- Steam explosion pretreatment followed by hydrolysis using the RUT-C30 fungal organism
- Direct microbial conversion of the cellulose to ethanol using bacteria in a single or mixed culture.

Modeling studies, including parametric and sensitivity analyses, have recently been completed and give an overview of the economic potential of these processes. The results of these studies will lead to a better definition of the present state-of-the-art for these processes and provide a framework for establishing the research and process engineering issues that still need resolution. In addition to these modeling studies, economic feasibility studies are being carried out by commercial engineering firms. Their results will supplement and add commercial validity to the program results. The feasibility contractors will provide input at two levels:

- Technical and economic assessment of the current state of the art in alcohol production from lignocellulosic biomass via thermoconversion to produce methanol and higher alcohol mixtures and bioconversion to produce ethanol

- Identification of research areas having the potential to significantly reduce the cost of production of alcohols.

The results of the feasibility and modeling studies should aid in further refinement of these processes.

#### **NEXT CONTRACTOR REVIEW MEETING**

The next Alcohol Fuels Contractors' Review Meeting will be held in Golden, Colo., on

November 8-9, 1984. The contractors will review the research results from the last six months.

The meeting will be open to principal investigators and others directly involved in the DOE Alcohol Fuels Program but will be closed to the public. Results of the discussions and executive summaries of all the contractors' reports will be published in the next issue of the Alcohol Fuels Program Technical Review.

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# Contractors' Executive Summaries

## Acid Hydrolysis Research

### DARTMOUTH COLLEGE

#### Acid Hydrolysis of Cellulosic Biomass

##### I. Introduction

The main focus of our work is on the acid-catalyzed hydrolysis of biomass. At severe conditions this hydrolysis leads to the production of sugars, principally glucose and xylose, and decomposition products such as furfural. We have found that under milder conditions, which cause the xylan but not the glucan to react, the pores in the biomass are enlarged. As a result, the rate of enzymatic hydrolysis is increased, and nearly quantitative yields are obtained.

Both direct-acid hydrolysis and mild-acid hydrolysis are of potential commercial interest when followed by enzymatic hydrolysis. Direct-acid hydrolysis is currently limited by glucose yield (~55%) and sugar concentration, but the rate is very rapid (residence time ~7 s). With enzymatic hydrolysis the yield can be quite high (>95%) but the rate is low (residence time ~24 h). Our goals are to find processing conditions that overcome those restrictions and to determine kinetic parameters that can be used in process design and optimization. The kinetics of the formation of valuable decomposition products such as furfural are also included in the project.

##### II. Pretreatment with Subsequent Enzymatic Hydrolysis

###### A. Steam Explosion

To obtain a direct comparison of steam-explosion and mild-acid hydrolysis pretreatments, a steam exploder has been developed. (Mild acid

hydrolysis takes place in a flow reactor and also involves an explosive decompression. Its effectiveness decreases when the acid is removed.) Poplar was steam exploded at various conditions and subjected to 24-h enzymatic hydrolysis using RUT C-30 strain at 92.5 mg/100 mL and NOVO 250L 0.1 mL. Samples were kept moist prior to enzymatic hydrolysis. Several runs are summarized in Table 1. Low-pressure steam (<450 psi) was investigated because of its general availability. The results show good yields at the longer pretreatment times. Further investigation of the pretreatment conditions is under way, and further work will be done to determine the reproducibility of all experiments.

With mild-acid hydrolysis, the residence time is less (7 s) and the yields are higher (>95%), but the steam pressure is much greater (~700 psi) and the feed must be pumped. By combining the data being obtained with existing data on the mild-acid hydrolysis pretreatment, we can make an economic comparison.

###### B. Disc Refinement

Table 2 shows the hydrolysis yield from a sample of disc-refined hardwood chips compared with the yield obtained from the same material after being subjected to the mild-acid hydrolysis in the flow reactor. The disc refiner as operated at Groveton is not a very effective pretreatment, probably because of the low steam pressure. When compared with the steam explosion results in Table 1, the glucose yields are in reasonable agreement, although outside the experimental range covered.

###### C. Cellobiase Kinetics Study

To obtain high yields of glucose, it is necessary to add cellobiase to the enzyme from

**Table 1. Glucose Yield Achieved through Enzymatic Hydrolysis of Steam-Exploded Wood (%)**

Feed	Steam Pressure (psig)	Residence Time (min)	Duration of Hydrolysis (h)		
			1	7	24
Untreated poplar	--	--	5.0	9.0	14.9
Steam-exploded poplar	250	5	1.6	34.8	60.5
Steam-exploded poplar	350	5	15.9	63.6	76.5
Steam-exploded poplar	450	5	a	70.1	79.2
Steam-exploded poplar	250	20	17.4	67.5	80.4
Steam-exploded poplar	350	20	8.0	62.3	82.0
Steam-exploded poplar	450	20	5.2	49.0	87.2

<sup>a</sup>No reading.

**Table 2. Glucose Yield Achieved through Enzymatic Hydrolysis of Disc Refined Hardwood (%)**

Sample	Duration of Hydrolysis (h)		
	1	7	24
Slurry from Groveton papermill <sup>a</sup> after steaming for 12 min at 160 psig and discharge through a disc refiner	9.4	32.1	51.9
The above slurry after mild acid pretreatment at 1% H <sub>2</sub> SO <sub>4</sub> , 200°C, 8 s	26.6	76.3	98.9

<sup>a</sup>James River Company, Groveton, NH. Prior to going through the refiner the wood was cooked in a solution of CaCO<sub>3</sub> and dilute NaOH.

T. reesei. We have used NOVO 250L cellobiase, which is obtained from A. niger.

To determine the appropriate amount of NOVO 250L to add, it is necessary to have a kinetics model. Although much of this work was supported under a previous NSF contract (CPE-792-1074), it is reported here because of

its potential use in process design. The rate expression and parameters are presented in Table 3. It is necessary to use two sets of parameters to cover the concentration range studied because more than one enzyme is involved. Figures 1-4 show predicted data compared to actual data. The cellobiase in NOVO 250L is much more active than that

**Table 3. Parameter Values in Cellobiase Kinetics Models**

Parameter	<i>T. reesei</i> , Rutgers' C-30 Strain		NOVO 250L	
	$\leq 27.3$	$\geq 27.3$	$\leq 21.1$	$\geq 21.1$
Initial cellobiase concentration, mM	$\leq 27.3$	$\geq 27.3$	$\leq 21.1$	$\geq 21.1$
Rate expression <sup>a</sup>	A	A	A	B
K, mmol/(g enzyme h)	0.623	13.0	59.7	59.7
K <sub>m</sub> , mM	0.153	472	1.66	1.66
K <sub>i</sub> , mM	0.212	32.6	2.87	--
K <sub>s</sub> , mM	--	--	--	43.4

<sup>a</sup> $v = 2k_3 SE/[Km(1 + G/Ki) + S]$ , rate expression A

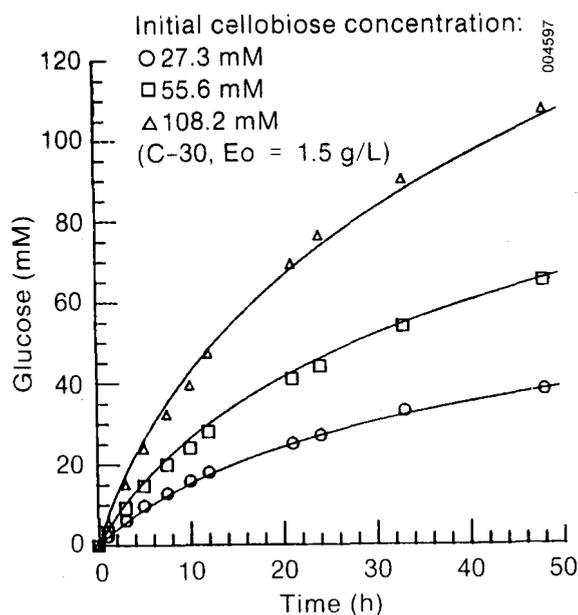
$v = 2k_3 E/[(Km/S) + 1 + S/Ks]$ , rate expression B

S = substrate

SE = substrate/enzyme complex

E = enzyme concentration

G = glucose concentration



**Figure 1. Comparison of Predicted (Solid Curves) vs. Measured Glucose Concentration as a Function of Time**

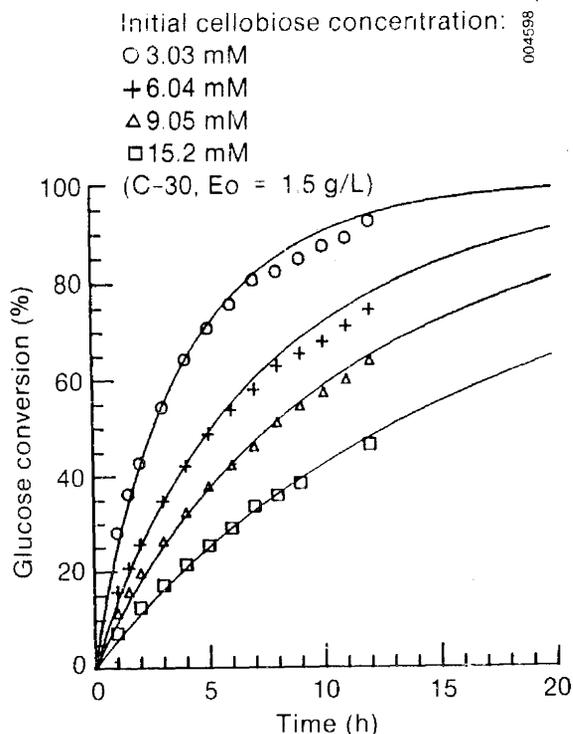
contained in the enzymes from *T. reesei*; note that the time scale of the former is in minutes, while the latter is in hours. The models fit the data well over a wide range of initial cellobiase concentrations.

### III. Reactor Design and Development

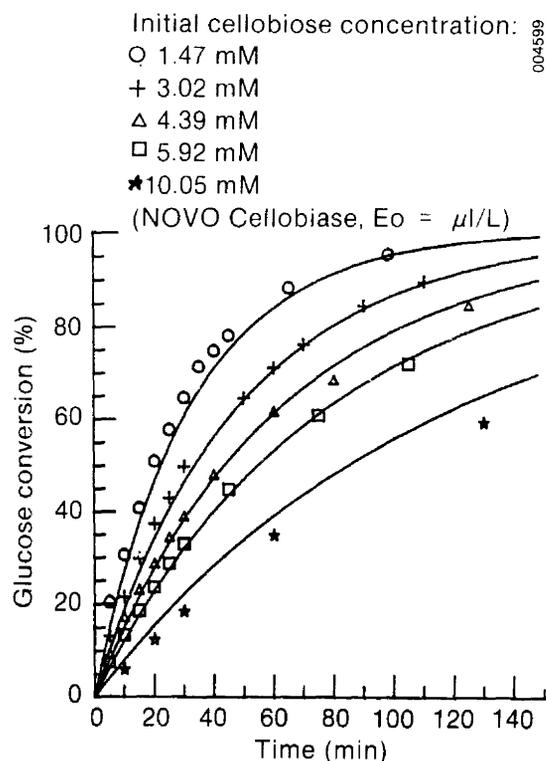
#### A. Vortex Letdown Device

In order to process larger wood particles in our continuous-flow, acid hydrolysis reactor, we have developed a vortex letdown device. For a given pressure drop, a larger orifice diameter can be used.

The reactor effluent is channeled so that it enters tangentially into a cylindrical chamber. The exit orifice senses a smaller pressure drop because of the flow field. For a given pressure drop, the orifice area has been increased from 1.2 mm<sup>2</sup> to 1.6 mm<sup>2</sup>.



**Figure 2. Comparison of Predicted (Solid Curves) vs. Measured Glucose Conversion as a Function of Time**



**Figure 3. Comparison of Predicted (Solid Curves) vs. Measured Glucose Conversion as a Function of Time**

Two devices are now used in series in the flow reactor, which raises the reactor pressure somewhat. This is important at the higher temperatures in order to obtain complete condensation of the steam. (Drawings are included in the Winter 1983 Technical Review.)

### B. Steam Injector

Originally, steam was injected from a tube running along the center line radially into an annulus through a large number of ports. This tube has been replaced by a small chamber in which the feed and steam enter tangentially. The stability of the reactor operation has increased and the reactor operation should be easier to scale up.

### C. Pumping of Larger Particles

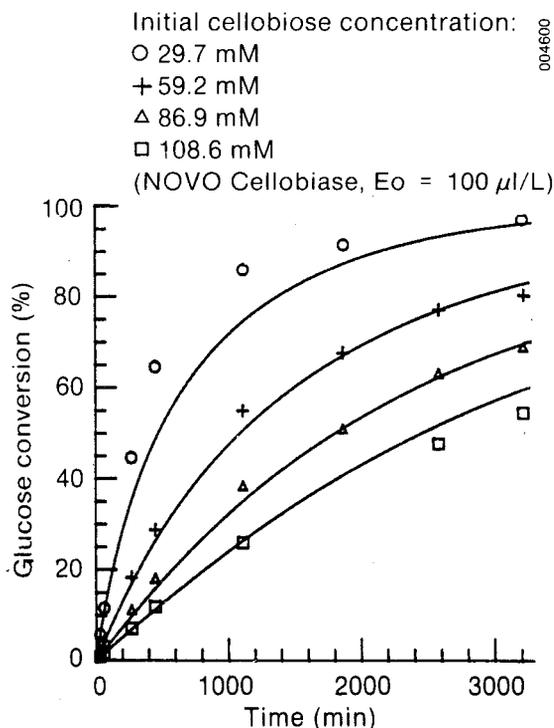
Slurry from the disc refiner at the James River paper mill (Groveton, N.H.) was success-

fully pumped through the reactor under reaction conditions. This slurry forms a mat when dried.

SERI researchers provided a sample of the 18- to 40-mesh (1- to 0.425-mm) fraction of ground aspen wood. The particles were needle-shaped, and some were as long as 10 mm. This feedstock plugged the 1/4-in. tubing that feeds the 1/2-in. reactor. This result suggests that a larger Moyno pump be used for this material.

### D. Vortex Reactor

The feed and steam are to be injected tangentially into a doughnut-shaped reactor causing the fluid in the reactor to rotate. The resulting radial acceleration causes the solids to have a longer residence time than the liquids.



**Figure 4. Comparison of Predicted (Solid Curves) vs. Measured Glucose Conversion as a Function of Time**

This should increase the glucan conversion but decrease the glucose decomposition. The reactor is under construction and is to be tested this summer.

#### IV. Acid Hydrolysis of Biomass Catalyzed by Sulfur Dioxide

We investigated hydrolysis using sulfur dioxide for the following reasons:

- The critical temperature of sulfur dioxide, 431 K, is in the range of normal acid hydrolysis temperatures. Hence, it is possible to carry our acid hydrolysis using sulfur dioxide under supercritical conditions. Other solvents and reactants have beneficial properties in the supercritical state.
- By carrying out the hydrolysis in a fluid predominantly composed of sulfur dioxide with only a relatively small amount of water present, it is possible to produce

sugar solutions that are much more concentrated than when water is used as the only conveying fluid.

The glucose yields obtained from Solka Floc are presented in Figure 5 as a function of temperature, reaction time, and water content. The highest yield is 41%, and the corresponding glucose concentration is 9%. For concentrated-acid hydrolysis, this yield is low. However, the 9% glucose concentration demonstrates the ability to obtain high concentrations.

The conditions under which this result was obtained ( $181^{\circ}\text{C}$ , 10 min, and 23% water) are not supercritical. Supercritical conditions did not produce high yields possibly because the pressure limit of the reactor required that the water content be less than 2%. Furthermore, the batch reactor has long heating and cooling times (45 and 30 min, respectively). This limitation will be overcome in the small-scale flow reactor that is being assembled.

Similar experiments have been run using mixed hardwoods. The maximum yield obtained to date is 25%.

#### V. Acid Hydrolysis in the Presence of Acetone

This research was undertaken to determine whether the presence of acetone will complex with the sugars formed and reduce the rate of decomposition. Experiments have been run in the batch and flow reactors. To date the yields have not exceeded the values obtained when the acetone is not present. The experimental program, however, has not been completed.

#### VI. Distillation

Although this work is not supported by SERI, it is of potential interest. Through the novel use of heat pumps, Lee Lynd and Hans Grethlein have developed the ethanol distillation process

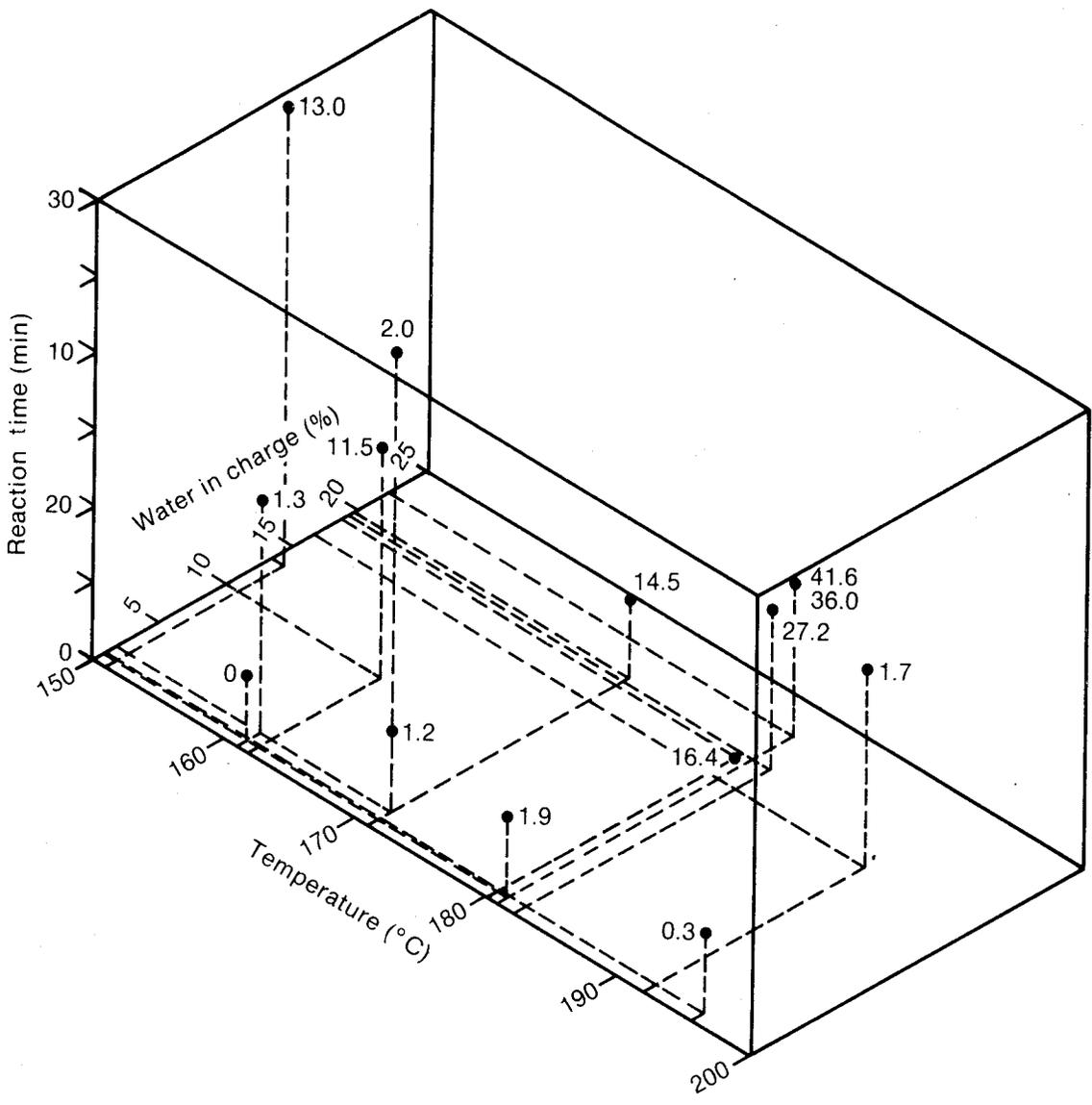


Figure 5. Glucose Yields as a Function of Reaction Time, Temperature, and Water Content for the SO<sub>2</sub> Hydrolysis of BW-200 Solka Floc

shown in Figure 6. A portion of the vapor from the feed tray is removed, compressed, condensed in the reboiler, and returned to the tower as reflux. Vapor from the first tower is condensed in the reboiler of the second.

Potassium acetate is used to break the azeotrope. It is recovered for recycle by evaporation followed by spray drying. Vapor from evaporation is returned to the first column to serve as a stripping medium.

Anticipated advantages of this system for ethanol recovery are lower energy requirements, the ability to produce anhydrous ethanol, and distillation columns with fewer stages and smaller diameters. Figure 7 presents energy and stage requirements for separating ethanol/water mixtures consisting of saturated liquid of 1-10 wt % ethanol. The heights of the uppermost rectangles relative to the horizontal axis represent the sum of the heat requirement and three times the work requirement ( $Q + 3W$ ). The  $Q + 3W$  requirements displayed in Figure 7 range

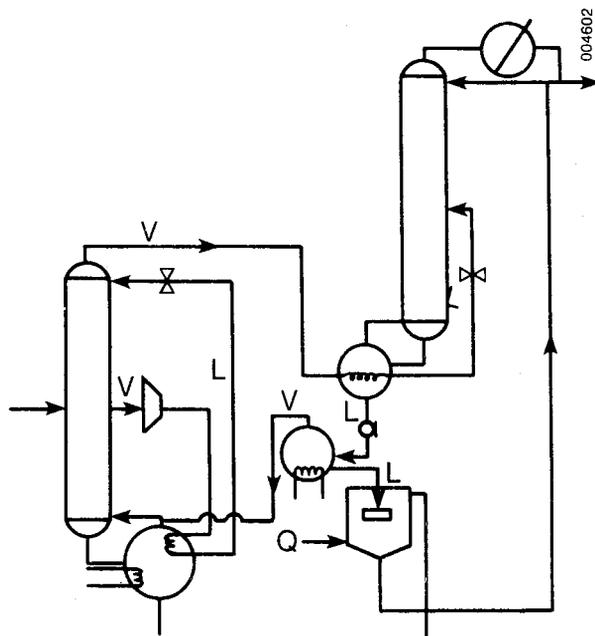


Figure 6. Flow Sheet for Separation of Ethanol/Water Mixtures via the Dartmouth Process

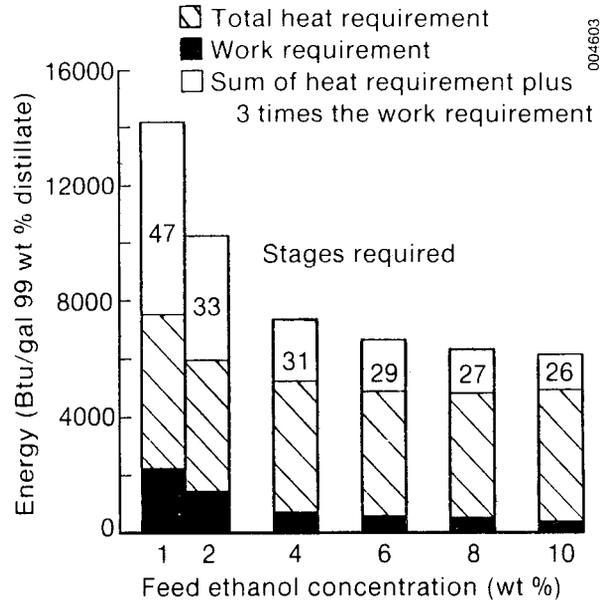


Figure 7. Stage, Heat, Work, and Heat-Equivalent Requirements for Separation of Ethanol/Water Mixtures via the Dartmouth Process

from ~14,000 Btu/gal for the 1 wt % case to ~6000 Btu/gal for the 10 wt % case.

For comparison, we calculated that the heat necessary to separate a 1 wt % feed is 60,000 Btu/gal by conventional distillation. The energy-efficient production of fuel-grade anhydrous ethanol (Katzen et al., U.S. Patent No. 4,217,178) requires 19,340 Btu/gal to separate an 8.54 wt % feed. State-of-the-art methods using vapor recompression heat pumps have a  $Q + 3W$  requirement of ~15,000 Btu/gal to separate a 10 wt % feed to anhydrous ethanol (Busche 1983, Proceedings of the Fifth Symposium on Biotechnology for Fuels and Chemicals). The theoretical stage requirements presented in Figure 7 range from 47 for the 1 wt % case to 26 for the 10 wt % case. Conventional ethanol distillation requires 86 theoretical stages for separating a 5 wt % feed according to Griffith et al. (1982, paper presented at the winter meeting of the AIChE). The Katzen process requires 140 trays for an 8.54 wt % feed. These comparisons need to be refined and a detailed economic evaluation prepared.

## TENNESSEE VALLEY AUTHORITY

### Ethanol from Herbaceous Cellulose Feedstocks

This report summarizes the facilities engineering phase of a project to build a large-scale (10 gal/h) experimental facility to produce ethanol from herbaceous cellulose feedstocks. The process uses a two-stage, low-temperature, concentrated-sulfuric-acid hydrolysis. Flowsheets, component specifications, and costs are given.

#### I. Facilities Engineering Summary

Based on the low-temperature, two-stage, concentrated-sulfuric-acid process (Figure 1) reported by TVA personnel at the last DOE Contractors' Review Meeting, an experimental facility has been built on the TVA Reservation at Muscle Shoals, Ala. The facility is sized to convert 300 lb/h of feedstock to 190-proof ethanol at about 10 gal/h if both xylose and glucose sugars are fermented. Existing fermentation and distillation systems will be incorporated into the facility.

This report summarizes the facilities engineering phase of the project. Included are component cost summaries (Table 1) and general component specifications.

Filters (F-1 and F-2): The filter presses have recessed chamber plates. The stand and all nonwetted parts are made of carbon steel, and the plates and wetted parts are made of polypropylene. The presses have a double-acting, hydraulic, open/close system capable of closing the plates at not less than 3,000 psi and are semiautomatically controlled.

Tanks: T-1, T-2, T-3, T-4, T-6, T-8, N-1, L-1, L-2, and L-3 are made of FRP (Hetrion 700 Resin) compatible with sulfuric acid (40%) at 212°F. T-5 is a concentrated-acid (93.4%) storage tank made of carbon steel. T-15 is a polypropylene hot-water storage tank. N-1 is equipped with an agitator for neutralization.

Reactors (R-1, R-2A&B): R-1 and R-2A are insulated, vertical-cylinder, 45° cone-bottom

tanks made of Hetrion 700 FRP. R-2B is an insulated, vertical-cylinder, 45° cone-bottom tank made of Hetrion 980 FRP. R-2A&B are equipped with agitators to maintain solids suspension.

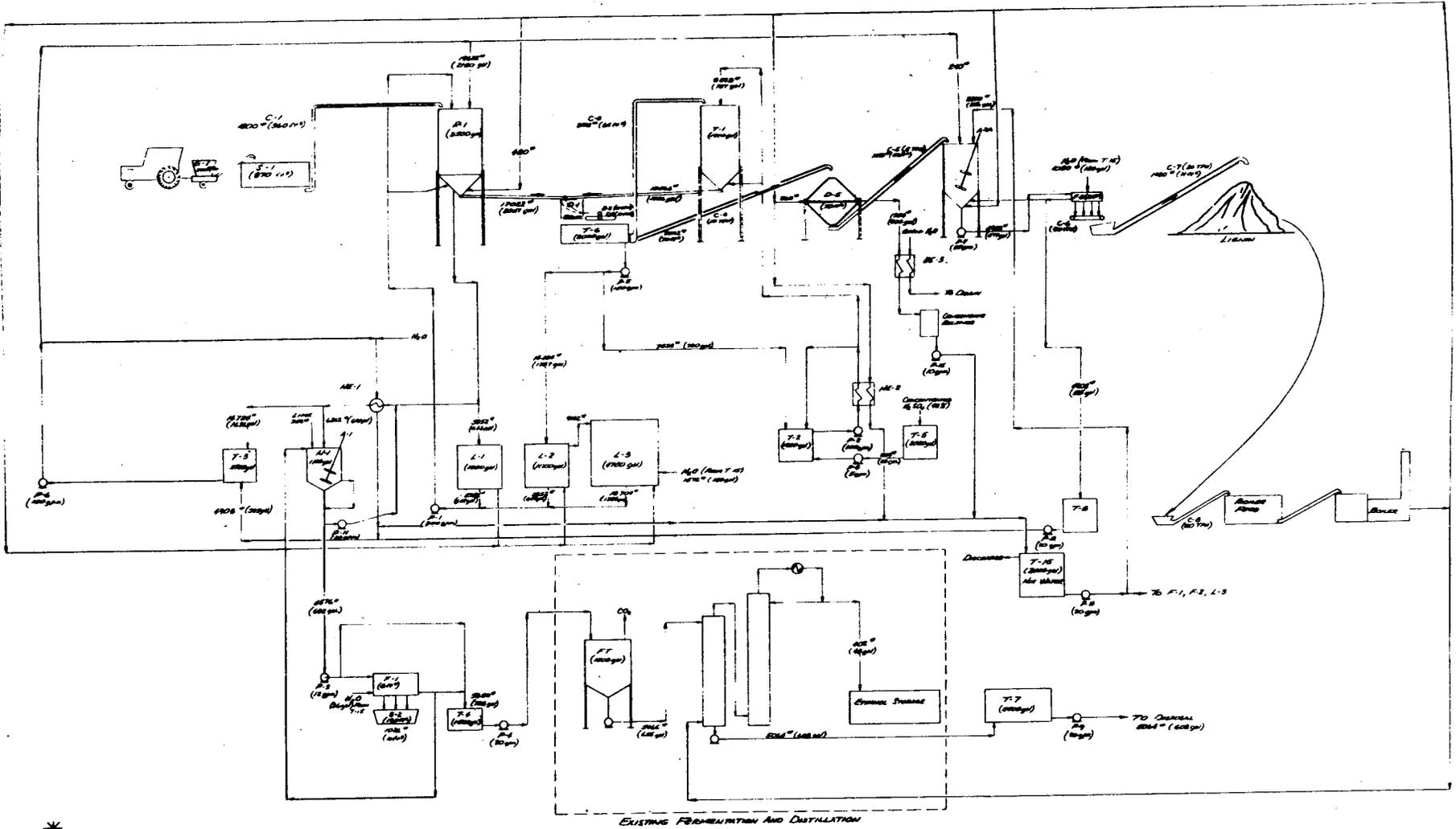
Pumps: P-1, P-2, P-4, P-5, P-6, P-9, P-11, P-12, P-13, P-14, and P-15 are all horizontal, centrifugal pumps made of FRP, cast iron, bronze, and carbon polysulfonate on carbon steel with Teflon linings. P-8 is a double-diaphragm metering pump for concentrated acid. P-3 and P-7 are air-driven diaphragm pumps made of cast iron and polypropylene and are used to feed the filter presses.

Conveyors: C-1 and C-3 are pneumatic conveyors designed around farm forage blowers modified to electric motor drive. C-2 is a custom-made belt conveyor to feed the roller press D-2. Belting is 3-ply white hylar with Teflon-covered rolls. C-4, C-5, and C-6 are troughing belt conveyors with PVC belting. C-7 is a single-chain, rubber-paddle conveyor.

Heat Exchangers: HE-1 and HE-2 are used for process temperature control and heat recovery. They are made of 8-in.-diameter CPVC shell with 3/4-in. Carpenter 20 internal coiled tube.

Moisture Removal Equipment: D-1 is a stationary hydrasieve consisting of a fiberglass housing with a 316-stainless steel screen. D-2 is a roller press with a nip pressure of 4,000 lb. Nip rolls are 6-in. diameter Nitule rubber over solid steel barstock. D-5 is a double-cone rotary vacuum dryer. The dryer is made of mild steel, coated with Kynar, and insulated with 1 in. of fiberglass. Teflon gaskets and 316-stainless steel valves and seats are also used.

Stover Grinder and Storage: G-1 is a portable agricultural tub grinder powered by a farm tractor. The grinder can receive large, round bales of feedstock material in the large circular tub. The unit has interchangeable hammermill screens and an integral discharge conveyor. Ground feedstock (S-1) is stored in a farm forage wagon (without running gear) modified to electric motor drive.



\*  
NOTE  
CAPACITY GIVEN FOR EACH SYSTEM COMPONENT  
WITH EQUIPMENT CODE (F-1, D-1, etc.)

Figure 1. Process Flow Diagram

**Table 1. Cost of Equipment for a Two-Stage, Low-Temperature, Concentrated-Acid Hydrolysis Plant**

Component	Designation	Cost
CaSO <sub>4</sub> filter	F-1	\$25,000
Cellulose/lignin	F-2	35,000
Hydrasieve	D-1	7,200
Roller press	D-2	9,600
Dryer	D-5	51,000
Pretreatment tank	T-1	5,500
Pretreatment acid tank	T-2	3,100
Sugar/acid solution tank	T-3	4,200
Sugar product tank	T-4	2,300
Sulfuric acid storage tank	T-5	3,800
Filtered-liquid tank	T-6	5,500
Hydrasieve hold tank	T-8	2,500
First leach tank	L-1	2,200
Second leach tank	L-2	2,700
Third leach tank	L-3	6,400
Neutralization tank	N-1	5,200
Leach pump	P-1	5,100
Prehydrolysis pump	P-2	5,300
CaSO <sub>4</sub> filter feed pump	P-3	600
Sugar product solution pump	P-4	200
Cellulose reactor pump	P-5	3,500
Sugar/acid solution recycle pump	P-6	6,000
Cellulose/lignin filter feed pump	P-7	920
Acid supply pump	P-8	1,400
Stillage disposal pump	P-9	200
Neutralization tank pump	P-11	2,400
Sugar/acid solution transfer pump	P-12	1,800
Sump	P-13	700
Hot water pump	P-14	300
Hot water pump	P-15	100
Hemicellulose reactor	R-1	9,100
Cellulose reactors	R-2A&B	9,100
Tub grinder	G-1	9,400
Pneumatic conveyor	C-1	1,400
Belt conveyor	C-2	2,500
Pneumatic conveyor	C-3	1,500
Belt conveyor	C-4	4,500
Belt conveyor	C-5	5,100
Belt conveyor	C-6	3,400
Belt conveyor	C-7	1,500
Heat exchanger	HE-1	3,600
Heat exchanger	HE-2	2,300
Stover storage	S-1	6,000

Piping: The following piping materials were used:

Material	Application
Carbon steel	Steam and concentrated acid
PVC	Portable water supply
CPVC	Hot water distribution and CaSO <sub>4</sub> slurry recirculation
PP	Corrosive drain
Kynar	Instrument fittings subjected to hot (> 200°F) corrosive environments
FRP	Process lines containing sulfuric acid

Electrical: Electrical service of single-phase 120 VAC and three-phase 230 VAC and 480 VAC is available. Most equipment is operated on 480 VAC. Conduits, enclosures, and connections meet NEMA 1 standards except for the external sump pump, which meets NEMA 4 standards. All motors are TEFC or explosion proof and have manual on/off controls.

Instrumentation: Material Flows--All process steps are batch operated and load cells are

used under tanks, reactors, and filter presses to measure mass flows. Flowmeters are used to monitor steam and water flows. A metering pump is used to quantify sulfuric acid flow. Process temperatures--Thermometers and thermocouples are used in tanks and pipelines as required. Pressures--Pressure gauges are used to monitor steam pressure and pump heads. Diaphragm isolators are used in acid lines. Acidity--A portable pH meter is used to monitor the acidity of N-1 during neutralization. Controls--The experimental plant requires the use of few automatic controllers. Most control operations involve only an on/off adjustment and routine monitoring of locally mounted temperature sensors, pressure gauges, and weight indicators. A single automatic control loop for producing 180°F water at HE-1 is designed into the plant. The control loop is composed of a filled thermal element integrated with a pneumatic controller and control valve. Data Collection--A 32K RAM memory microcomputer will be used for data storage and retrieval.

## II. Future Plans

After facility shakedown in late spring 1984, the plant will be operated for 2-3 consecutive 24-hour days per month. Initial tests with corn stover will verify material balances, process efficiencies, materials of construction, and materials handling procedures.

## Support Research

UNIVERSITY OF CALIFORNIA AT SANTA  
BARBARA

### Electrochemical Transformation of Levulinic Acid and Other Biomass-Derived Products

#### I. General Discussion

The features of biomass-derived levulinic acid and its simple derivatives I that make them attractive for conversion to other organic chemicals, either by accretion or by degradation, are the difunctionality at C-1 and C-4, as shown in the following equation:



5    4    3    2    1

I

Oxidative decarboxylative dimerization of I (R=H) focuses on C-1, while reduction and cyclization focus on C-4. Various photochemical degradations of I (R=H) have also been carried out at SERI.

The aim of our work is to investigate synthetic possibilities that arise because I has yet other

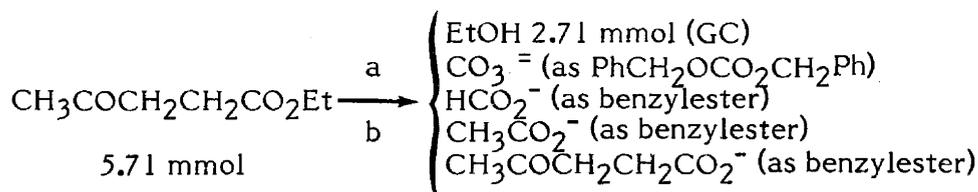
functionalities not exploited. At C-2, C-3, and C-5 a carbon acid can be deprotonated by appropriate electrogenerated bases (EGBs). The anions formed as a consequence of this deprotonation must engage in intra- and inter-molecular reactions.

Our early work with I involved deprotonating it by an EGB and then alkylating. Alkylation could have occurred at C-3 or C-5 or both; we found reaction only at C-3.

Using electrogenerated  $\text{O}_2^-$  as an EGB and  $\text{O}_2$  as a reactant or both, both I and ethyl-3-methyllevulinate gave a variety of fragmentation products described in this and other reports. It is now of interest to determine the product that will be formed on treating I with EGB and no other reagent.

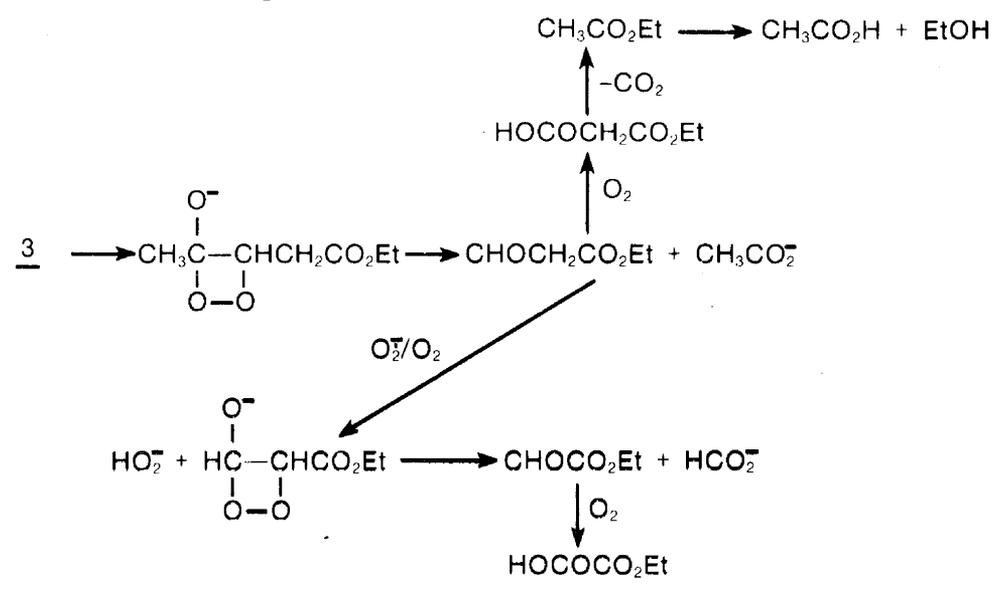
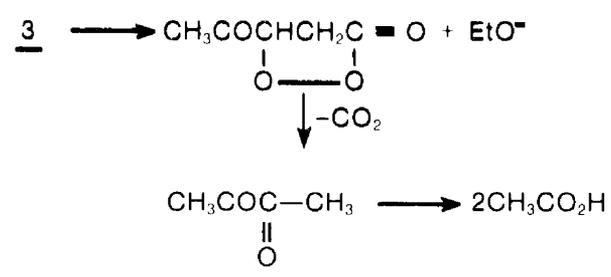
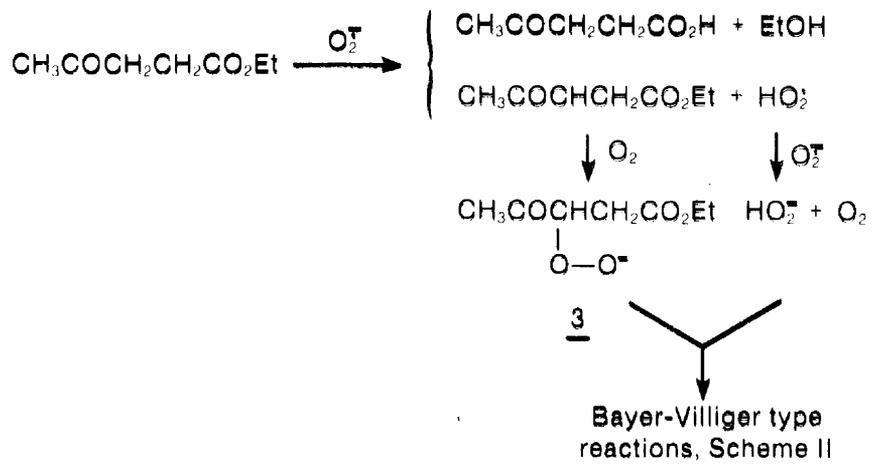
#### II. Work Accomplished

Ethyllevulinate (1), upon treatment with electrogenerated super-oxide/ $\text{O}_2$ , provides a set of products similar to those produced from ethyl-3-methyllevulinate (2). However, the notable absence of ethyl acetate contrasts with the reaction of 2. The reaction is shown here:



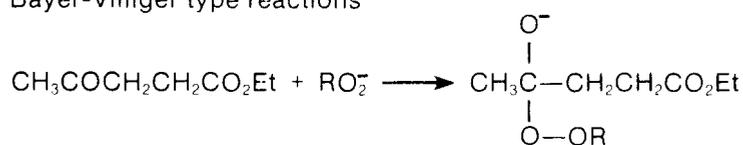
- $\text{O}_2$ , -1.0 V vs. SCE (Hg), DMF,  $(\text{n-Bu})_4\text{NB}_r$
- The nonvolatile residue obtained in the work-up procedure was treated with  $\text{C}_6\text{H}_5\text{CH}_2\text{Cl}$  to convert all  $(-\text{COO}^-)$   $(\text{n-Bu}_4\text{N}^+)$  to  $-\text{COOCH}_2\text{C}_6\text{H}_5$ .





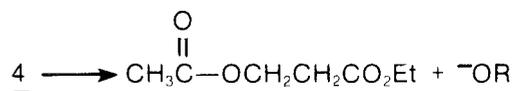
Scheme I.

Bayer-Villiger type reactions

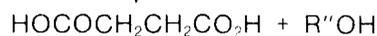


(R = CH<sub>3</sub>COCHCH<sub>2</sub>CO<sub>2</sub>Et, H)

4



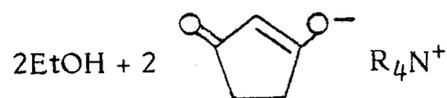
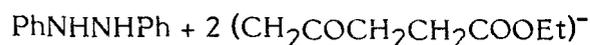
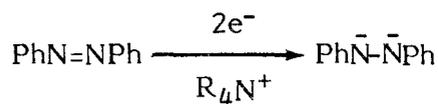
( R' = Me, R'' = Et )  
( R' = Et, R'' = Me )



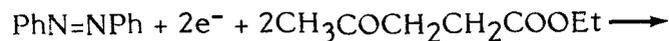
Scheme II.

- Use one of several probases (such as azobenzenes, tetraalkyl ethenetetracarboxylates) that have been used many times to produce EGB-catalyzed condensations.

For the initial experiments we have used azobenzene as the probase. In the ideal case the following sequence of reactions may be expected:



### B. Overall



Note that the dione is expected to be present at the end of the electrolysis in the form of its tetraalkyl-ammonium enolate. This should enable us to (1) acidify strongly to obtain the

dione itself, or (2) treat the catholyte with a trace of HOAc to discharge any residual PhNNPh or PhNPh and then with an electrophile to convert the dione enolate directly in a one-pot reaction to a derivative (at O- or C- or both).

The first exploratory experiment is described below; subsequent ones are in progress.

### C. Experiment

The cathode material was mercury and the anode material platinum. The cell was charged under nitrogen with the electrolyte (0.22 M Bu<sub>4</sub>NBr/DMF). To the catholyte was added some alumina (to trap traces of water) and the azobenzene (1.1 equiv. wt). The ethyllevulinate (1 equiv. wt) was dissolved in 3 mL of DMF and added to the catholyte during 4 h. To prevent hydrostatic pressure at the same rate, 4 mL of DMF were added to the anolyte. The reaction was performed at -1.2 V vs. SCE, and the disappearance of starting material was monitored by TLC and finally checked by GC.

After 0.74 F/mol the current was shut off and the reaction mixture was stirred until all ethyllevulinate had disappeared (55 min). The catholyte still contained an excess of the alkaline azobenzene anion (brown color), which was

neutralized with AcOH. The catholyte was poured on ice and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic phase was dried with MgSO<sub>4</sub> and the solvents (CH<sub>2</sub>Cl<sub>2</sub> and DMF) were evaporated off. The residue was acidified with a small amount of NaHSO<sub>4</sub>/H<sub>2</sub>O and stirred with DME. The presence of 1,3-cyclopentanedione was detected by GC; the retention time was the same as that of an authentic sample.

The experience gained in this run will enable us to make quantitative determinations of products in subsequent experiments.

### IV. References

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2. Šraga, J.; Hrnčiar, P., Z. Chem., Vol. 15, p. 189, 1975; and Czech., 172,020 April 15, 1978.
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## GEORGIA TECH RESEARCH INSTITUTE

### Fuel-Grade Ethanol Recovery by Solvent Extraction

The goal of this program is the development of a cost-effective and energy-efficient solvent extraction process that can recover fuel-grade ethanol (i.e., 98-99 wt %) from dilute aqueous mixtures (2-10 wt %). The ethanol product must be suitable for use in the manufacture of gasohol, and the energy and cost requirements for recovery should be less than for an equivalent distillation process. In addition, the solvents used should have a low toxicity so they

will not interfere substantially with any fermentation processes that may be used to produce the aqueous ethanol feed to the recovery system.

Currently, beer stills and azeotropic distillation systems can produce a fuel-grade product from an 8 wt % beer with energy expenditures of about 24,000 Btu/gal. Our current solvent extraction concept offers the possibility of producing a fuel-grade product with an energy expenditure of about 8200 Btu/gal. This process uses a single cycle of liquid/liquid extraction to produce at least an 80% wt ethanol product from the extract as recovered from a

5 wt % ethanol/water mixture. According to UNIFAC calculations, the extract may then be processed using extractive distillation with a total energy expenditure of about 8200 Btu/gal to produce a 99 wt % ethanol product. We currently estimate that the capital expenditures for this combined solvent extraction/extractive distillation system will be less than for an equivalent distillation system. Hence, the expected savings are about 15¢/gal, including the energy savings and the reduced capital expenditures.

During this reporting period, the primary emphasis has been on the implementation of the extractive distillation/solvent regeneration cycle. We have designed and constructed one six-stage bubble-cap column that will serve as the extractive distillation column. In addition, another five-stage bubble-cap column is being fabricated to use as a solvent regeneration column. In this concept, the extract is first processed through an extractive distillation column in which the water is removed from the organic phase. According to our UNIFAC calculations, this can be accomplished by a mild heating of an atmospheric column with the regenerated solvent being added to the top of the column and no more than three theoretical stages. The energy expenditure to operate this column is approximately 3200 Btu/gal.

The dehydrated extract is then passed into a second column where the ethanol is removed. In this case, UNIFAC predicts that the column may be operated at about 500 mm Hg and

heated to about 220°C to effectively regenerate the solvent. The estimated energy expenditure is about 5000 Btu/gal. Both of these columns require the use of economizers and heat-exchanger matching to minimize the overall energy use.

During this period we have also evaluated the use of a new extractant, a methyl ester. This material costs about \$4/gal and consists of a methylated coconut oil middle cut. Our distribution coefficient correlations for this solvent indicate that we can obtain an 80 wt % ethanol product from a 5% ethanol-and-water mixture in a single solvent extraction cycle operating at 70°C. Actually, at 70°C and in the presence of inextractable dissolved solids such as dextrose, the extract appears to be above the azeotrope on a solvent-free basis. We have also tested this solvent in our 1-in. Karr reciprocating plate column and confirmed its basic feasibility, although the solvent forms relatively stable hazes in the organic phase. Hence the extract must be carefully filtered to achieve the anticipated equilibrium concentrations. Actual tests have led to an 82% product based on the filtered extract.

Batch fermentation tests suggest that our latest solvent choice does not affect the rate of ethanol production by Saccharomyces cerevisiae. Therefore, we anticipate that the methyl ester will be relatively nontoxic to microorganisms.

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## LEHIGH UNIVERSITY

### Catalysts for Alcohols from Biomass

During the last six months of research, our efforts have been specifically directed toward determining the influence of experimental reaction conditions on the activity and selectivity toward the synthesis of low molecular weight alcohols over a Cs/Cu/ZnO = 0.4/30/70 mol % catalyst. Parameters that have been studied include (1) the H<sub>2</sub>/CO ratio of the inlet synthesis gas, (2) the reactor temperature, (3) the contact

time of the synthesis gas with the catalyst, and (4) the pressure under which the alcohol synthesis reactions are achieved.

The binary Cu/ZnO = 30/70 mol % catalyst was prepared by coprecipitation, drying, calcination at 350°C, pelletization, and reduction at 250°C in a flowing H<sub>2</sub>/N<sub>2</sub> = 2/98 vol % gas stream. Impregnation of the catalyst with cesium was achieved by adding the reduced catalyst to an aqueous solution, under a nitrogen atmosphere, that contained cesium hydroxide. The solution was then evaporated, and the resultant catalyst was returned to the 316 stainless steel reactor for testing. The

yield of methanol over the Cs/Cu/ZnO catalyst at 235°C and 75 atm with  $H_2/CO = 2.33$  vol % synthesis gas at GHSV =  $5000\text{ h}^{-1}$  was observed to be  $290\text{ g kg catalyst}^{-1}\text{ h}^{-1}$ .

During previous research, we observed that increasing the temperature of the reaction and decreasing the GHSV (thus increasing the residence time of the synthesis gas over the catalyst) led to the formation of higher molecular weight alcohols. Table 1 shows the effect of varying the  $H_2/CO$  synthesis gas ratio on the activity and selectivity observed over the Cs/Cu/ZnO catalyst at 325°C and GHSV =  $860\text{ h}^{-1}$ . It is evident that as the  $H_2/CO$  ratio decreases, the activity toward the synthesis of oxygenates decreases. The selectivity toward methanol synthesis also decreases. However, the overall selectivity toward the production of oxygenates, and in

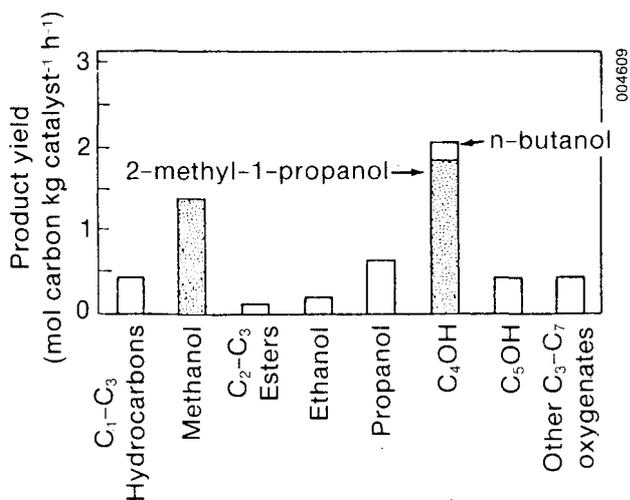
particular the formation of 2-methyl-1-propanol (isobutanol), increases as the  $H_2/CO$  ratio declines.

Utilizing the  $H_2/CO = 0.45$  synthesis gas ratio but decreasing the temperature to 310°C yielded the results shown in Figure 1. The percentage of carbon conversion to products, exclusive of the formation of carbon dioxide, was 18.45%, and the product mixture contained 38.09 wt % methanol and 29.26 wt % 2-methyl-1-propanol. The product selectivity as a function of temperature between 288°C and 325°C is indicated in Figure 2. While the product selectivity shifted toward the branched  $C_4$  and  $C_5$  alcohols at the higher temperatures, it would be desirable to produce the  $C_1$ - $C_5$  alcohols at temperatures below 300°C to ensure the long-term stability of the catalyst.

**Table 1. Composition of the Alcohol Mixtures Produced at 75 atm, 325°C, and GHSV =  $860\text{ h}^{-1}$  over 2.42 g of CsOH/Cu/ZnO = 0.4/30/70 mol % Catalyst (wt %)**

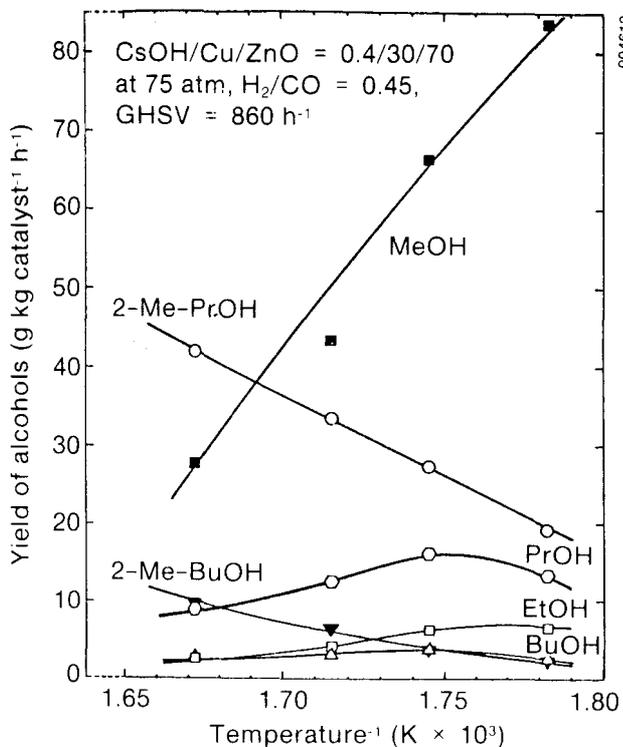
Products	$H_2/CO$ Synthesis Gas Ratio		
	0.45	0.75	1.00
Methanol	23.83	35.13	40.06
Ethanol	2.22	3.18	3.60
Propanol	7.62	11.12	11.48
Butanol	2.37	2.32	2.51
2-butanol	1.29	1.33	1.10
Isobutanol	35.93	26.98	26.71
2-methyl-1-butanol	7.86	4.82	4.45
Pentanol	0.94	0.94	0.76
$C_2$ - $C_3$ formates	1.35	1.22	1.23
$C_3$ - $C_4$ aldehydes	2.33	1.79	0.91
$C_4$ - $C_5$ ketones	1.14	1.63	0.92
Other $C_5$ - $C_7$ oxygenates	12.61	9.54	6.52
Percentage of oxygenates in product <sup>a</sup>	98.6	95.0	94.5
Percentage of carbon conversion to products <sup>a</sup>	20.25	23.6	29.0

<sup>a</sup>Exclusive of  $CO_2$ .

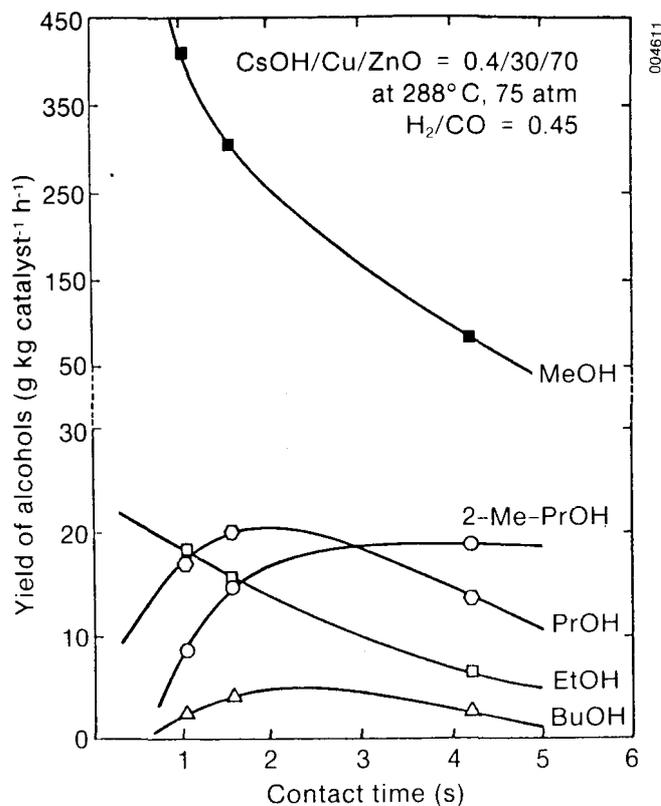


**Figure 1.** Yields of Products Obtained over a Cs/Cu/ZnO = 0.4/30/70 mol % Catalyst at 310°C with a H<sub>2</sub>/CO = 0.45 Synthesis Gas at 75 atm and GHSV = 860 h<sup>-1</sup>

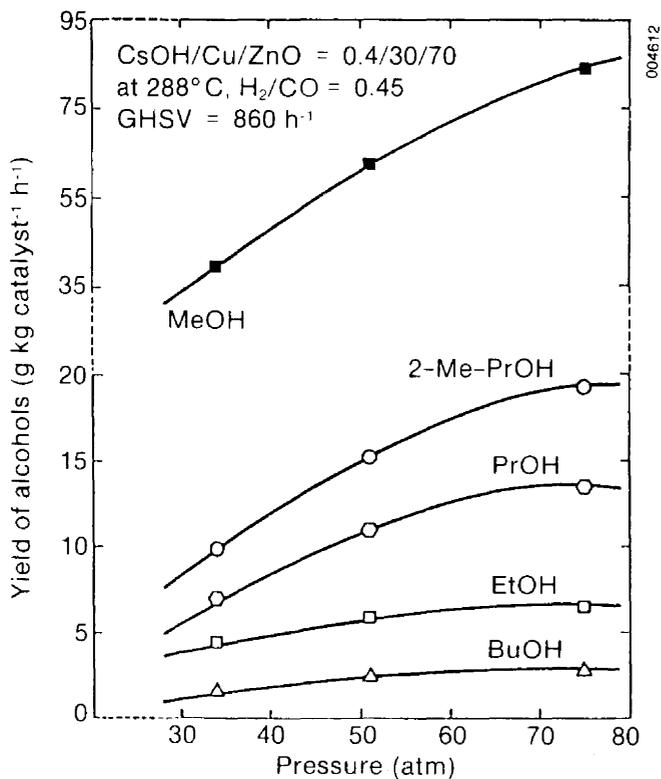
At the lowest temperature used to obtain the data shown in Figure 2, the H<sub>2</sub>/CO = 0.45 synthesis gas contact time was varied by controlling the space velocity (GHSV) of the inlet gas stream. The experimental results are shown in Figure 3. As the residence time for the reactants and products increased, the lower-molecular-weight alcohols that were formed reacted further to produce higher-carbon-number compounds. A constant contact time of 4.2 s and a stepwise decrease in the pressure yielded the data shown in Figure 4. The overall catalytic activity decreased with decreasing pressure, but the 2-methyl-1-propanol/methanol ratio remained constant. Thus, at 288°C the synthesis gas contact time is the more important parameter to control in shifting the product selectivity toward the higher-molecular-weight, branched alcohols.



**Figure 2.** Alcohol Selectivities Expressed as Weight Yields per kg of Catalyst per h as a Function of the Reaction Temperature



**Figure 3.** Yield of Alcohols at 288°C as a Function of the H<sub>2</sub>/CO = 0.45 Synthesis Gas Contact Time with the Cs/Cu/AnO Catalyst



**Figure 4. Yield of Alcohols at 288°C as a Function of the Reactor Pressure**

From these observations, we concluded that the alcohol synthesis activity increases over this Cs/Cu/ZnO catalyst with

- Increasing H<sub>2</sub>/CO ratio
- Increasing temperature
- Increasing pressure
- Decreasing contact time.

At the same time, the 2-methyl-1-propanol/methanol selectivity increases with

- Decreasing H<sub>2</sub>/CO
- Increasing temperature
- Increasing contact time.

However, it is independent of the reaction pressure. Therefore, an adaptable pathway to the synthesis of a high-grade alcohol fuel mixture has been found, and further reaction engineering studies of this catalytic system will be carried out.

## UNIVERSITY OF ILLINOIS

### Corn Stover Feedstock Supply Analysis

The objective of this subcontract is to develop methods for estimating the supply (quantity and price) of corn stover as a feedstock to large acid hydrolysis plants for the production of ethanol. The development of methodologies for estimating supply curves and a site-specific application of the methodologies will provide data needed to determine the technical and economic status of acid hydrolysis processes under evaluation by SERI. Optimization of overall plant economics requires an examination of the primary factors of production and product markets in a setting representative of commercial potential.

The research program will involve the sequential review of pertinent technical issues, the

development of techniques to quantify the factors affecting corn stover supply, and the design of a methodology to be applied to a specific region or county with extensive corn production. Process design parameters and input and output requirements of the production facility will be supplied by SERI. The research will focus on the factors affecting the required supply of corn stover and markets for the ethanol and by-products produced. The technical and institutional components of the corn stover supply relationship will be evaluated as a basis for the development of quantitative methods to estimate corn stover supply.

The initial step in the program will involve a description of the basic plant physiology of varieties of corn grown in the primary corn-producing regions of the United States and the production of corn for grain. To determine corn biomass (grain and whole plant) yields,

factors that affect crop yields will be identified, including varieties grown, nutritional requirements, soil type and cultivation effects, and weather and climatic effects. Subsequently, a method or methods for estimating the composition of corn plants at harvest will be presented that will provide a means of estimating the total biomass yield and the component yields represented by grain, husks, cobs, leaves, stalk, and roots based on the physiological and agronomic data obtained initially. The accuracy of these methods will be evaluated through experimental or secondary data analysis to determine the advantages and disadvantages of each. Finally, a recommendation will be made as to the preferred method for estimating the fractional yields and the total potential biomass yields of corn stover.

Given potential biomass yields, the research will survey available techniques for harvesting corn stover and propose new schemes for low-cost harvest. Techniques reviewed will include whole plant and stover systems. Important institutional arrangements affecting harvest methods, such as sole equipment ownership, cooperative equipment ownership, and local customs will be identified. The review will evaluate each system with regard to capital and operating cost, efficiency of harvest of stover, storability of harvested stover, and compatibility of equipment with typical farm operations (multiple use and compatibility with existing machinery).

A quantifiable methodology for estimating the supply relationship for corn stover can then be developed. This relationship will functionally relate deterministic factors such as corn variety, cultivation factors, and harvest efficiency to the quantity of stover per unit area. In addition, stochastic or other factors that shift the supply curve will be identified, and the effect of these factors on the supply curve will be quantitatively or qualitatively evaluated.

The next step requires the establishment of criteria for evaluating potential sites for a case study on corn stover supply and the selection of a particular site. The criteria will reflect the objective of analyzing acid hydrolysis technology in a commercially attractive setting. Therefore, the case study will involve an area with extensive corn production, with conditions conducive to the harvest of corn stover, and with potential markets or access to markets for ethanol and coproducts produced by acid hydrolysis technology.

This case study will use design requirements for the acid hydrolysis plant, to be supplied by SERI, and determine the supply and the effects of corn stover harvest on the agricultural economy in general and on farm incomes. In addition, the study will evaluate potential markets for both ethanol and coproducts in the site area and in wider markets. Impacts on the local economy of locating a hydrolysis plant will also be summarized.

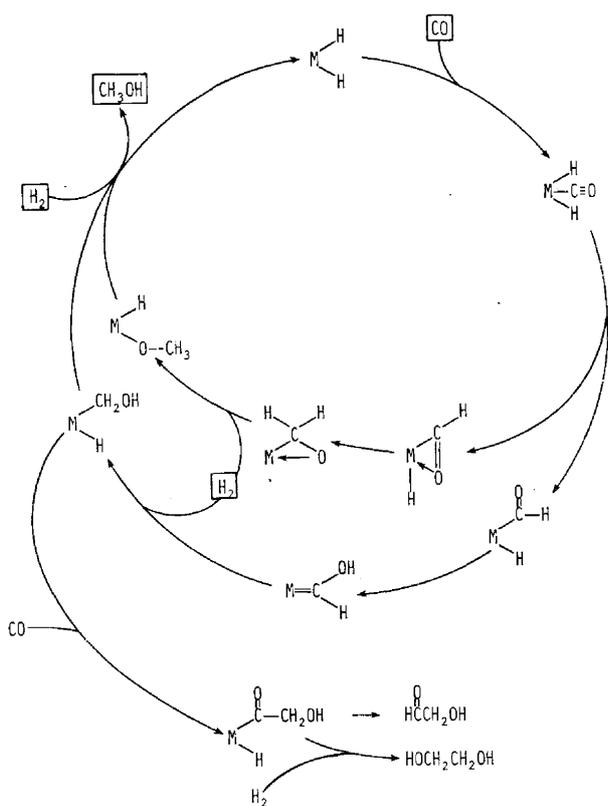
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## SOLAR ENERGY RESEARCH INSTITUTE

### Homogeneous Catalysts for Alcohols from Biomass Derived Syngas ( $H_2/CO$ )

In an effort to develop soluble transition metal complexes that will actively catalyze the hydrogenation of carbon monoxide at the moderate temperature and low pressures of  $H_2/CO$ , we are exploring the reaction chemistry of early transition metal compounds. Figure 1 illustrates the molecular pathways currently thought to be available for  $H_2$  addition to CO at a transition metal center. The use of early transition metals will increase the "hydric" nature of the hydride ligands ( $MH_2$ ) to promote addition from metal carbonyl hydride complexes  $[M(CO)(H)]$  to yield an intermediate

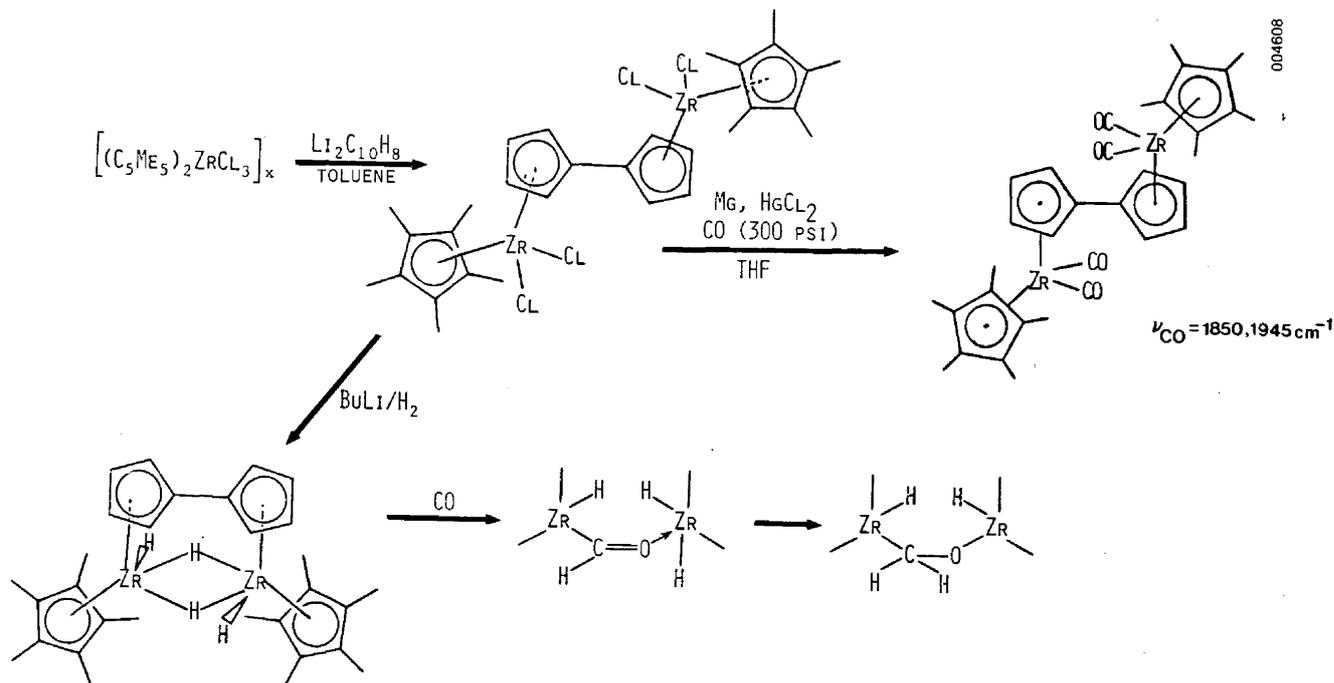
formyl  $[M(CHO)]$  complex that is bonded either to carbon and oxygen atoms or to only the carbon atom. These two intermediates lead to formaldehyde ( $MCH_2O$ ) or hydroxycarbene  $[MC(H)OH]$  complexes. The latter will add hydrogen to yield a hydroxymethyl ( $M-CH_2OH$ ) complex that can either undergo hydrogenolysis to yield methanol or carbonylation to yield hydroxycarbonyl ( $M-COCH_2OH$ ) precursors to higher alcohols. During the last six months we have completed the synthesis and characterization of several dinuclear zirconium complexes, and are analyzing their  $H_2/CO$  reaction chemistry. These studies indicate that carbon monoxide can be stoichiometrically hydrogenated under mild conditions to yield a metal bound oxygenated hydrocarbon.



**Figure 1. General Mechanism of H<sub>2</sub>/CO Conversion to Alcohols at a Transition Metal Center.**

Figure 2 outlines the synthesis of dinuclear complexes starting from a newly developed synthesis of pentamethylcyclopentadienyl zirconium trichloride,  $[(C_5Me_5)ZrCl_3]_x$ , which is probably dimeric in solution. This reacts with the bridging ligand dilithiofulvalene,  $Li_2C_{10}H_8$ , prepared in situ to give good yields of  $(C_5Me_5)_2(C_{10}H_8)Zr_2Cl_4$ .

This dinuclear zirconium(IV) complex can be reduced with magnesium under carbon monoxide (300 psi) to give moderate yields of a zirconium(II) carbonyl complex,  $(C_5Me_5)_2(C_{10}H_8)Zr_2(CO)_4$ . Infrared (IR) spectral studies indicate the presence of two terminal carbonyl ligands ( $\nu_{CO} = 1850, 1945\text{ cm}^{-1}$ ) coordinated to each zirconium atom. This complex reacts in solution with hydrogen at low pressures ( $\sim 100\text{ psi}$ ), but only at somewhat elevated temperatures ( $\sim 200^\circ\text{C}$ ) to give a mixture of as yet uncharacterized products. It appears that a loss of coordinated carbon monoxide at elevated temperatures involves the generation of an extremely reactive complex that oxidatively adds an Me group from the  $C_5Me_5$  ligands at a rate comparable to the expected



**Figure 2. Synthesis and Reactions of Dinuclear Zirconium Complexes**

addition of hydrogen. Reaction studies with hydrogen will be pursued in an effort to suppress the nonproductive reaction path. In addition, studies are in progress to prepare the analogous  $(C_5H_5)_2(C_{10}H_8)Zr_2(CO)_2$  complex that has no ring-substituted Me groups.

The  $(C_5Me_5)_2(C_{10}H_8)Zr_2Cl_4$  complex reacts with methyllithium to yield a stable  $Zr_2Me_4$  derivative. A less stable  $Zr_2(n-Bu)_4$  derivative can be generated in situ and reacted with hydrogen (1 atm) to yield an interesting  $(C_5Me_5)_2(C_{10}H_8)Zr_2H_4$  complex. Combined IR and Fourier-transform nuclear magnetic resonance (FT-NMR) studies indicate the presence of a terminal hydride ligand on each zirconium atom in addition to two bridging

hydride ligands between zirconium atoms. The  $Zr_2H_4$  derivative reacts with carbon monoxide at low pressure ( $\sim 100$  psi) to yield products that contain oxygenated hydrocarbons bound to zirconium. Preliminary FT-NMR studies on this uncharacterized mixture of products suggest the presence of formyl groups ( $-CH_2-O-$ ) that may be bridging two zirconium atoms.

If further carbon monoxide insertion and hydrogenation reactions can be made to continue from such an intermediate, it will enable precursors to higher alcohols to be generated. If these can be hydrogenolyzed to regenerate the starting  $Zr_2H_4$  complex and yield alcohols, a catalytic cyclic will be established. These studies are in progress.

# Enzymatic Hydrolysis Research

## LAWRENCE BERKELEY LABORATORIES

### Bioconversion of Cellulose and Production of Ethanol: Summary of Research Results

#### I. Introduction

This report summarizes research activities in four main areas that are part of the proposed overall scheme for conversion of cellulosic materials to sugars by enzymatic hydrolyses, and the subsequent fermentation of these sugars to ethanol. The first involves an examination of methods for production of cellulase enzymes from low-cost raw materials such as corn stover and poplar. The cost of enzyme production is a significant factor in overall process economics. The second area is the development of a kinetic model of the enzymatic hydrolysis, based on the activities of the individual enzymes comprising the cellulase complex. The model must predict rates of reaction on actual raw materials; also, it must be sufficiently detailed so that variations in composition of feedstock can be incorporated. The third area is that of fermentation of sugars from the hydrolysis process to ethanol. A detailed study of the nutritional requirements for yeast growth and alcohol production has been completed. The levels of supplemental nutrients required for the lowest cost of ethanol production can be determined for any specified feedstock. Also, an understanding of the sugar conversion to ethanol requires an examination of xylose fermentation by Clostridia, since problems such as ethanol and xylose inhibition and by-product formation need to be resolved. The fourth area focuses on ethanol recovery from fermentation broth, using liquid-liquid extraction and other low-energy-consuming techniques.

#### II. Kinetics of the Enzymatic Hydrolysis of Cellulose

##### A. Enzyme Purification

The major extracellular proteins elaborated by Trichoderma reesei RUT-C30 grown on Solka

Floc have been purified by ion-exchange and gel permeation chromatography. The proteins obtained were homogeneous as determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis, but the proteins contained species of differing isoelectric points when subjected to isoelectric focusing.

The  $\beta$ -glucosidase obtained was purified from 1.75 to 27.3 to 90.5 U/mg specific activity (p-nitrophenolglucoside [P-NPG] units); the major endoglucanase from 6.0 to 24.8 to 29.0 U/mg (carboxymethylcellulose bonds broken, measured by viscometry). Because of the synergism between exo- and endoglucanases, the specific activity of exoglucanase decreased upon purification if measured by traditional methods (i.e.,  $C_1$  assay); but with a small amount of purified endoglucanase present, the specific activity increased from 6.0 to 17.0 to 30.6 U/g.

We obtained two endoglucanases and two cellobiohydrolases (exoglucanases). In each case the lesser component showed approximately 75% of the specific activity of the major component, but it was present in much smaller concentrations and was neglected in the following analysis.

##### B. $\beta$ -glucosidase Kinetics

With this homogeneous reaction system, the kinetics were readily determined. Glucose production from cellobiose is a function of cellobiose concentration and glucose concentration; thus we could determine  $K_M$ ,  $V_M$ , and  $K_i$  (with competitive inhibition by glucose). The stability of this enzyme was determined by the decrease in activity with respect to p-NPG as a function of pH and temperature.

##### C. Cellobiohydrolase Kinetics

Walseth (amorphous) cellulose showed no increase in production of reducing sugars or incubation with exoglucanase with a decrease in mesh size. We also determined the maximum cellobiose production rate possible by

cellobiohydrolase. Competitive self-inhibition (substrate inhibition) was not seen at the relatively low concentrations used.  $K_m$ ,  $V_m$ , and  $K_i$  (competitive inhibition constant) for cellobiose were determined.

#### D. Endoglucanase Kinetics

The activation of endoglucanase action seen on CMC may not hold for action on cellulose. Since one subsite is filled by glucose, the remainder are more easily filled by CMC, resulting in an effective increase in substrate concentration and hence activation. Thus the hydrolysis of CMC depends on the distribution and degree of substitution.

From our observation of these enzymes on the substrate (and from other experiments), the kinetics of the action on crystalline and actual substrates may be described.

### III. Nutritional Requirements for Alcohol Production by Yeast

Sugars produced by the enzymatic hydrolysis of agricultural residues or whole tree biomass lack the supplemental nutrients found in molasses. It is necessary to add these nutrients to the fermentation to achieve high rates of ethanol production, although yeast is capable of ethanol production on a completely synthetic medium. The most important nutrients, in order of effectiveness, were biotin, pantothenate, thiamine, pyridoxine, inositol, nicotinic acid, and p-amino-benzoic acid. The levels required for a 100 g/L glucose feed are shown in Table I. Yeast extract or corn steep liquor can supply these nutrients to some extent, with biotin being the first limiting nutrient in yeast extract. The effects of yeast extract and corn steep liquor on ethanol productivity have been quantified.

Yeast also requires oxygen in low concentrations for biosynthesis. Earlier work indicated 0.07 mm Hg oxygen tension was optimal for ethanol productivity. The effect of oxygen

**Table I. Growth Factor Requirements for Fermentation of 100 g/L Glucose**

Nutrient	Quantity (mg/L)
d-biotin	1.0
Ca pantothenate	12.5
thiamine HCl	10.0
pyridoxine HCl	12.5
myo-inositol	250.0
nicotinic acid	10.0
Na p-aminobenzoate	2.0

supply (flux) and concentration at very low levels was investigated by supplying  $\text{CO}_2$ . The specific oxygen uptake ( $q_{\text{O}_2}$ ) has been determined as a function of growth rate, and the effect of oxygen concentration on growth rate analyzed. The following linear relationship was established:

$$q_{\text{O}_2} \text{ g O}_2 (\text{g cells}^{-1} \text{ h}^{-1}) = 0.018 + 0.14 h^{-1}$$

The maintenance requirement is thus  $0.018 \text{ g O}_2 \text{ g cells}^{-1} \text{ h}^{-1}$ . The oxygen demand for biosynthesis is linear in growth rate.

#### A. Xylose Fermentation of Clostridium

C. thermohydrosulfuricum has been examined as a potential organism for the conversion of xylose to ethanol. The original strain examined (39E) was inhibited by moderate levels of ethanol, and mutagenesis and selection in a chemostat have been used to develop an ethanol-tolerant strain suitable for use in flash-fermentation.

A yield of 0.43 g ethanol/g xylose fermented has been found at low (less than 5 g/L) xylose concentrations. Above 1% xylose the yield decreases because of inhibition by the other main by-products--lactate and acetate. Continuous culture of the ethanol-tolerant strain UC42L shows an increase in lactate production at the expense of ethanol. Work is under way to develop a low-lactate-producing mutant.

## B. Liquid-Liquid Extraction for Ethanol Recovery

Liquid-liquid extraction represents one possible alternative to distillation as a means for reducing energy consumption in recovery. Various potential solvents have been examined for distribution coefficients and selectivity. Empirical models for predicting water and ethanol distribution coefficients have been developed, and a generalized model based on

UNIFAC/UNIQUAC can predict trends in  $K_W$  and  $K_E$  for a variety of solvents.

Analysis of the experimentally obtained  $K_D$  and  $S$  values shows that a single solvent extraction offers no advantages over distillation. Thus, a liquid-liquid extraction, followed by selective water adsorption, has been examined. The fixed-bed adsorber allows passage of ethanol while retaining water; the process is regenerated by hot solvent. This scheme appears to offer economic advantages over optimized distillation.

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## RUTGERS UNIVERSITY

### Production and Characterization of High-Cellulase-Producing Mutants of Cellulolytic Microorganisms and Genetic Engineering of Cellulolytic Zymomonads

#### I. Immobilization of Trichoderma reesei for Cellulase Production

##### A. Cellulase Production

Cellulase production by T. reesei cells immobilized Celite has continued to yield high enzyme productivities; average yields are  $175 \text{ FPU L}^{-1} \text{ h}^{-1}$  (high yields are  $270 \text{ FPU L}^{-1} \text{ h}^{-1}$ ). Several nutrient feeding ranges have been studied, and maximum cell loading of 107 g/L with 2 g of cells for each gram of Celite has been achieved. Reduction in the carbon supply perturbed the steady-state population, which caused lower cell populations but higher cellulase productivities. These may simply be artefactual and a result of some cell lysis. Cellulase production by mycelial pellets was compared to that using Celite immobilized cells. Pellets (5-8 mm in diameter) formed readily, and we monitored cellulase production for roughly three weeks. Yields averaged  $50 \text{ FPU L}^{-1} \text{ h}^{-1}$  and are probably a result of lower cell loadings (70 g/L compared to 100 g/L of Celite immobilized cells).

A Celite-immobilized culture was subjected to stepwise decreasing carbon-to-nitrogen (C:N) ratios from 8:1 (routine cellulase production) to 22:1, while maintaining a constant lactose feed. Cellulase production was reduced under these conditions, but sporulation became marked at the 12:1 feed-rate. Phialospore counts reached  $2 \times 10^7/\text{mL}$  effluent and were equivalent to  $2 \times 10^9 \text{ spores L}^{-1} \text{ h}^{-1}$ . Over 90% of the phialospores were viable. A direct descending lactose gradient resulted in thinner, less-branched hyphae, and large, intercalary, thick-walled chamydospores were also formed. These findings can have application in projects that use massive spore inocula, such as control of plant pathogens.

#### II. Glycoprotein Nature of Trichoderma reesei Cellulase

The interest in cloning Trichoderma reesei cellulase genes into prokaryotes has spurred research into the role of the oligosaccharide moieties of these glycoprotein enzymes. Routinely, prokaryotic DNA in vivo systems do not glycosylate enzymes of eukaryotic origin. Because one objective is the insertion and transcription of Trichoderma genes in a bacterial system, it is important to know if the protein portion of cellulase is active and stable without addition of its glycosyl moieties. T. reesei cellulases contain two types of oligosaccharide-protein linkages:

- N-linked through the amide nitrogen of asparagine.
- O-linked through the hydroxyl group of serine or threonine.

We have used two approaches in our laboratory to prepare a cellulase enzyme deficient in N-linked oligosaccharide: through the *in vivo* action of tunicamycin, and application of *in vitro* endo- $\beta$ -acetylchitobiose residues in the N-linked glycoprotein, leaving one N-acetylglucosamine residue attached to the asparagine in the protein and releasing the glycosyl variety. Cellulase production in the presence of TM prevents all N-linked glycosylation; Endo H treatment of an intact enzyme will cleave N-linked oligosaccharides whose cleavage sites are exposed to the attack. In the case of both TM and Endo H, only partially deglycosylated enzymes are formed since neither treatment affects the O-linked oligosaccharides. Partially N-deglycosylated cellulase has been prepared from two strains of *T. reesei*, the parent QM6a, and the hypercellulolytic, catabolite-repression-resistant mutant RUT-C30, using both TM and Endo H protocols.

Protein production by *T. reesei* QM6a is not affected by cellulase induction in the presence of tunicamycin (10  $\mu$ g/ml), the same amount of protein occurring in extracellular, intracellular, and mycelial bound locations. For RUT-C30, less protein was secreted and greater protein remained cell bound in the TM-treated cells. The specific activities of carboxymethylcellulase (CMCase) and acid-swollen cellulase (ASCCase) were the same for both control and TM-treated QM6a cultures. For RUT-C30, specific activities are higher for the enzymes from the TM-treated cells. Intracellular activities are the same for both control and TM-treated RUT-C30 strains, and the specific activities of mycelial bound CMCase and ASCCase are higher in the control culture than in the TM-treated cells.

Nondenaturing "PAGE" electrophoresis of extracellular proteins of both TM-treated QM6a and RUT-C30 showed different protein

patterns than the controls. All of the proteins reacted positively when stained for glycoprotein. Several of the TM-treated components show a slightly faster electrophoretic mobility than proteins in the control and were identified as active exosplitting glucanases. Four other bands of slower mobility have been identified as active endoglucanases, but no change in mobility was noted in control and TM-treated enzymes. In RUT-C30, four protein bands of slower mobility are present only in the control culture. These glycoproteins do not exhibit activity against carboxymethyl cellulose, cellulo-oligosaccharides, or arylglucosidase substrates with "in gel" assays. Isoelectric focusing indicated that all of the proteins are acidic (pH 3.9 to 5.10). To assess the role of glycosylation in temperature stability, endoglucanases from control and TM-treated QM6a and RUT-C30 were heated and residual activities compared. No differences in heat stability were observed.

Endo H cleavage of N-linked oligosaccharide of cellobiohydrolase I (CBH I) of RUT-C30 resulted in a protein with approximately the same activity as the control. The Endo H-treated protein had a slightly faster electrophoretic mobility than the control enzyme and had the same specific ASCase activities.

In summary, cellulase components that have been deglycosylated via enzymatic cleavage (Endo H treatment), or by synthesis in the presence of tunicamycin, continue to show activity equivalent to that of the fully glycosylated enzyme. No change in temperature stability of the deglycosylated enzymes was noted. These results are considered promising in relation to prokaryotic processing of *T. reesei* RNA and with regard to the apparent lack of a need for glycosylation to maintain enzyme activity.

### III. Cloning of Carbohydrate Utilization Genes in *Zymomonas mobilis*

We have focused on cellobiase genes, new vector systems, and transformation, which are described as follows.

## A. Cellobiase

Cloning and coordinate expression of genes of the total cellulase system are complex, and the use of *E. coli* strains that synthesize any of these genes would be most advantageous. Thus if *E. coli* produced cellobiase, it would not be necessary to clone further foreign cellobiase genes into it. We have also obtained strains of *E. coli* that are capable of growth on cellobiose and other strains that are capable of growth on aryl-glucosides, arbutin, and salicin. Cellobiase is active against methyl-umbelliferyl- $\beta$ -D-glucoside, yielding a characteristic fluorescent aglycone that can be seen under ultraviolet light. The cellobiose fermentation can be detected by colorimetric changes on McConkey Agar with cellobiose as well as by growth on cellobiose as a sole source of carbon. These colorimetric reactions will be helpful in screening for the cellobiose utilization genes once they are cloned on pULB113 and in subsequent subcloning.

## B. Vectors

The plasmid pULB113 is a self-transmissible, chromosome-mobilizing vector with broad host range replicating ability. Thus, this plasmid can carry genes from one bacterial species to another and also allows expression of the carried genes. It is best maintained in *recA*<sup>-</sup> hosts such as *E. coli* MXR or HB101. We have characterized the rate of transfer of plasmids

pULB113 and the rate of mobilization of chromosomal genes by pULB113 (formation of R' plasmids). In *E. coli* x *E. coli* matings, the rate of transfer of pULB113 from donor to recipient is 10<sup>-1</sup> to 10, while the rate of transfer of chromosomal genes (R' plasmids) is about 1 x 10<sup>-5</sup> as observed through complementation of auxotrophy. Thus pULB113 has proven an excellent tool. Transfer of pULB113 from *E. coli* to *Z. mobilis* was also accomplished. Rates of transfer were much lower (2 x 10<sup>-5</sup>) but are to be expected in interspecific matings. However, the transfer of genes from *E. coli* to *Zymomonas* is possible.

## C. Transformation

Direct transformation of DNA into *Zymomonas* would be a most useful genetic approach, and we have attempted this transformation during the last year. This has now been accomplished with transfer of the vector plasmid pKT230 Cm<sup>r</sup> to *Z. mobilis* strain CP4 through modification of standard pseudomonas transformation protocols. Yields are low (4.2 x 10<sup>2</sup>/μg DNA). However, transformation will allow introduction of foreign DNA into *Zymomonas* without the dependence on the self-transmissibility of foreign broad host-range plasmids or the presence of mobility sites on the cloning vectors. Selection via transformation can yield clearer selection, since there is no donor organism to eliminate during the selection process.

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## MASSACHUSETTS INSTITUTE OF TECHNOLOGY

### Degradation of Lignocellulosic Biomass and Its Subsequent Utilization for the Production of Liquid Fuels

#### I. Introduction

This project is a coordinated effort to develop process technology for the degradation of lignocellulosic biomass to produce liquid fuels.

In previous efforts, we have demonstrated the feasibility of a direct, single-step, microbiological process for the conversion of lignocellulosic materials to ethanol. This process uses a mixed culture of *Clostridium thermocellum*, a thermophilic cellulolytic anaerobe, which degrades cellulose and hemicellulose to fermentable sugars, and *Clostridium thermo-saccharolyticum*, a thermophilic anaerobe capable of producing high concentrations of ethanol from both pentoses and hexoses. The proposed research program is a continuation of our efforts on direct fermentation of lignocellulosic biomass. Based on prior experience,

we have identified three cost-sensitive areas--improved conversion yield, alcohol productivity, and alcohol concentration--which require further research. The proposed program will address the problems of lignocellulosic biomass pretreatment using a thermal, dilute-acid treatment that greatly facilitates fermentative conversion of pentoses and hexoses to liquid fuels. Because the rate and extent of hemicellulose and cellulose hydrolysis are so important to the economics, considerable effort focuses on overproduction, optimization, and recycle of the cellulases from C. thermocellum. Studies on increasing alcohol tolerance are directed at improving this aspect of the technology.

## II. Dilute-Acid Pretreatment

Various cellulosic biomass pretreatment methods have been examined to improve microbiological utilization and conversion to ethanol. Using the dilute-acid hydrolysis process we have attempted to achieve the maximum production of soluble sugars from natural biomass materials such as corn stover and aspen wood. Our approach is to modify native biomass but not completely hydrolyze the cellulose. Using a dilute acid (1% H<sub>2</sub>SO<sub>4</sub>) at temperatures around 175°C for short reaction times (1 min) corn stover and aspen wood were pretreated at 300 g/L. Soluble reducing sugars ranging from 66.5 to 87 g/L were obtained. The residual solid material contained 63 wt % solids following high-pressure dewatering. The soluble sugars were fermented using C. thermosaccharolyticum, which effectively utilized up to 83% of the five- and six-carbon sugars. The residual solids, which were mostly cellulose, were fermented by C. thermocellum

at a 65% utilization rate. This pretreatment of lignocellulosic biomass was found to be equally effective on agricultural residue such as corn stover and aspen wood.

## III. Overproduction and Characterization of Cellulase

The extracellular cellulase of C. thermocellum ATCC 27405 has been partially purified based on the activity toward Avicel. "Avicelase" came out at the void volume of an Ultrogel A column, indicating that the enzyme has a molecular weight higher than  $1.5 \times 10^6$ . The enzyme was separated better on a sepharose 2B column. When examined by SDS-polyacrylamide gel electrophoresis, the active fraction was resolved into six protein bands with molecular weights ranging from 60 to 220. These results suggest that avicelase is a multiunit enzyme complex. The purification results are summarized in Table 1.

The enzyme will be further purified by high-pressure liquid chromatography (HPLC) or agarose electrophoresis. Separated fractions will be examined for endo- and exo- $\beta$ -glucanase activities. The conformation of multi-subunit enzymes is currently being examined by electron microscopy.

## IV. Ethanol Production from Acid Hydrolyzate

Some preliminary experiments were carried out to examine growth and product formation by C. thermosaccharolyticum HG8 on corn stover hydrolyzate provided by Dr. Phillip Badger of TVA. The NaOH and Ca(OH)<sub>2</sub> neutralized TVA hydrolyzates were analyzed

Table 1. Partial Purification of Clostridial Cellulase

Step	Total Volume (mL)	Total Activity (U)	Total Protein (mg)	Yield (%)	Specific Activity (U/mg)
1. Crude broth	130	3900	15.6	--	250
2. Acetone concentration	25	3750	15.6	96	240
3. Sepharose-2B	32	2300	4.5	60	510

by HPLC and found to contain 58 g/L xylose, 12.8 g/L glucose, and 8.8 g/L arabinose as the major sugars. Because the high initial levels of sugars in the hydrolyzate are inhibitory to C. thermosaccharolyticum, dilutions were used to test growth and product formation. At lower initial sugar concentrations, all four sugars were completely utilized, and we obtained a favorable ratio of ethanol to by-products. When the results for the NaOH and Ca(OH)<sub>2</sub> neutralized TVA hydrolyzate are compared, we note that the latter shows better growth of HG8 as evidenced by the higher levels of products. This is probably because of the reduced levels of both SO<sub>4</sub> and Na ions in the Ca(OH)<sub>2</sub>-reduced medium. C. thermosaccharolyticum HG8 can utilize Ca(OH)<sub>2</sub> neutralized TVA hydrolyzate provided the initial sugar levels in the medium do not exceed about 10-12 g/L. A lower initial sugar not only promotes complete utilization of all the sugars on the TVA hydrolyzate but also provides a higher yield of ethanol. This suggests that a continuous culture system operating under sugar limitation would provide the desired condition for complete utilization and product selectivity.

#### V. Increased Tolerance to Ethanol

In earlier studies, we showed that glyceraldehyde phosphate dehydrogenase (GAPDH) was the point of alcohol sensitivity in C. thermocellum. In alcohol-resistant mutants, this enzyme was shown to be less sensitive to ethanol. Realizing the importance of improv-

ing alcohol tolerance in C. thermocellum and C. thermosaccharolyticum, we have begun a program to increase solvent tolerance through a better understanding of solvent sensitivity. Our approach is to cleave the gene for GAPDH from both solvent-sensitive and solvent-resistant variants of C. thermocellum and then determine the sequence of the gene. This will tell us what structural changes are responsible for the alcohol tolerance and will provide us with a gene for an enzyme that is alcohol tolerant.

#### VI. Ethanol Production in Continuous Culture

One of our objectives is to develop a high-productivity system for ethanol production from biomass. We have been conducting a series of studies using continuous culture with cell recycle using C. thermosaccharolyticum HG8 grown on xylose. The advantage of cell recycle is that a higher cell mass concentration can be maintained in the fermentor, and both cellulase and undegraded biomass can be retained in the system for longer retention times to increase degradation. We have demonstrated that recycling of up to 75% of the cells leads to higher ethanol productivity. A continuing problem, however, has been a poor selectivity for ethanol and production of considerable amounts of lactate when high sugar concentrations are used. Recently, we have found that the selectivity can be improved by higher iron concentration in the medium, and we are proceeding with studies at higher ethanol concentration.

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### PURDUE UNIVERSITY

#### Production of Alcohol and Chemicals from Cellulosic Biomass

##### I. SUMMARY

##### A. Cellulose Hydrolysis Using Both Phosphoric and Sulfuric Acid

We began this work only recently. In March and April 1984 we conducted some preliminary

experiments to provide some basic information regarding reactions between H<sub>3</sub>PO<sub>4</sub> and cellulose. Our approach is to use concentrated H<sub>3</sub>PO<sub>4</sub> first to swell and then dissolve cellulose. If this is done at room temperature, dilution with water will reprecipitate essentially all the cellulose. The reprecipitated cellulose is still resistant to hydrolysis. If the H<sub>3</sub>PO<sub>4</sub> and cellulose are heated together for a short time, the cellulose is derivatized. We hope to find an optimal level of derivatization that is enough to decrystallize cellulose but does not yield much soluble sugar. At this point, we

can perhaps add water to recycle the acid and precipitate out the partially derivatized cellulose. We hope that the partially derivatized, decrystallized cellulose can be hydrolyzed easily.

Preliminary work has shown that heating at 90°C for 20 min will give a cellulose phosphate with a degree of substitution (DS) of 0.5. This DS level is too high for our processing purpose as evidenced by the observed solubility in water (i.e., no precipitate is formed immediately after dilution with water). We will try to reduce the temperature and the duration of the reaction in further experimentation. Meanwhile, we have determined that the DS 0.5 cellulose phosphate has the phosphate group on C6 position according to the <sup>13</sup>C-NMR analysis.

## B. Genetic Engineering for Improving Ethanol Fermentation

We hope to clone xylose isomerase genes in yeast to make it capable of fermenting xylose to ethanol. Once this is achieved, yeast can be used to ferment most of the carbohydrates in cellulosic materials, including xylose from hemicellulose and glucose from cellulose, to liquid fuel.

An alternative system also under development is a cloning system for Candida species. Candida cultures can utilize pentoses under aerobic conditions. Some species can even produce ethanol.

Another alternative approach is to overproduce xylose isomerase by Bacillus and E. coli so that we can make the enzyme cheap enough to use in a xylose-to-xylulose-to-ethanol process. Progress made in the last few months in the genetic engineering work includes the following (details follow in this report):

- Construction and characterization of plasmids containing the xylose isomerase gene with its ribosomal binding site partially or totally removed to improve the expression of the xylA gene in yeast.

- Insertion of xylA into Leu2 gene cloned on pLR2 to study the expression of xylA by the Leu2 controlling signals.
- The construction of a cloning vector for Candida utilis by using its DNA sequences capable of autonomous replication in yeast and by using a bacteria gene that can inactivate antibiotic G418 as a selection marker.

## C. The Water Process for Cellulose Hydrolysis

Due to reductions in funding, this work was held up for 12 months, and this report includes one month's progress. The preliminary data look encouraging. After a high-temperature water pretreatment, the enzymatic hydrolysis of the pretreated Avicel gave essentially 100% yield, while the control of unpretreated Avicel under identical hydrolysis conditions gave a yield of only 76%.

## II. Use of Phosphoric Acid in Cellulose Hydrolysis

### A. Introduction

Various techniques have been used to phosphorylate cellulose. Cellulose containing 16%-18% phosphorous has been prepared using phosphoric acid and acid catalysts and by reacting cellulose with oxygen-containing chlorides of phosphoric acid in the presence of benzene [1,2]. A cellulose derivative with 7%-9% phosphorous was produced by researchers at LORRE by treating microcrystalline cellulose with concentrated phosphoric acid and heat. Investigation of this compound is the subject of this research. <sup>13</sup>C-NMR and other analytical techniques were used to illustrate the cellulose phosphate structure.

### B. Principle

Adding concentrated acid to cellulose induces "staged activation." This three-step process may be described as follows: initial sorption

by the cellulose, swelling, and solution. Extensive swelling disrupts the inter- and intramolecular weak Van Der Waals forces and hydrogen bonding, thus forming a solution. If the polymeric solution is formed at normal temperature and pressure, with a strong swelling agent such as phosphoric acid, cellulose II will quickly recrystallize upon addition of water. The acid is believed to be adsorbed only by the cellulose, with an additional compound resulting. Dilution of the acid complex allows hydrogen bonds to reform. Hence, recrystallization ensures favorable entropy and enthalpy for a more stable polymer. The cellulose is returned as cellulose II with only slight modification in structure. While the cellulose is in the liquid phase, heating modifies the molecular structure significantly. Besides hydrolytic degradation and dehydration, esterification occurs without a reagent or catalyst.

### C. Observations

Heating a solution of phosphoric acid and cellulose for short periods (20-60 min) and at an elevated temperature (90°C) yielded a cellulose derivative on which experimental analysis was performed. Diluting this reaction mixture with water resulted in a clear amber solution. At room temperature this solution remained clear for approximately 10-15 minutes, when a cloudy precipitate was observed. This precipitate continued to form until a flocculated material was left. A water-soluble phosphate ester was probably produced when the cellulose solution was heated. A strong nucleophile (H<sub>2</sub>O) readily hydrolyzed the ester back to its original acid and insoluble cellulose (Figure 1).

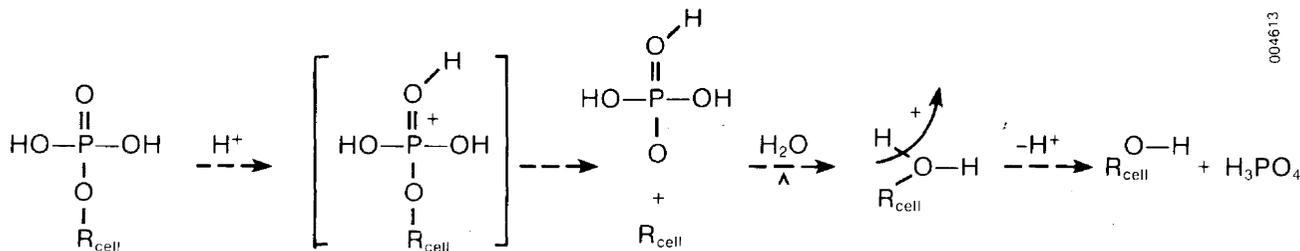
Adding DMSO to the reaction mixture also produced a clear solution; however, the cellulose did not precipitate with time. Table I shows other solvent effects.

**Table I. Solvent Plus Reaction Mixture (20 min at 90°C)**

Precipitate Solvent (%)	Collected (e)	Dielectric Constant <sup>a</sup>
Water	0	78.54
DMSO	0	--
Methanol	13.13	32.63
Ethanol	27.19	24.30
Acetone	60.73	20.70
THF	56.07	--
Ethyl ether		133.80
		4.34

<sup>a</sup>Ref. 3.

Immediate solubility of the compound in water and DMSO suggested that a highly polar solvent was required for total dissolution. It seemed reasonable that nonpolar solvents produced a white-flaked precipitate. The general trend of Table I is evidence of this observation. This behavior may be a consequence of a low degree of substitution on the cellulose hydroxyl groups [4]. The more groups available for hydrogen bonding, the more likely an aqueous medium will dissolve it. Also, introduction of a small number of substituents decreases crystallinity and likewise increases solubility in polar solvents.



**Figure 1. Reaction to Heating Phosphoric Acid and Cellulose; Also Addition of a Strong Nucleophile that Hydrolyzed the Ester Back to the Original Acid and Cellulose**

Tetrahydrofuran was chosen as the solvent for product isolation because of its aprotic and nonpolar characteristics. An aprotic solvent was used to prevent premature hydrolysis of the derivative. Maximum quantities of unreacted cellulose, cellulose derivative, and degradation products were desired. However, as heating time increased, less material was collected (Figure 2). As more degradation took place, the concentration of water-soluble cellodextrins also increased (Figure 3).

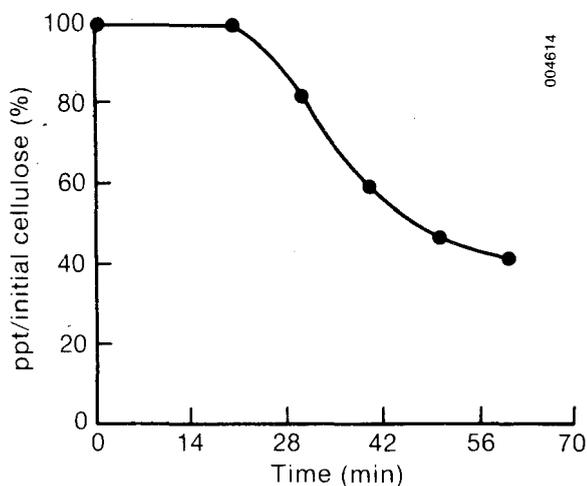


Figure 2. Percentage of THF Precipitate vs. Heating Time

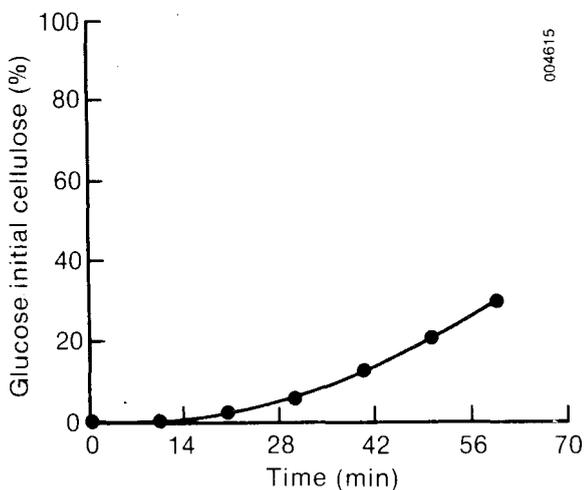


Figure 3. Percentage of Glucose vs. Heating Time

Figure 3 shows the amount of  $\beta$ -glucose only. The most noticeable effect is the decrease in glucose and cellobiose content when using the THF precipitate. Since the acid contained 15% water, some of the derivative was probably lost when the THF was added. Besides glucose and cellobiose, other important sugars including glucose 6-phosphate are located in this water.

The precipitate collected using THF and ethyl ether only partially redissolved in water; an insoluble gel remained. This gel perhaps indicates strong intramolecular bonding and diesterification leading to cross-linking (Figure 4).

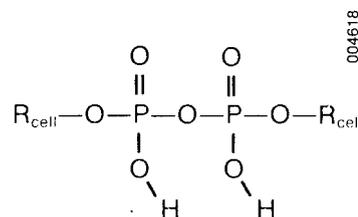


Figure 4. Intramolecular Bonding after Redissolving THF and Ethyl Ether in Water

A 5% aqueous NaOH solution essentially dissolved and kept solubilized all of the precipitate; a plain water solution eventually hydrolyzed the derivative to cellulose. The stability of cellulose phosphate in alkaline solution can be explained using electrostatic considerations (Figure 5).

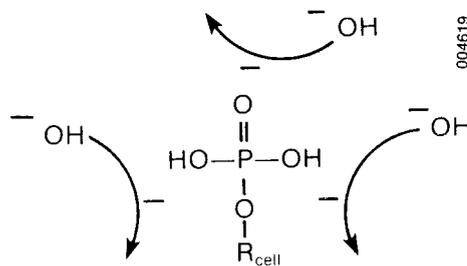


Figure 5. Electrostatic Patterns Indicating the Stability of Cellulose Phosphate in Alkaline Solution

It is well known that mono- and dialkyl phosphate esters are inert in basic solution because of the opposing charges of these anions. Thus, alkaline hydrolysis is impossible[5].

Results seem to conclude that the THF precipitate is probably a phosphate ester with a low degree of substitution. Elemental analysis of three THF samples at 90°C is shown in Table 2. The instability of the precipitate in nucleophiles was explained and the degree of degradation demonstrated. Nuclear Magnetic Resonance gives a precise definition to the actual structure.

**Table 2. Elemental Analysis**

Heating Time (min.)	Element (%)			
	C	O	H	P
0	40.0	53.3	6.67	0
20	36.8	48.6	6.74	7.91
40	37.5	47.4	6.66	8.46
60	36.8	49.3	6.32	7.64

## D. Experiment

### 1. Materials

The cellulose solution was prepared by mixing Avicel PH 101 cellulose (15 g) with 85% concentrated phosphoric acid (150 mL) in a Warren blender for 30 min. This liquid was poured into a 250-mL round bottom flask and heated in an oil bath at 90°C ( $\pm 0.10^\circ\text{C}$ ). At 5-min intervals 1-mL aliquots were quickly withdrawn. They were allowed to cool, and THF was added to each sample until precipitation was complete. A ground-glass Buchner funnel was used for filtration. A second cycle of washing, soaking, and filtering was then done on each sample.

## 2. Glucose Determination

A Beckman glucose analyzer was used to find the number of grams per liter of  $\beta$ -glucose in neutral solutions.

## 3. Liquid Chromatograms

We prepared chromatograms that illustrate the following conditions:

- Dilute sulfuric acid eluent (0.125 mL acid/L H<sub>2</sub>O) at 85°C
- Flow rate = 0.35 mL/min
- 20  $\mu\text{L}$  injection volume.

## E. <sup>13</sup>C-NMR

The powerful utility of <sup>13</sup>C-NMR was employed to assign a structure to the THF precipitate cellulose phosphate. To accurately identify chemical shifts, it was necessary to compare cellulose phosphate with the largest water-soluble cellulose, which is polysaccharide-cellohexaose. In this way, cellohexaose would, ideally, represent cellulose[6]. This cellulose spectra would display characteristic chemical shifts without confusion from the  $\alpha$ ,  $\beta$  reducing and nonreducing end groups of the smaller polysaccharides. Ester formation could then be clearly monitored and studied.

The importance of maximizing the degree of polymerization (DP) when the derivative was produced became evident when we observed spectra of various heating times (Figure 6). A reduction in heating lowered monomer production, and subsequent analysis resulted in unobstructed spectra of cellulose phosphate. The spectral assignments for selected cello-oligosaccharides in D<sub>2</sub>O are given in Table 3. Examination of the line spectra in Figure 7 and assignments in Figure 8 reveal two significant peaks at the C-6 position. The absence of any peaks at 92.6 and 96.6 ppm indicates that no

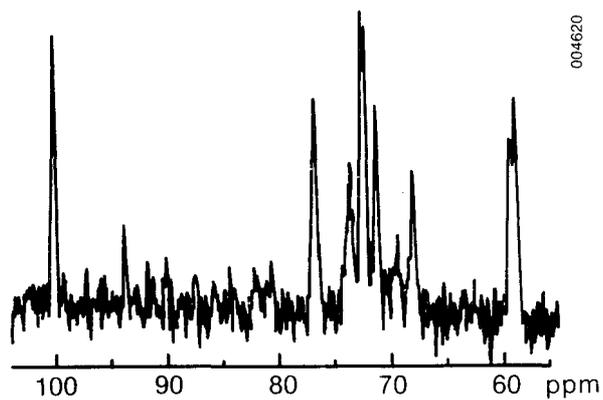


Figure 6. Cellulose Spectra at Various Heating Times

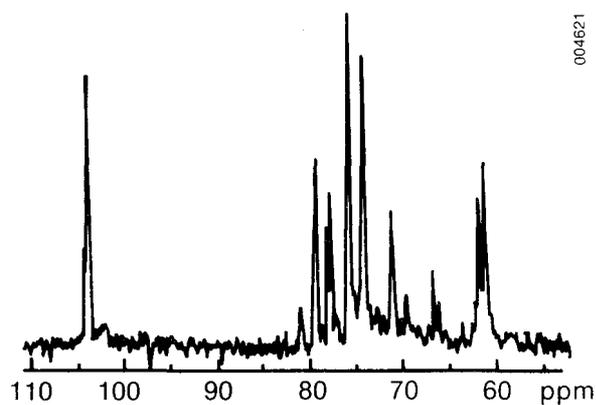


Figure 7. Line Spectra for Selected Oligosaccharides in D<sub>2</sub>O

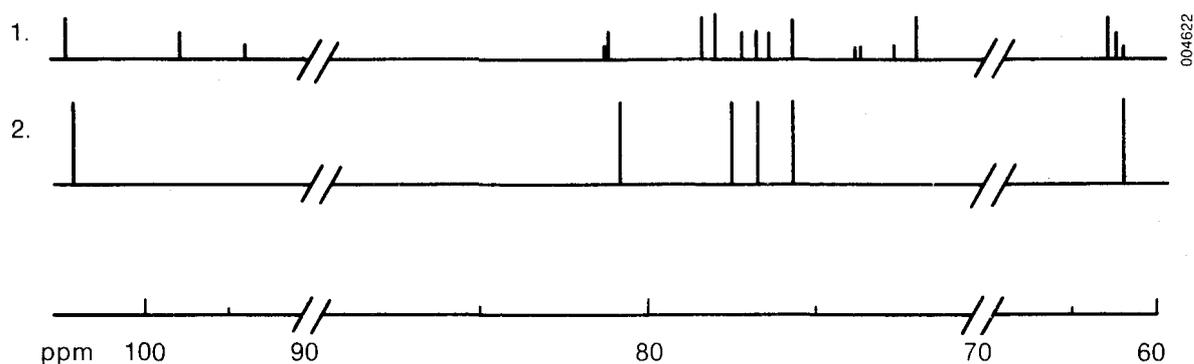


Figure 8. Assignments for Selected Oligosaccharides

Table 3. <sup>13</sup>C-NMR Chemical Shifts in H<sub>2</sub>O Solution

Compound	Residue of Group		C-1	C-2	C-3	C-4	C-5	C-6
Cellobiose	Reducing end group	$\alpha$	92.6	72.2	72.3	79.7	70.9	61.0
		$\beta$	96.6	75.1	74.8	79.5	75.6	61.1
Cellulose (hexoase)	Reducing end group	$\alpha$	92.0	a	a	80.7	a	60.6
		$\beta$	96.8	a	74.9	80.7	75.1	60.6
	Internal residues nonreducing end group		102.7 a	73.2	74.9 76.7	80.1 70.3	75.1 76.9	60.6 61.2 <sup>b</sup>

<sup>a</sup>Small peak.

<sup>b</sup>Refs. 7 and 8.

"small" saccharides are present. The obvious conclusion is that an ester has shifted the peak on the C-6 carbon.

## F. Summary

By using liquid chromatography, NMR, and various other techniques, we can obtain a clear explanation of the formation, stability, and identification of cellulose phosphate. Figure 9 shows the resulting structure. We hope that a better understanding of this compound will assist further work on cellulose hydrolysis. The motivation for this research was development of an alternative method for cellulose hydrolysis. By producing a water-soluble cellulose derivative, a homogeneous hydrolysis could take place. Besides favorable kinetics, excess acid could be recycled after precipitation with THF was complete.

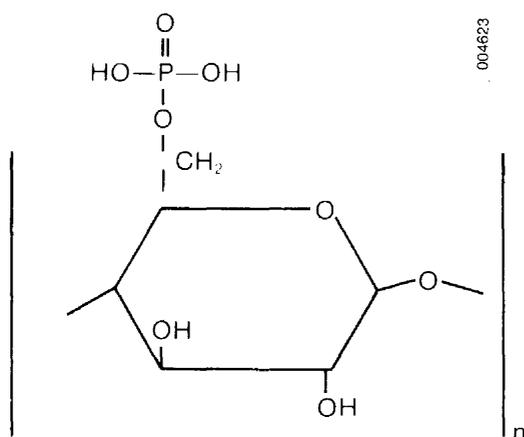


Figure 9. Phosphate Ester on C-6

## G. References

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## III. IMPROVEMENT OF MICROBIAL ALCOHOL FERMENTATION PROCESSES VIA GENETIC ENGINEERING

We used the following genetic engineering techniques to improve microbial alcohol fermentation processes:

- Cloning the xylose isomerase gene in yeast (Saccharomyces cerevisiae) to make the yeast capable of fermenting xylose to ethanol
- Development of a cloning system for Candida species so that their capability for the fermentation of xylose can be improved by gene cloning
- Overproduction of xylose isomerase in E. coli and B. subtilis to improve the xylose-to-xylulose-to-ethanol process.

## A. Research Performed

1. We constructed and characterized plasmids containing xylose isomerase gene with its ribosomal binding site partially or totally removed to improve the expression of the xylA gene in yeast.
2. We inserted xylA into a Leu2 gene cloned on pLR2 to study the expression of xylA by the Leu2 controlling signals.
3. We constructed a cloning vector for Candida utilis by using its DNA sequences capable of autonomous replication in yeast and by

using a bacteria gene, which can inactivate antibiotic G418, as a selection marker.

4. We conducted a preliminary investigation of the overproduction of xylose isomerase in E. coli and B. subtilis.

## B. Results

1. The nucleotide sequence data of the xylA gene upstream from its initiation codon (see Alcohol Fuels Program Technical Review, Winter 1983) showed that twelve more ATG nucleotides were upstream from the "real" initiation codon. In yeast, translation most likely will be initiated at the first ATG, resulting in the production of an inactive fused protein. This is probably the major reason that yeast transformants that acquire the cloned xylA gene do not functionally express the gene. By examining the nucleotide sequence of that region, we designed a specific experiment to remove the nucleotides up to four bases from the second ATG (the real initiation codon for xylA). This was accomplished by treating pLX10-14 with a klenow fragment of Pol I under conditions that favored the 3→5 exonuclease activity. Specifically pLX10-14 was digested with XhoI, incubated with klenow and d-GTP, treated with S<sub>1</sub> nuclease, and ligated with XhoI linkers. The resulting plasmids were used to transform xylA mutants, and pLX10-14D was isolated from one of the transformants that can no longer complement the xylA mutation.

The fact that pLX10-14D could no longer complement xylA mutation already indicated that part of an essential region of the xylA gene had been removed from pLX10-14. In order to prove that the loss of xylose isomerase activity was due to the removal of the ribosomal binding sequences of the xylA, and not due to other parts of the gene being altered, we analyzed the nucleotide sequence of the xylA gene in pLX10-14D. As we expected, fourteen nucleotides have been deleted between the XhoI site and the initiation codon of the xylA gene. The nucleotide sequences in the region upstream from the initiation codon of the xylA

gene in pLX10-14 and pLX10-14D are listed below for comparison:

pLX10-14

TCGAGGTGGATTATGGAGTTCAATATG . . .

pLX10-14D TCGAGCAATATG . . .

The underlined sequences are those being specifically deleted from pLX10-14, and therefore they are not present in pLX10-14D.

2. The XhoI-EcoRI fragment containing the promoterless xylA structural gene, isolated from the plasmid pLX10-14, was inserted into the pLR2 plasmid, which has been digested with restriction endonucleases XhoI and EcoRI to generate the proper ends for the insertion of the xylA structural gene. The resulting plasmid containing the hybrid gene was used to transform E. coli xylA mutants. The transformants that acquire the hybrid gene formed light pink colonies on McConkey/xylose/Amp plates, which indicated that the xylA gene fused within the Leu2 gene can be expressed by the yeast Leu2 promoter. Analysis of the xylose isomerase activity of the E. coli transformants containing the cloned hybrid Leu2-xylA gene indicated that the xylA mutants that acquired the hybrid gene only had one-tenth of the xylose isomerase activity that wild-type E. coli normally has. The hybrid Leu2-xylA gene still contains a small portion of the 5' terminal sequences of the Leu2 gene as well as the E. coli ribosomal binding site upstream of the xylA structural gene. So that the E. coli xylA gene can be properly expressed in yeast, these unnecessary sequences, particularly the E. coli ribosomal binding site, have to be removed.

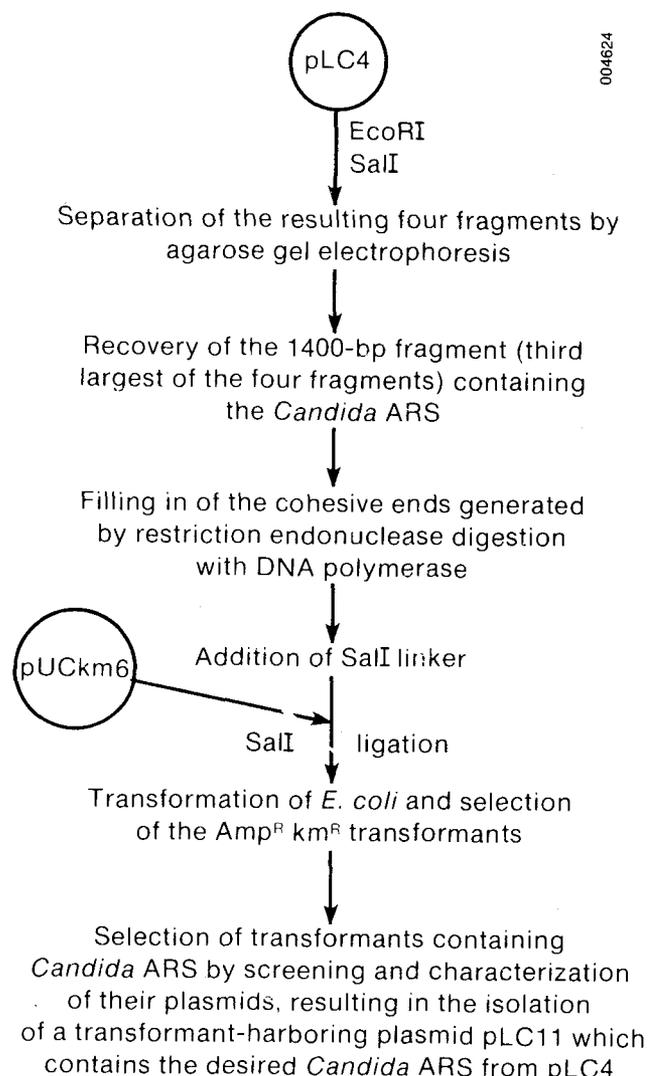
3. As we reported in Section I, the protocol used for the regeneration of protoplasts to viable cells for S. cerevisiae was not suitable for Candida species. Subsequently, we have successfully developed reliable procedures for the production of protoplasts for Candida utilis and for the regeneration of the Candida protoplasts to viable cells. Based on the development of these procedures, we believe that a

transformation system for Candida species could be developed. Furthermore, we have been able to isolate a number of Candida utilis DNA fragments that can function as autonomous replication sequences (ARS) in yeast. These ARS fragments can be used as the replication origin for the construction of a cloning vector for C. utilis. They were obtained by the insertion of partially digested Candida DNA into the BamHI site of the E. coli plasmid pLARS1 we constructed, which contained the yeast Leu2 gene as the selection marker. The resulting hybrid plasmids were used to transform yeast, and plasmids recovered from yeast transformants were those presumably containing Candida ARS. pLC4 is one hybrid plasmid that contains a 1400-bp Candida ARS, the smallest Candida DNA fragment containing ARS that has been isolated by our laboratory.

A detailed restriction map has been constructed for pLC4 plasmid. From the restriction map, we found that the Candida ARS fragment was located on a 1400-bp EcoRI-SalI fragment. The 1400-bp fragment was then isolated and reinserted into the pUCkm6 plasmid, which contains the km resistance gene. The km resistance gene is expressible in yeast (S. cerevisiae) to inactivate the antibiotic G418. The km resistance gene may also be able to be expressed in Candida. The detailed protocol for the construction of such a plasmid (pLC11) is shown in Figure 10. Preliminary analysis showed that the pLC11 contained the pUCkm6 with an insert corresponding to the 1400-bp ARS, although some rearrangement occurred during the construction of pLC11.

We then used pLC11 to transform Candida and isolated G418-resistant clones. Although Candida can also be induced to become resistant to G418 without the presence of the km resistance gene, resistant clones that acquired pLC11 have been obtained. This was verified by the successful recovery of plasmids from some of the G418-resistant transformants. By analyzing restriction patterns of these recovered plasmids, we found that some of them are identical to the original pLC11. However, plasmids with an altered restriction pattern were also obtained.

4. Preliminary investigation of the overproduction of xylose isomerase in both E. coli and B. subtilis has also begun. Our strategy for overproduction of the enzyme in E. coli is to subject the promoterless xylA gene under the control of various strong E. coli promoters to determine the maximum enzyme activities being allowed to be produced in E. coli. The advantages of using B. subtilis as a host for the overproduction of enzymes produced by genes cloned on plasmids are (1) nonpathogenicity, (2) lack of endotoxin, and (3) ability to secrete



**Figure 10. Protocol for the Subcloning of the Candida ARS from pLC4 into pUCkm6**

enzymes or proteins directly into the medium. Hence, we believe that it is also desirable to clone the *E. coli* xylose isomerase gene into *B. subtilis* to study the overproduction of the enzyme in this host.

### C. Information of Public Interest

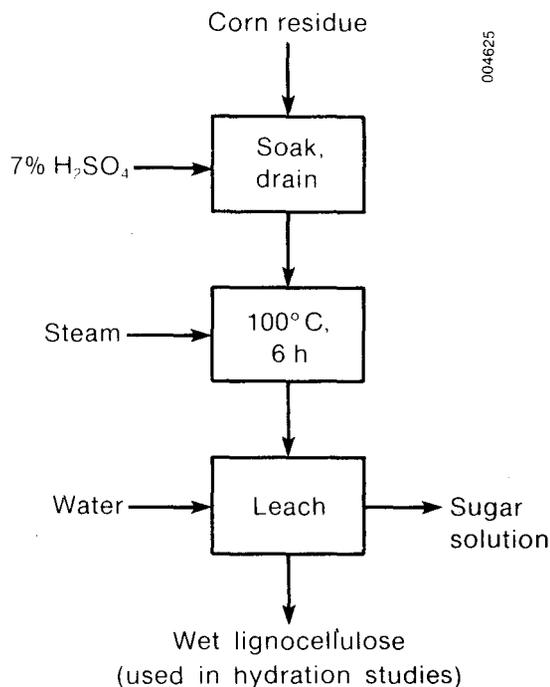
1. An abstract entitled "The Construction of a Cloning Vector for Eukaryotic Microorganism by Using its DNA Sequences Capable of Autonomous Replication in Yeast" has been accepted for presentation in a poster session at the Annual Scientific Meeting of the American Society of Biological Chemists, St. Louis, MO, June 3-8, 1984.

2. An abstract entitled "The Development of a Cloning System for *Candida* Species" has been accepted for presentation at a slide session at the 6th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May 15-18, 1984.

### IV. The Role of Water in Cellulose Hydrolysis

Research has begun on the role of water in cellulose hydrolysis of corn residue. The first step is to hydrolyze the hemicellulose fraction of corn residue and to leach the resulting sugars. This procedure (outlined in Figure 11) was carried out in our scale-up facility to yield corn residue lignocellulose (LIC), which is then used in subsequent research. Since the purpose of the procedure is to generate LIC, we do not take special steps to obtain a concentrated hemicellulose hydrolyzate. Rather, the primary objective is to wash the sugars from the hydrolyzed biomass as quickly as possible. The procedure is as follows:

1. Pack 42 lb of corn residue (at 35% moisture and 3.5%  $H_2SO_4$ ) into the reactor.
2. Inject steam to bring the reactor to 100°C and hold for 7 hours.
3. Fill the reactor with water (71.4 lb) and drain (56 lb).



**Figure 11. Process Schematic of Hydrolysis Procedure to Obtain LIC from Corn Residue**

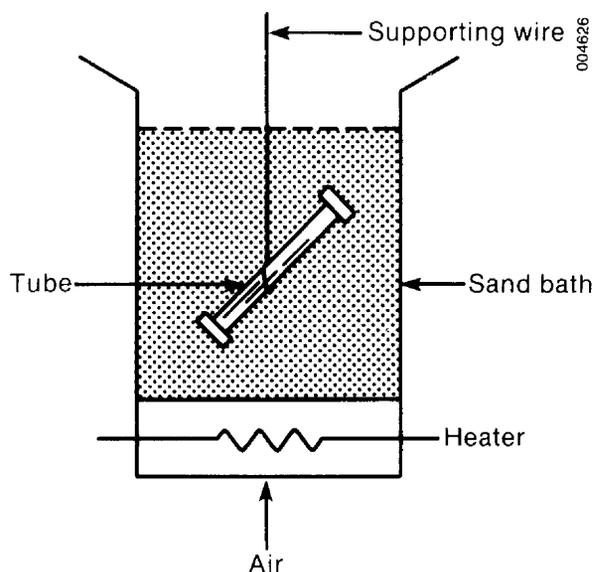
4. Repeat, adding approximately 61 lb water and draining two more times.
5. Remove corn residue lignocellulose and store in a refrigerator until further use.

The corn residue is currently being analyzed for cellulose content. The concentrations of the three fractions obtained from washing the hydrolyzed biomass are given in Table 4.

**Table 4. Analysis of Hydrolyzate Solutions from Hydrolyzed Corn Residue (%)**

	First Wash	Second Wash	Third Wash
Glucose	0.46	0.12	0.07
Xylose	3.20	0.83	0.48
Arabinose	0.81	0.22	0.12
Acetic acid	0.83	0.16	0.07
$H_2SO_4$	1.60	0.40	0.26
<b>TOTAL</b>	<b>6.10</b>	<b>1.73</b>	<b>1.00</b>

While the corn residue was being hydrolyzed, we were setting up the apparatus for the laboratory-scale runs on the role of water in cellulose hydrolysis (see Figure 12). The procedure consists of filling thick-walled, 316 stainless steel, 0.275-in. (OD) tubes with the cellulose slurry, capping the tubes, and submerging them in a sandbath as indicated in Figure 12. The tubes are removed, quenched in water, and capped. The solids, if any, are separated by centrifugation, and the resulting supernatant is analyzed by either liquid chromatography or the glucose analyzer. Shakedown runs were carried out with Avicel using a slurry containing 450 ppm  $H_2SO_4$ . The preliminary results summarized in Table 5 show that a high glucose-to-acid ratio is possible.



$t = 0$       Tube submerged  
 $t = 2-3 \text{ min}$       Complete heat-up  
 $t = t'$       Tube removed and  
    quenched in water

**Figure 12. Illustration of Apparatus for Laboratory-Scale Runs on the Role of Water on Cellulose Hydrolysis**

**Table 5. Avicel Shakedown Runs**

Run Number	Temperature (°C)	Time (min)	Concentration (%)	Ratio of Glucose to $H_2SO_4$
1	200	10	7.6	17.7
2	200	15	8.2	19.1
3	220	7	16.6	38.8
4	220	10	22.5	52.6
5	240	5	23.6	55.2

## ARGONNE NATIONAL LABORATORY

### Ethanol Production via Fungal Decomposition and Fermentation of Biomass

#### I. Introduction

The purpose of the program is to isolate and develop Fusarium strains that can produce ethanol by decomposing cellulose and hemicellulose (from natural sources) to monosaccharides and fermenting five- and six-carbon sugars. Between December 1983 and April 1984, our research efforts were restricted (for financial reasons) to isolating new Fusaria and screening them for cellulase production and xylose fermentation. Additional studies focused on relevant areas of Fusarium activities. The results were evaluated, and preparations for further experiments are now under way. Research findings and approaches for future studies are summarized in this report.

#### II. Isolation, Development, and Selection of Fusarium Strains

Several new Fusarium strains were isolated from soil samples and anaerobic digester sludge with the "dextrose-peptone-PCNB-rose bengal-neomycintetracycline" medium, which is very effective for selectively isolating Fusaria. The isolated strains were cultured for identification on "carnation leafagar", which aids in the observation of spore morphology (for species determination). Fusarium oxysporum was most frequently isolated, along with isolates of F. solani, F. tricinctum, and F. moniliforme.

UV-irradiation, chemical mutagenesis, and parasexual recombination seem to be suitable for developing new Fusarium strains. Work with UV-irradiated microconidia has developed a few strains that are superior to the wild strains in decomposing cellulose and fermenting simple sugars. Future efforts will focus on the areas of mutant induction by appropriate chemicals and UV exposure, as well as by

exploring and utilizing the parasexual behavior of Fusaria.

#### III. Screening for Lignolytic Fusaria

One of the most important and complex biopolymers in woody biomass is lignin, which is not easily degraded by most microbes. It has been recorded that among other fungi, certain Fusarium strains are able to decompose lignin. In order to study lignin degradation, either the decomposition of lignin model compounds (mostly dilignols) must be investigated or the degradation products must be identified.

Based on preliminary experimentation, future studies of lignin degradation by Fusarium strains will focus on the following:

- Woody biomass lignolysis. Pretreated and sterilized wood will be inoculated with Fusarium strains (mainly F. solani) under mesophilic temperature and a high moisture regime. Incubation will take several weeks, and the decayed material will be collected for further processing.
- Extraction of lignin degradation Products. Fractions from the decayed wood will be obtained by successive extractions with petroleum ether, chloroform, methyl alcohol, and dioxane.
- Characterization of Lignolysis Products. Further treatment of the fractions will separate them into more specific materials. Chemical characterization and quantitative determination of the specific fractions will identify the ability of the investigated Fusaria to degrade lignin.

#### IV. Cellulose Decomposition by Fusarium Strains

Fusarium species are microorganisms that produce cellulase and hemicellulase enzymes. Our studies have indicated that strains of Fusarium oxysporum consistently produced higher amounts of extracellular cellulase in submerged cultures in the presence of insoluble cellulose than did any strains from other

Fusarium species. Several mutants isolated from UV-irradiated samples of F. oxysporum cultures produced twice the amount of extra-cellular cellulase enzymes produced by their parent isolates. Nearly all the Fusarium isolates have shown cellulolytic activity.

Preliminary screening for cellulose decomposition by Fusarium isolates was done by the test-tube-clearing assay. A plate-clearing assay was also used, but growth inhibitors (necessary for a plate-clearing assay) appeared to suppress cellulase production, rendering this screening ineffective. Further tests were conducted in liquid culture with cellulose as the sole carbon source. Most Fusarium isolates produce detectable levels of cellulase enzymes, and several isolates have produced up to 1.0 IU/mL of cellulase after two weeks of growth. The optimum temperatures for cellulase production were in the range of 28<sup>o</sup>-30<sup>o</sup>C. Fusarium isolates have also been screened for cellulase production at temperatures of 35<sup>o</sup>-40<sup>o</sup>C, and several isolates were found to produce equal amounts of cellulase at 30<sup>o</sup> and 37<sup>o</sup>C. Other optimum conditions found for cellulase production were pH 4-5, 0.75%-1% Solka Floc BW-40 as a carbon source, and 1.0-2.0 g/L ammonium nitrate as a nitrogen source. Further studies will investigate the cellulase complex of Fusaria and will isolate and develop such enzyme-producing strains.

#### V. Glucose Fermentation by Selected Fusarium Strains

Fusarium strains, besides releasing polysaccharide-splitting enzymes, seem to be equipped with an effective enzymatic mechanism that enables them to readily ferment monosaccharides. Therefore, one of the main objectives of the program is to isolate and develop effective pentose- and hexose-fermenting Fusarium strains and to optimize the fermentation parameters. Several of the Fusarium isolates have yielded up to 4.3 mg/mL ethanol within 48 h in 1% glucose solutions, as reported previously, and further work will focus on improving this yield.

It was noted that inoculum consisting of spores, germinated spores with short germ tubes, and fusoid-to-spheroid hyphal cells showed increased ethanol yield and was easier to handle in the fermentor than inoculum composed mainly of mycelium. In addition to studies of the nature of inoculum, more research has focused on the effects of glucose concentration, xylose concentration, and glucose-xylose mixtures.

#### VI. Xylose Fermentation by Selected Fusarium Strains

One of the main monosaccharide components of hemicellulose is xylose, which must be fermented to make the ethanol process economically feasible. Selected Fusarium strains have been found to be effective xylose fermenters. These strains have been tested and have produced up to 4.2 mg/mL ethanol from 1% xylose solutions, and up to 8.0 mg/mL from 2% xylose in 48 h. Increased concentrations were fermented less efficiently, but ethanol was produced in solutions of up to 8% xylose, with a maximum ethanol yield near 25 mg/mL. Attempts to increase ethanol production further will concentrate on more efficient and more ethanol-tolerant strains immobilized in a bioreactor.

#### VII. Ethanol Tolerance Studies

Restricted research efforts have focused on the identification of ethanol-tolerant Fusarium strains. Although little is known about the mechanism of ethanol tolerability by microbes, this characteristic is vital to a highly efficient ethanol fermentation system. Ethanol denatures proteins and therefore deactivates enzymes. Some research has focused on the effect of ethanol on cell lipids as an important component of tolerance. It appears that increased ethanol concentration in a medium affects the fatty-acyl unsaturation of cellular phospholipids. Certain ethanol-tolerant microbial strains are able to withstand high intracellular concentrations of ethanol. Experiments have shown that selected

Fusarium strains can tolerate up to 5% ethanol concentrations, based on dry weight production, ethanol yield, and viability. Further research will be directed toward determining the effects of medium composition, temperature, and growth rate on ethanol tolerance.

### VIII. Experimentation with Fusarium Mixed Cultures

We plan to study the coexistence and cooperation of Fusarium strains and those from other fungal genera in producing ethanol from sugar fermentation. The study will be restricted to binary population experiments, because triculture systems are more complicated to investigate. This work will be conducted in a chemostat-type apparatus to provide a controlled environment. Experiments with Fusarium and Saccharomyces cerevisiae strains will be initiated in this investigation. The successful establishment of this system will be further tested to determine fermentation ability on glucose and on glucose-xylose solutions.

### IX. Fusarium Cell Immobilization

We have observed that in shaken liquid cultures of Fusaria the developing hyphae become entangled and form sphere-like pellets. It has been pointed out that this pelletization creates high viscosities and restricts fungal growth and product formation. Limited nutrient transport and assimilation have a serious impact on the metabolic activities within the fungal pellet. However, we observed hyphal fragmentation at the end of the growth phase, possibly due to a decrease of cytoplasmic content and an increase in vacuolation. Germinating micro-

conidia were found to be more enzymatically active than nongrowing hyphae and chlamydo-spores. Immobilization of fungal cells could mitigate undesirable limitations arising from freely suspended mycelia and spores and could increase ethanol production efficiency.

The immobilization of fungal cells growing within gel matrices seems to be very efficient in producing fuels and chemicals, because the immobilized fungal cells are growing biocatalysts that act for a long time under the protection of the gel matrix. Various biosupport materials have been tested for fungal cell entrapment, including calcium alginate, aluminum alginate, k-carrageenin, and others. Some methods employ prepolymer matrices that can be photo cross-linked. Our preliminary studies of Fusarium cell immobilization employed calcium alginate. Future research will test other entrapping materials and will include comparative studies.

Immobilized growing cells are often more efficient than immobilized enzymes or treated cells because the entrapped growing cells can perform more enzymatic catalyses than entrapped enzymes. Spore (mainly microconidial) immobilization is the most appropriate method for Fusaria. The immobilization of Fusarium cells will focus on the development of an immobilized cell reactor for efficient and continuous fermentation of glucose and xylose to produce ethanol. It will also determine the effects of biomass loading (number of spores and cells encapsulated within a specific amount of biosupport), sugar concentration, ethanol concentration, temperature, pH, cell type and age, and dilution rate on ethanol production.

# Feasibility Studies

## Methanol Gasification

### STONE AND WEBSTER ENGINEERING CORPORATION

#### Economic Feasibility Study of a Gasification-Based Methanol Plant

##### I. Introduction

This report summarizes the progress of two tasks in our contract: Task I, "Process Selection and Integration," and Task II, "Plant Design, Specification, and Cost." The objectives of this feasibility study are to evaluate the current commercial potential of a modular methanol plant (MMP) using the SERI oxygen-blown, pressurized, downdraft gasifier technology and to identify areas in which further research and development are required. The integrated base-case conceptual design will utilize commercial operations except for the gasifier.

Several major assumptions have guided the site selection and process integration for the base-case design and have been carried through to the plant design. The guidelines are as follows:

- The price of conventional methanol, delivered to the selected site, should be higher than methanol produced at an MMP.
- The product methanol will be used as an octane enhancer or as a neat fuel (90% methanol-10% gasoline).
- All unit operations except the gasifier should be commercial or near commercial.
- The plant will purchase raw materials and services, rather than generating them, when practical; i.e., oxygen, water treatment, etc.
- A goal is the complete utilization of internally available energy through integration.
- Maximum utilization of shop fabrication and modular construction have been specified.

##### II. Task I, Process Selection and Integration

Task I included the following subtasks:

- Site definition
- Site determination and selection
- Determination of MMP size
- Oxygen costs
- By-products
- Process design and selection.

Some of these subtasks are discussed briefly here.

##### A. Site Selection

The selected site is Spokane, Wash. This site satisfied the criteria of

- High delivered cost of methanol
- Availability of raw materials at a reasonable cost
- Demand in the region for the product
- Potential for by-product sales.

##### B. Design Basis

The major parameters of the design of the MMP are set forth in Table I. The SERI gasifier has been scaled up to have a flow diameter of 8.5 ft and a throughput of 12,000 lb/h of dry wood. This design produces an output of 77 tons per day of methanol.

Analysis of the potential problems in scale-up of the SERI reactor concentrated on oxygen distribution, uniform wood distribution, and nonuniform gas flow. The nonuniform gas flow is the result of bridging of the bed (sticky wood or ash fusion) or plugging of the bed (fines generation). The reactor size was limited to what would easily fit on a rail transportable skid. The present size of 8.5-ft ID and 10-ft OD was chosen after considering

**Table 1. Basis of Design of the Modular Methanol Plant**

Item	Basis
Raw wood feed	Whole tree chips, 50% moisture content, approximately 311 tons per day
On-site storage	7 days
Processed wood feed	Pin chips, 3/8-in. diameter by 1-1/2 in. long, 16% moisture content
Gasifier pressure	150 psia
Gasifier size	8.5 ft internal diameter
Gasifier heat loss	2% of input HHV
Wood carbon conversion to gas	98%
Gasifier throughput	1.78 MBtu/ft <sup>2</sup> h
Gasifier output	See Figure 2
Acid gas removal	Benfield process: hot potassium carbonate followed by zinc oxide guard catalyst
Methanol synthesis	Lurgi low-pressure process
Methanol yield	77 tons/day
Energy integration	Steam: all generated internally Electricity: part internally generated, part purchased
Solid, liquid, gaseous wastes	Systems designed to meet local/federal regulations

other solid fuel gasifiers, the SERI pilot plant design, and transportation limitations.

### C. Process Design

The overall process flow diagram is shown in Figure 1, with the corresponding material and energy block diagrams in Figures 2 and 3, respectively. Several unique energy and process integration steps have been incorporated to increase yield and reduce energy demand. The more important design innovations are described here.

- The use of indirect heat in the wood dryer. This allows the dryer exhaust steam to be reused via vapor recompression as the heat source for the acid gas reboiler.
- The use of direct water injection to supply steam for shifting. Water is injected after the raw synthesis gas cooler to supply the shift steam. This heating and mixing method is efficient and cost effective.
- The use of a pressure swing adsorption (PSA) unit for increased methanol production. Approximately 75% of the hydrogen from the methanol purge gas is recovered and recycled to the methanol synthesis unit, increasing the yield approximately 7% (from 72 to 77 tons/day). The remaining purge gas is fed to a diesel engine generator to produce power and steam.
- The use of a small, fluid-bed combustion system to burn char and waste wood (undersized particles). This eliminates most of the solid waste disposal problem and allows the generation of additional process steam in addition to start-up steam.
- The use of solvent extraction to remove phenolic compounds from the syngas wash to reduce environmental costs.

### III. Task II, Plant Design, Specification and Cost

Task II included the following subtasks:

- Startup, shutdown, and turndown procedures and requirements

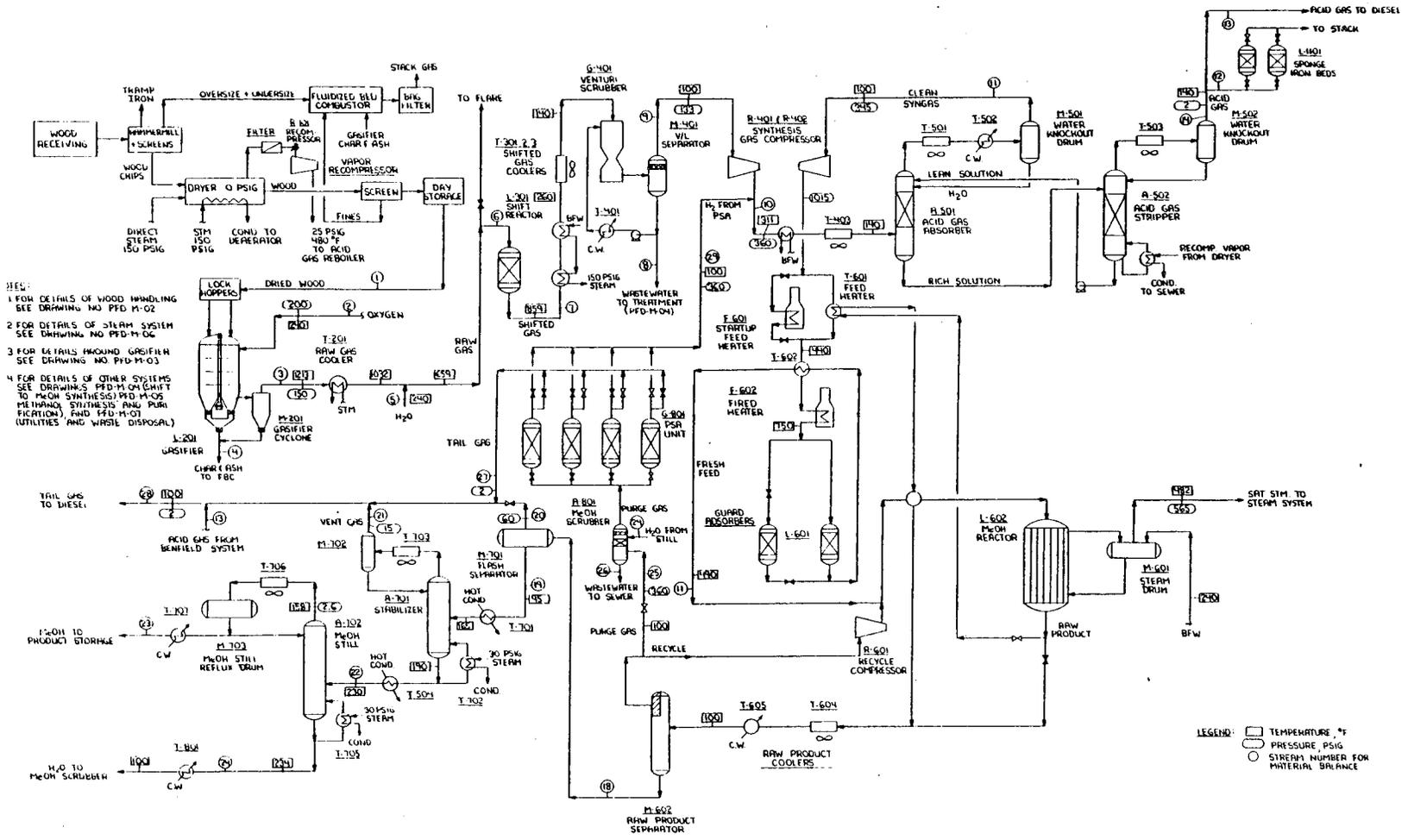
- Environmental requirements
- Plant operating requirements
- Equipment duty specifications
- Module and plant layout
- Capital cost estimation.

The engineering effort of Task II has produced a series of engineering flow diagrams, equipment duty specifications, and plant layouts that are being used to estimate the capital cost of the facility. The overall and process area plot plans are shown in Figures 4, 5, and 6. The modular methanol plant (MMP) consists of eleven sections in which maximum utilization of shop fabrication and skid mounting of equipment has been used to minimize field installation. Only Section 100, "Wood Handling and Preparation," has not been designed for skid mounting because the size and elevations of the conveyors, screens, and other equipment make field installation more practicable.

In the remaining ten sections, most of the equipment is skid-mounted except for the major towers, which are free standing. The system comprises 46 individual skids, of which 13 are vendor supplied packages and 7 are air cooled heat exchangers placed on top of several other skids. The total number of skids is large, but many skids are of moderate size. Standard size allows an envelope of 12 ft wide by 12 ft high by 80 ft long. Use of Low Boy rail cars could add several feet to the allowed height.

During the process design, we identified several areas for investigation in Task III to improve system economics. The following potential improvements will be investigated in detail:

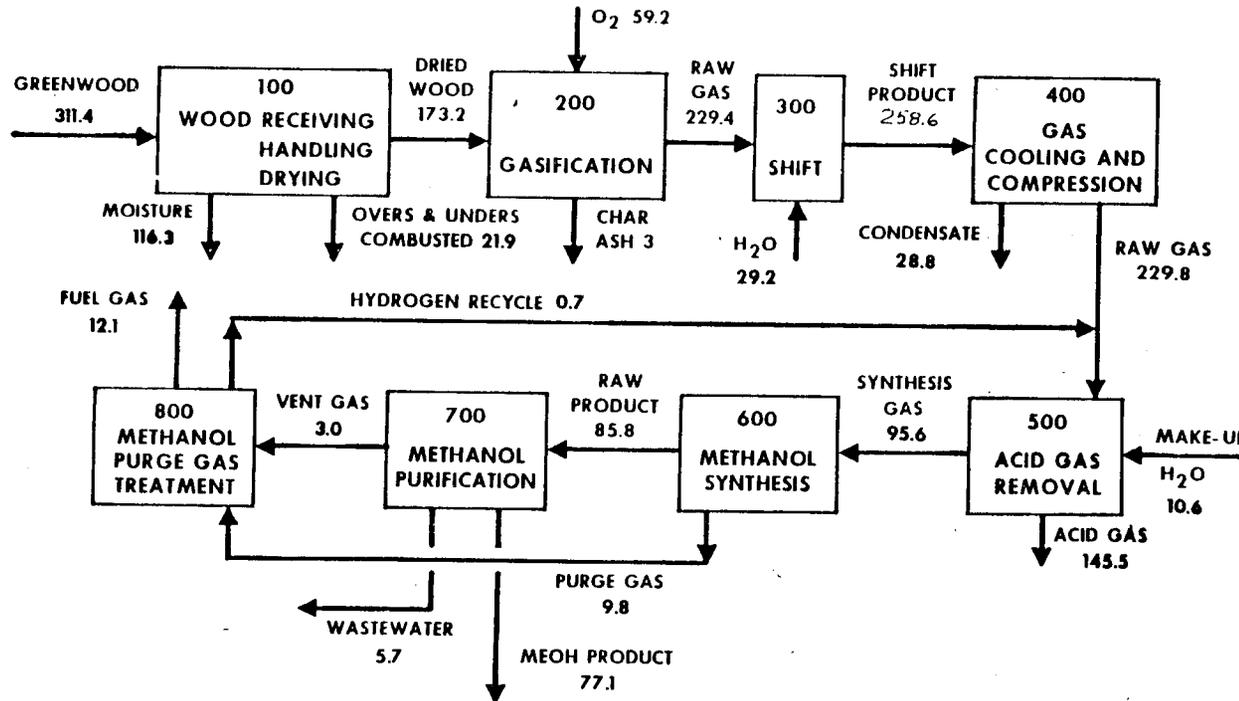
- Higher-pressure gasifier operation
- Higher moisture content of wood going to the gasifier
- Reforming purge gas methane for increased yield
- Increased gasifier throughput to increase methanol production.



1. FOR DETAILS OF WOOD HANDLING SEE DRAWING NO PFD-M-02  
 2. FOR DETAILS OF STEAM SYSTEM SEE DRAWING NO PFD-M-06  
 3. FOR DETAILS AROUND GASIFIER SEE DRAWING NO PFD-M-03  
 4. FOR DETAILS OF OTHER SYSTEMS SEE DRAWINGS PFD-M-04 (SHIFT TO MOOH SYNTHESIS), PFD-M-05 (METHANOL SYNTHESIS AND PURIFICATION), AND PFD-M-07 (UTILITIES AND WASTE DISPOSAL)

LEGEND:  
 □ TEMPERATURE °F  
 ○ PRESSURE PSIG  
 ○ STREAM NUMBER FOR MATERIAL BALANCE

Figure 1. Overview Process Flowsheet for Modular Methanol Plant



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Figure 2. Final Material Balance (in tons/day)

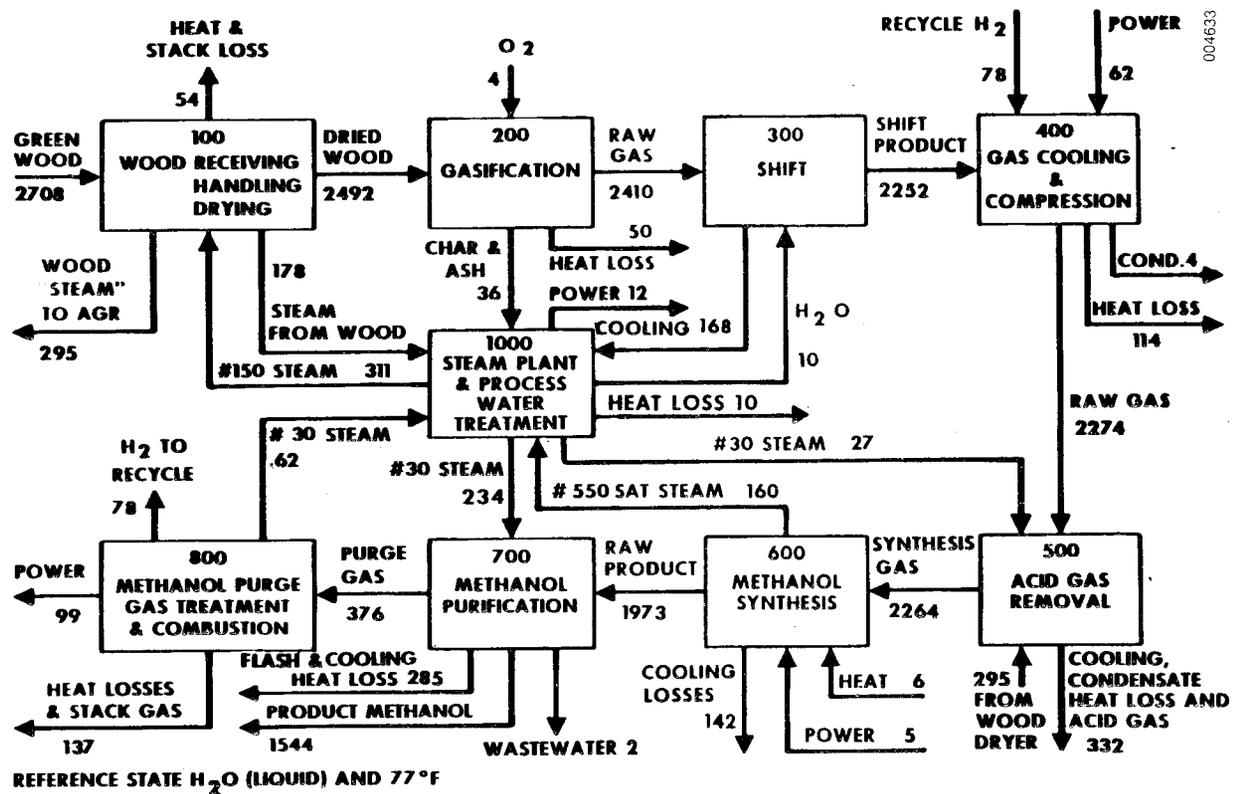
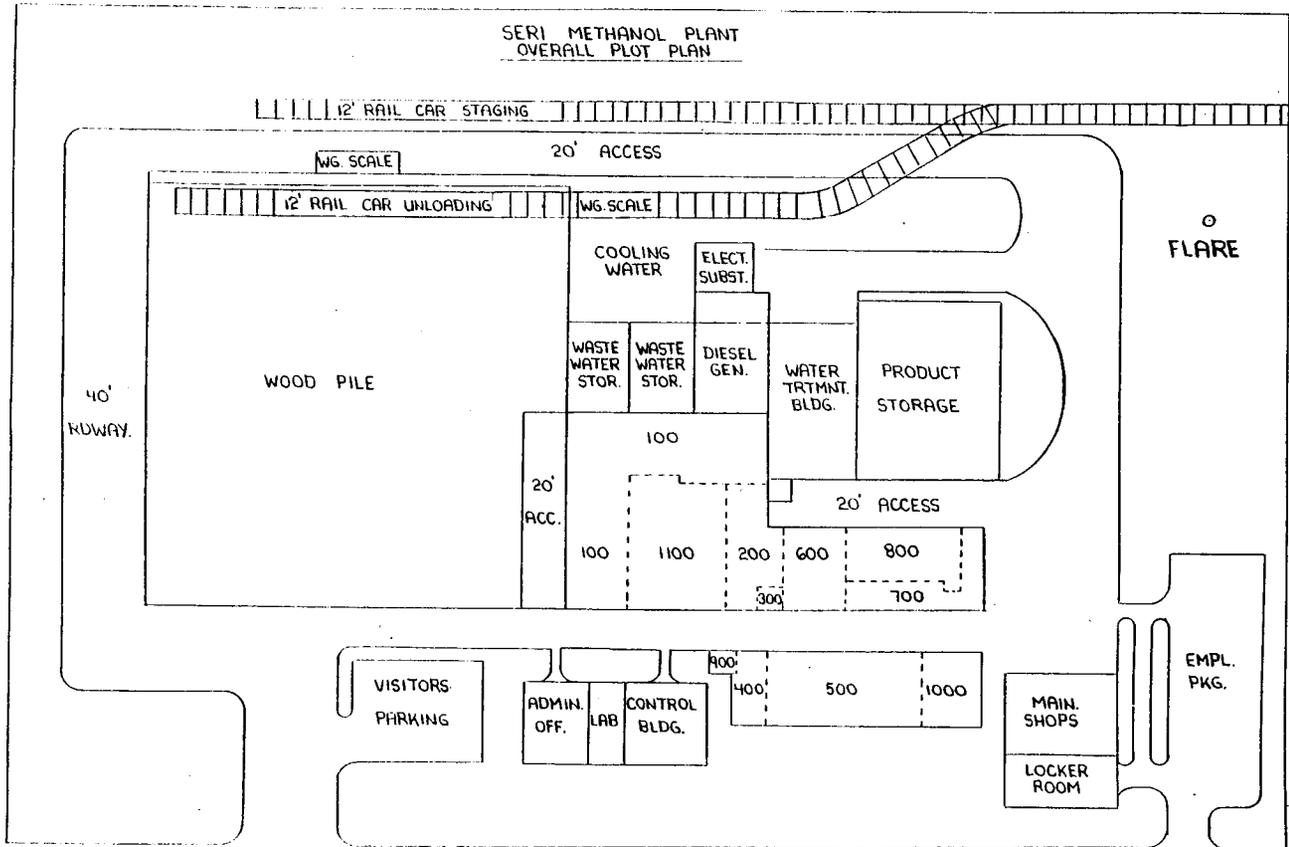


Figure 3. Final Energy Balance (in  $10^6$  Btu/day)



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Figure 4. Overall Plot Plan for the SERI Methanol Plant

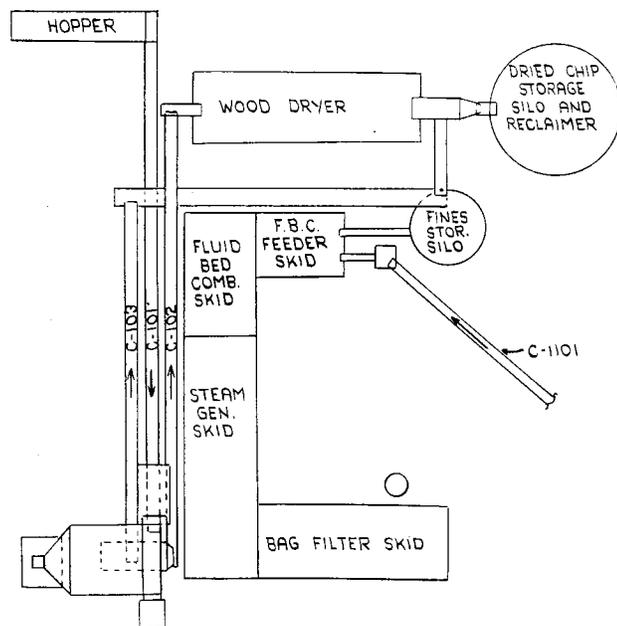


Figure 5. Section 100 of the SERI Methanol Plant

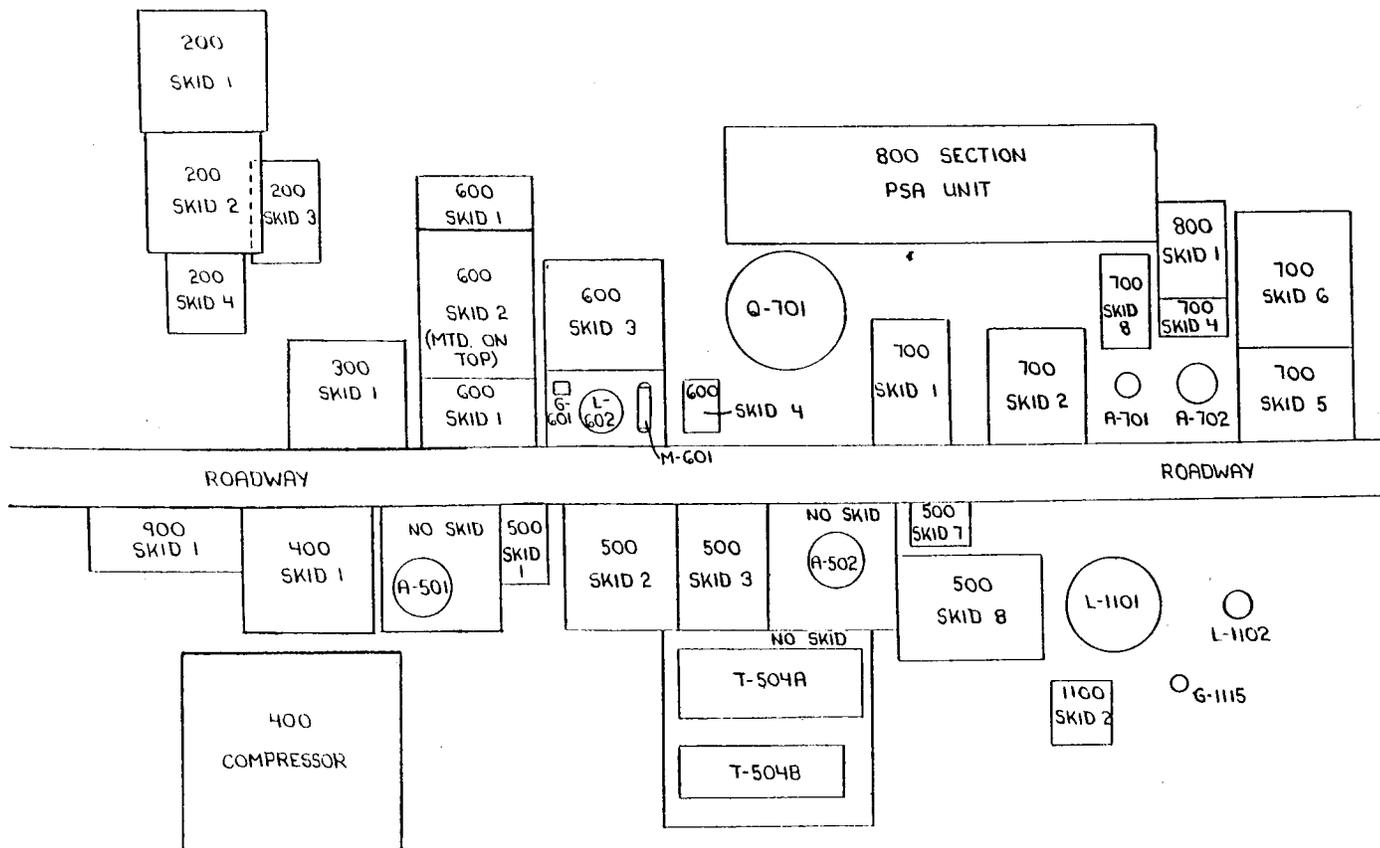


Figure 6. On-Site Process Unit Skid Layout for the SERI Methanol Plant

The major plant sections of the MMP are listed in Table 2. The plant is designed to process approximately 311 tons of wood per day (50%

moisture content) and produce 77 tons per day of methanol product.

When Task II is completed, the economic analysis for Task III will begin.

**Table 2. Plant Sections**

Section	Description	Number of Skids
100	Wood handling and preparation	0
200	Gasification	4
300	Shift	2
400	Gas cooling and compression	2
500	Acid gas removal	8
600	Methanol synthesis	4
700	Methanol purification and storage	7
800	Methanol purge gas treatment	2
900	Wastewater treatment	1
1000	Steam plant and process water treatment	10
1100	Utilities and buildings	6

## Acid Hydrolysis Processes

### STONE AND WEBSTER ENGINEERING CORPORATION

#### Economic Feasibility Study of an Acid-Based Ethanol Plant

##### I. Introduction and Definition

The objectives of the study are to determine the economic feasibility of a commercial-size facility for the conversion of wood to ethanol via acid hydrolysis and to recommend areas of continued research and development that will allow transfer of this technology to the public sector. The study is divided into three tasks. Task I is the selection of a preferred processing sequence and the development of a basis of design for a base-case facility. Task II consists of engineering the base-case processing sequence into an integrated facility in sufficient detail to determine the facility's capital and operating costs. Task III will establish the economics of the base case and evaluate the

effect of technological changes, process modifications, or economic trade-offs of the base-case economics. One of the primary goals of Task III will be to determine the requirements for further research, development, and demonstration that are needed to make a commercial facility an economically and technically attractive means of producing a fuel-grade product.

##### II. Definition of Geographic Plant Location

Consultations with researchers at the Hawaii Natural Energy Institute have resulted in the selection of the island of Hawaii as a suitable site for the eucalyptus tree plantation and energy plant. Two specific sites--one near Hilo and the other near Honokaa--have been identified as suitable plant locations. Site selection is based primarily on the following features:

- The availability of abandoned sugarcane refineries

- The close proximity (economic trucking distance) to tested eucalyptus plantations capable of supporting the feedstock requirement (1,900 tons/day) for a plant capacity up to 15 million gallons of ethanol per year.

### III. Process Design Basis

We selected the base-case process after evaluating various processing options. The final selection was based primarily on the following criteria:

- Availability of process information
- Compatibility of process segments in an integrated facility
- Commercial viability of processing options.

The integrated facility is designed using good engineering judgment that is optimistic in assumed performance but conservative in equipment adaptation. Process integration was performed considering energy consumption, water reuse, and capital cost advantages.

### IV. Base-Case Process Description

The process can be divided into six sections:

- Wood handling and pretreatment
- Hydrolysis
- Fermentation preparation
- Fermentation
- Product purification
- Offsites.

Within these sections, the process can be divided into several operations, which are listed and discussed here.

#### A. Wood Chipping and Cleaning

The wood feed is chipped at the tree plantation and trucked to the plant site. The chips are cleaned using standard cleaning techniques.

#### B. Chip Defibrating

The clean wood chips are processed in a standard Sunds defibration unit with dual heating vessels to recover process heat. The chips are held at 374°F to solubilize the xylose sugars and break the wood fiber bonding. The "soft" wood chip fibers are then mechanically separated in a defibrator, which discharges to a flash cyclone to recover 50 psig steam.

#### C. Xylose Sugars

The hot fibers are washed to recover xylose. The xylose sugars are treated in an anaerobic digester to produce a methane-rich gas.

#### D. Acid Hydrolysis

The cellulose stream is acidified to a 1%  $H_2SO_4$  concentration and heated to 479°F. The hydrolysis kinetics are based on Grethlein's plug-flow reactor system operating with a 7-second residence time.

#### E. Process Heat Recovery

The acidic hydrolyzate is quenched to 356°F by flash cooling. The flash vapors are used for wood chip heating, water makeup heating, and evaporation heat. A secondary flash at 266°F produces steam for the beer still. The hydrolyzate is further cooled prior to lignin removal.

#### F. Lignin Removal

The lignin and insoluble solids are removed in a two-stage solids separation process. The solids from the first stage of separation are pH adjusted and diluted with evaporator condensate. They are then concentrated in the second stage, which produces a solid feed that is sent to a lignin boiler and a clear stream. The clear overflow is mixed with the first-stage liquid overflow, reheated, and sent to neutralization.

#### G. Lime Softening

The glucose stream is adjusted to pH 10 to reduce the fermentation inhibitors and precipitate sulfate ions as calcium sulfate. The

precipitate is removed in a gravity settler followed by a rotary vacuum filter. The remaining calcium salts are then reacted with sodium carbonate to reduce the soluble calcium carbonate, thereby preventing scaling in downstream processing equipment. The glucose-rich stream is then adjusted to pH 4.5 for fermentation.

#### H. Evaporator

The neutral, delignified process steam is concentrated in a four-stage multieffect evaporator to approximately 15 wt % glucose.

#### I. Fermentation

The glucose stream is fermented with immobilized yeast using the Kyowa Hakka Kogyo Co., Ltd., continuous fermentation process.

#### J. Ethanol Recovery

Ethanol is recovered in a standard distillation system (beer still) followed by azeotropic distillation using cyclohexane as the azeotrope breaker. The beer is reboiled by direct injection of recovered flash vapors.

#### K. Offsites

In addition to normal offsite equipment, the acid hydrolysis process will include a fluidized bed boiler to burn the residual solids (lignin and unconverted cellulose) and the methane-rich gas from the xylose digesters.

#### V. Processing Options

A number of different processing options were considered in order to select the sequence of process operations for the base-case design. The major areas of consideration are described here.

##### A. Wood Size Reduction

The options considered for preparing the wood for hydrolysis were grinding, milling, steam explosion, and steam treatment followed by milling. In general, grinding to the size required is highly energy intensive due to the

necessity of drying the wood prior to grinding and the large power requirements of the grinding itself. Wet milling, or mechanical pulping is also highly energy intensive if the wood is not pretreated. Steam explosion uses substantial amounts of high-pressure steam. The procedure is not continuous nor is it considered energy efficient by the wood industry. Unlike enzyme hydrolysis, acid hydrolysis does not require an exploded feedstock. The heat treatment followed by disc milling offers the following advantages:

- Lower power requirements for milling the wood
- Energy efficiency through integration with steam generated within the process
- Commercial, continuously operating equipment
- Recovery of hemicellulose sugars.

##### B. Solids Concentration in Hydrolysis

The base-case design assumes a wood fiber feed to the hydrolysis reactor of 17 wt % total solids (TS). This value was chosen as the maximum solids that could be pumped into the hydrolysis reactor using existing wood slurry handling equipment. At higher solids loadings, slurry heating problems are envisioned. We believe that since the hemicellulose wood fraction has been extracted from the wood feed, this 17 wt % TS stream would become a thicker slurry to process than an equivalent 17 wt % TS pulp slurry. Pumping and heating characteristics of the stream area require more demonstration.

Material balance cases were investigated assuming higher wood feed concentrations. Results of these cases indicated that an increase in the solids concentration could (1) reduce steam requirements, (2) lower chemical costs, and (3) reduce equipment size in the hydrolysis section. However, wash requirements in the lignin recovery section increased and subsequently reduced the beneficial effect of higher concentrations downstream of the hydrolysis section. These trade-offs will be discussed in Task III of this study.

### C. Ethanol Fermentation

The options considered for ethanol fermentation of the 15% glucose sugar solution were batch and continuous process. Compared with batch fermentation, continuous fermentation has higher fermentation rates, better acclimation of the yeast to feedstream impurities, reduced operating labor, and continuous operation for up to 2,000 hours.

A number of alternative continuous fermentation processes were considered including Alfa Laval's Biostill and JGC and Kyowa's immobilized yeast systems. Kyowa's system is the most advanced continuous fermentation system that is compatible with the wood hydrolyzate stream.

### D. Use of Hemicellulose Sugars

The five options considered for utilization of the hemicellulose sugars were:

- Methane generation (anaerobic digester)
- Wet air oxidation
- Production of molasses
- Production of animal feed
- Furfural production.

Anaerobic digestion of the sugars to produce a methane-rich gas is the selected base-case option because it produces a fuel product needed at the plant and in the state of Hawaii. Both methane and electrical power can be sold on the island. Wet air oxidation of the organics could meet the plant energy needs by producing low-pressure steam that can be used by the process. However, this option is very capital intensive, and the low-pressure steam does not offer the flexibility of a methane-rich gas.

Products other than fuels have limited markets in Hawaii. Animal molasses is derived cheaply from sugar cane and sells for about half the mainland market price. The production of Torula yeast for single-cell protein (SCP) is also capital intensive and involves the inclusion of another complicated process operation. Furfural has no market in Hawaii; therefore, it

was not considered to be a valuable by-product. However, the present wood-to-ethanol process does allow for furfural production. Thus, if a furfural market is identified, the process can be modified to meet the furfural demand.

### VI. Secondary Trade-Offs

Secondary trade-offs considered in developing this process include fermentation pretreatment options; sequence of lignin removal, neutralization, and sugar concentration; and molecular sieves versus azeotropic distillation for product purification.

### VII. Areas of Future Work

The process areas that still require technical development include the following:

- Design of a high-pressure pump for severe conditions and high slurry concentrations
- Design and demonstration of a commercial-sized reactor able to withstand extremely high temperatures and handle erosive and corrosive conditions
- A device for rapid steam heating of the pulp prior to hydrolysis
- Improved and demonstrated methods of solid separation and distribution
- Extent of prehydrolysis in steam heating of wood (hemicellulose removal)
- Extent of removal of lignin and development of improved lignin separation techniques (such as flocculation, aeration, setting, and filtering)
- Kinetics of hydrolysis of steam-treated wood at commercial rates
- Ability to concentrate neutralized hydrolyzate by reverse osmosis
- Combustion characteristics of lignin cake in a fluidized bed boiler
- Suitability of the xylose stream for anaerobic digestion.

## **BADGER ENGINEERS, INC.**

### **Economic Feasibility of an Acid-Based Wood-to-Ethanol Plan**

This report describes our progress to date on the economic feasibility study of an acid-based wood-to-ethanol process. The report covers the present status of the study, the design basis, choice of by-products, a brief process description, and design considerations in critical process areas.

#### **I. Present Status of the Study**

The design of the base-case (single hydrolysis reactor configuration) process is complete. Process design of the alternative case involving a two-stage hydrolysis reactor is under development. The estimating phase leading to a plant capital cost estimate (accuracy of  $\pm 25\%$ ) for the base-case process is in progress. The completed process design includes specification of utilities, offsites, and environmental requirements. Process flow sheets for the major process sections have been prepared.

#### **II. Design Basis**

The process design basis specifies the production of 25 million gal/yr of fuel-grade ethanol from mixed hardwood chips. By-products produced for sale are furfural (129 million lb/yr) and electricity (12 MW). The base-case design uses 1.18 million metric tons of whole tree chips per year as feedstock.

The design basis for the plant has been reduced from 50 million gal of ethanol per year in the original proposal. Wood chip availability in areas such as New York State, Michigan, New England, and the South Central states was investigated. It is apparent that although in some areas enough wood is available to feed a 50-million-gal process, the smaller plant has many more potential locations. The 25-million-gal scale also represents a more realistic size for wood chip collection and

handling for at least the first two or three commercial plants.

### **III. Production of By-Products**

#### **A. Furfural**

Furfural is produced as a major by-product (129 million lb/yr). There is good reason to believe that the present limited furfural market will expand greatly in response to a major source of furfural at a price of around 30¢/lb. Furfural by-product revenue would provide a significant benefit at least to early plants that produce fuel ethanol from renewable wood resources.

Furfural is produced when pentose sugars degrade. Yields are about 60% of theoretical in the reactor system under study. An additional advantage is that the reactor exit flash system affords an easy primary separation.

#### **B. Electricity**

In the present study, lignin, tar solids, and anaerobic digester gas are burned to provide process heat and electricity. This process produces 12 MW of electricity in excess of process requirements, which is sold under the provisions of PURPA legislation and provides a significant source of revenue. As an alternative, methane gas generated in the anaerobic digester can be used to make methanol as an additional liquid fuel. Preliminary calculations indicate that there would be enough excess methane to produce over 17 million gallons of methanol per year.

### **IV. Process Description**

#### **100—Wood Chip Handling**

There are no critical process areas in this section. The system is similar to that in operation at the Burlington Electric wood-fired McNeil power station in Vermont. A chip-washing system has been added to remove rocks and large tramp metal items.

## 200—Acid Hydrolysis

Critical process areas in this section are chip grinding to "through 20 mesh" particles, wood slurry mixing and pumping, and lignin/tar separation and washing.

We used conservative figures based on vendor discussions and published literature for the process design of the grinding step. However, the economic analysis would benefit greatly if more accurate figures could be obtained from vendor tests. These tests are being scheduled.

In this process, a 20% wood slurry is prepared and pumped to the hydrolysis reactor. Samples of a 20% consistency "through 20 mesh" slurry were made up at Badger's Weymouth Laboratory and sent to both mixing equipment and pump equipment manufacturers. Assessment of the samples and the mixing and pumping requirements indicated that the VRIECO conical mixer and the Sier-Bath twin screw pump are suitable for this area of the process.

Lignin residue separation and washing are to be carried out using liquid cyclones, which allow many stages of washing and separation for little capital cost. An increase in the number of solids washing stages reduces the required wash water, thus improving the process water balance. Hydrolyzate samples produced at reactor temperatures of 482° and 500°F (250° and 260°C) have been sent to two hydroclone equipment manufacturers. Their initial laboratory assessment indicates that this is a suitable application for hydroclones. Larger scale (20-50 gal) testing will be scheduled.

Hydrolyzate slurry samples have also been sent to manufacturers of dissolved air flotation equipment. Dissolved air flotation also appears to be suitable for primary separation of lignin residue.

## 300—Fermentation

There are no critical process areas in the fermentation section, which was designed by JGC based on their experience with their demonstration unit in Japan. A liter sample of con-

centrated hydrolyzate was prepared at Badger's Weymouth Laboratory and sent to JGC for testing. Results show that the hydrolyzate did not contain any substances toxic to their yeast strains, nor did it contain substances which attacked their polymer support. Fermentability was not observed in this preliminary work. A full program of yeast acclimatization is planned for the near future.

## 400—Ethanol Distillation

There are no critical process areas in this section. The design is based on two-stage distillation to produce the azeotropic mixture and on molecular sieve dehydration to produce anhydrous ethanol. Studies of multicomponent vapor/liquid equilibrium carried out by Badger on the beer produced from fermentation of wood hydrolyzates show that the presence of extraneous organics has two effects. First, the relative volatility of ethanol is significantly reduced, and second, the composition of the ethanol-water azeotrope is reduced to 80 mol %. Both these effects increase the capital and operating cost of the ethanol purification process.

## 500—Furfural Recovery

There are no critical process areas in this section. The design is based on data from a thesis by Smuk (University of Wisconsin 1960).

## 600—Offsites

There are no critical process areas in this section, which includes product, raw material, and intermediate storage tanks and transfer pumps.

## 700—Environment

The critical cost item in this section is the anaerobic digester. This processes approximately 1 million lb/day of chemical oxygen demand and produces approximately 256,000 lb/day of methane gas. This methane is burned to produce electricity; however, it could be utilized as a feed for a methanol production reformer.

## 800--Utilities

The critical process item in this section is the lignin boiler. It will burn the lignin/tar sludge residue to satisfy process energy needs. The

residue is discharged at 50% moisture and must be dried to provide efficient fuel. To do this a Flakt drying system, which uses boiler flue gas as the heat source, will be used. The sludge will be spray-dried prior to being fluidized into the burner.

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## Enzymatic Hydrolysis Processes

### STONE AND WEBSTER ENGINEERING CORPORATION

#### Economic Feasibility Study of an Enzyme-Based Ethanol Plant

##### I. Introduction and Definition

The objectives of the study are to determine the economic feasibility of a commercial-size facility for the conversion of wood to ethanol via enzymatic hydrolysis and to recommend areas of continued research and development that will allow transfer of this technology to the public sector. The study is divided into three tasks. Task I was the selection of a preferred processing sequence and the development of a basis of design for a base-case facility. Task II consists of engineering the base-case processing sequence into an integrated facility in sufficient detail to determine the capital and operating costs. Task III will establish the economics of the base case and evaluate the effect of technological changes, process modifications, or economic trade-offs on the base-case economics. One primary goal of Task III will be to determine the requirements for further research, development, and demonstration that will make a commercial facility an economically and technically attractive means of producing a fuel-grade product.

##### II. Definition of Geographic Plant Location

Consultations with researchers at the Hawaii Natural Energy Institute have resulted in the

selection of the island of Hawaii as a suitable site for the eucalyptus tree plantation and energy plant. Two specific sites--one near Hilo and the other near Honokaa--have been identified as suitable plant locations. These selected sites are based primarily on the following features:

- The availability of abandoned sugarcane refineries
- The close proximity (economic trucking distance) from tested eucalyptus plantations capable of supporting the feedstock requirement (1,307 tons/day) for a plant capacity up to 15 million gallons of ethanol per year.

##### III. Process Design Basis

We selected the base-case process after evaluating various processing options. The final selection was based primarily on the following criteria:

- Availability of process information
- Commercial viability of processing options
- Compatibility of processes segments in an integrated facility.

The integrated facility is designed using good engineering judgment that is optimistic in performance criteria but conservative in equipment adaptation. Process integration was performed considering energy consumption, water reuse, and capital cost advantages.

#### IV. Process Description

Wood chips from the tree plantation are washed and fed to sulfuric acid soaking vessels. The acid-saturated chips are then steam exploded to render the cellulose suitable for enzymatic hydrolysis. The exploded chips are then flash cooled to 308°F. The flash vapors generated are used to reboil the beer in the ethanol recovery system. A subsequent vacuum flash in a washed flash vessel reduces the temperature to about 160°F.

The pentose sugars are now removed in a countercurrent wash and sent to anaerobic digesters to produce a methane-rich fuel gas. The pentose wash is followed by caustic extraction of the lignins. The lignin stream is adjusted to a certain pH level and burned to provide process steam.

A portion of the delignified cellulose stream is sent to a fed-batch enzyme production section, where nutrients are added for production of the cellulose enzyme complex. The remaining feedstock is split into two streams. One goes to enzyme recovery to adsorb and recycle enzymes to the hydrolysis reactors. The remaining delignified cellulose stream is sent directly to hydrolysis for conversion to sugars.

The dilute glucose stream from hydrolysis is evaporated in a five-stage multi-effect evaporator to a 15 wt % glucose solution. The concentrated glucose is cooled and fermented in an immobilized yeast continuous fermentation system. The alcohol is recovered in a standard distillation system followed by an azeotropic distillation using cyclohexane as the azeotrope breaker.

In addition to standard offsites, the plant will contain a fluidized lignin boiler and an anaerobic digestion system to produce a methane-rich fuel gas.

#### V. Processing Options

A number of process options were considered to arrive at the sequence of process operations selected for the base-case design. The major areas of consideration are described here.

#### A. Pretreatment Alternatives

The various pretreatment alternatives evaluated for enzymatic hydrolysis include the following:

- Physical pretreatments such as grinding and milling
- Chemical pretreatments such as organosolv and alkali soak
- Steam explosion.

Steam explosion has been chosen as the most favorable alternative for the following reasons:

- Experimental data indicate that the cellulose fraction is made accessible to enzymatic hydrolysis.
- The lignin fraction is rendered soluble in dilute alkali wash for recovery.
- Hemicellulose is rendered almost completely soluble in hot water.
- Steam explosion is the least energy-intensive pretreatment option when 50% steam recovery is considered.

Physical pretreatments were excluded primarily because the lignin/hemicellulose/cellulose matrix is not sufficiently disrupted to expose the cellulose for efficient hydrolysis and also because of the high energy requirements (at least 2-3 times that of steam explosion).

Solvent pretreatments employing organic solvents for delignification were excluded due to the high energy requirements and capital cost of solvent recovery and the operating cost associated with solvent makeup. Alkali swelling pretreatment was ruled out due to the high cost of alkali and the potential problems of salt buildup in the process.

#### B. Lignin Recovery

The trade-off between removing lignin prior to or after hydrolysis has been evaluated. The preferred option is to remove the lignin prior

to hydrolysis since the presence of lignin during hydrolysis will result in the following problems:

- The rate of hydrolysis and the overall yield of glucose is expected to be reduced.
- From 8% to 12% of total soluble sugar is effectively lost in the lignin cake upon liquid/solid separation after hydrolysis.
- Residual lignin solids are expected to interfere with enzyme recovery by adsorbing active enzymes.
- Process equipment must be larger to accommodate the higher solids load.

### C. Solids Concentration Fed to Hydrolysis Reactors

The two extremes of enzymatic hydrolysis reactions are (1) high solids concentration (15% cellulose) with low yields of glucose (70% of theoretical), and (2) low solids concentration (5% cellulose) with high yields of glucose (90% of theoretical).

A study evaluating utility consumption versus plant yields was performed using these boundary limits. It was found that the preferred operating condition is 7% cellulose concentration at 84% of theoretical yield. The equipment design is based on 48-h residence time in the hydrolysis reactors. Task III trade-off studies will consider the effect of residence time on capital equipment, enzyme recovery, and plant sterilization requirements.

### D. Use of Hemicellulose Sugars

The five options considered for utilization of the hemicellulose sugars were:

- Methane generation
- Wet air oxidation
- Production of animal molasses
- Single-cell protein (SCP) production
- Furfural production.

Anaerobic digestion of the sugars to produce a methane-rich gas is the preferred base-case

option because it produces a fuel product needed at the process and in the state of Hawaii. Both methane and electrical power can be sold on the island. Wet air oxidation of the xylose stream could meet the plant energy needs by producing low-pressure steam that can be used by the process. However, this option is very capital intensive, and the low-pressure steam does not offer the flexibility of methane fuel.

Products other than fuels have limited markets in Hawaii. Animal molasses is derived cheaply from sugar cane and sells for about half the mainland market price. The production of torula yeast for SCP is also an energy- and capital-intensive process with a limited available market. Furfural has no market in Hawaii and a limited market on the mainland.

Other options that have been considered in developing this process include:

- Utilization of lignin
- Batch versus continuous ethanol fermentation
- Use of an  $H_2SO_4$  impregnation step
- Number of washing stages and quantity of wash water specified versus percentage of hemicellulose sugar and lignin recovered from the water and alkali wash, respectively.

## VI. Areas of Future Work

By analyzing engineering work performed to date we have identified three major areas for future work.

A. Pilot/equipment evaluation tests should be performed to either verify engineering feasibility or accurately quantify the assumptions made on the current SWEC basis of design. We suggest the following tasks:

- Demonstration of high concentration solids handling equipment and verification of separation equipment design parameters
- Specific requirements for the degree of plant sterility and the effect of sterility on plant operations

- Enzyme recovery studies to maximize recovery of filter paper activity.

B. The following near-term R&D areas can make a significant cost reduction or improve operability on the current SWEC basis of design:

- Elimination of the H<sub>2</sub>SO<sub>4</sub> impregnation step or replacement with a more practical alternative
- Increase in the specific activity of the enzyme complex
- Replacement of evaporator with reverse osmosis to concentrate the glucose feed to hydrolysis
- Use of an alternative to NaOH for the solubilization of lignin.

C. The following long-term R&D areas can result in a novel alternative enzymatic process or a more economical plant design:

- Hydrolysis under higher solids concentration (with achievement of high glucose yields)
- Development of a  $\beta$ -glucosidase enzyme that is not inhibited by glucose
- Development of a simultaneous hydrolysis/fermentation process with a temperature-tolerant yeast that can produce  $\beta$ -glucosidase
- Use of lignin as a higher value chemical
- Fermentation of the hemicellulose sugars to ethanol.

## CHEM SYSTEMS INC.

### Economic Feasibility Study of Enzymatic Hydrolysis-Based Ethanol Plant

Chem Systems is undertaking an economic feasibility analysis of an enzymatic hydrolysis process for converting wood chips to 25 million gal/yr of anhydrous ethanol. This process uses dilute acid hydrolysis as a pretreatment for enzymatic hydrolysis following the developmental work of the Thayer School of Engineering at Dartmouth College (U.S. Patent No. 4,237,226, Dec. 2, 1980). This work has demonstrated that high glucose yields can be obtained from prehydrolyzed wood in relatively short residence times of approximately 24 hours using the RUT C30 enzyme supplemented by a small amount of  $\beta$ -glucosidase. The necessary enzymes will be produced on-site using a portion of the pretreated feedstock as a carbon source.

Another novel aspect of our design is the recovery of by-product furfural and the possible utilization of the remaining xylan fraction in the feedstock for the production of additional ethanol. The revenues derived from the

sale of furfural will reduce the cost of production for the main product. Additional ethanol production without substantial increase in either capital investment or raw materials would also lower the ethanol selling price.

Since project initiation, Chem Systems has concentrated on site and feedstock selection, acquisition of relevant process information, and flow sheet development. The current status of this feasibility study is outlined in the following sections.

#### I. Site and Feedstock Selection

One task in this feasibility study is the selection of a Midwest site for the ethanol facility and the selection of the wood feedstock based on available forest types. This selection process involved screening ethanol consumption patterns, gasoline demand, applicable tax laws, wood resource acreage, and cost elements for delivered wood including both current and future trends.

After a review of the above issues, we determined that Michigan is the best location for the proposed ethanol plant. The plant would

be located in the northern part of Michigan's Lower Peninsula, which contains about 1.8 million acres of aspen forest as well as significant quantities of other suitable hardwoods. If it is assumed that 80% of the plant feedstock will be hardwoods from aspen forests and the remainder from maple/birch forests, the average feedstock composition will be 57% aspen, 20% maple, and 23% other hardwoods. On a moisture-free basis, the plant feed will contain approximately 47.2% cellulose, 31.3% hemicellulose, and 18.5% lignin. The hemicellulose can be further broken down to 7.9% hexosans, 16.5% pentosans, and 6.9% other materials.

The Bay City and Midland area of Gladwin County has been chosen as the location for this facility. This county contains 7% of the total state aspen forest land; by itself, this county could supply approximately half the required plant feedstock. When combined with five surrounding counties, the total aspen forest resource base would be sufficient for a facility of twice the capacity of this project. Furthermore, this site provides convenient access to product and by-product markets within the state.

The major by-products from this facility are carbon dioxide and furfural. It is anticipated that the carbon dioxide can be sold locally for use in beverages or enhanced oil recovery. Furfural is currently used primarily in the Midwest and Southwest to produce furfural alcohol, furan resins, and THF. Low-cost furfural would provide additional THF capacity as well as adipic acid and 1,4-butanediol manufacture. A wide variety of chemicals could be produced from these building blocks if the furfural could be priced at approximately half its current market value. This price is feasible when the furfural is produced as a by-product from an ethanol-from-wood complex.

## II. Process Description

The ethanol facility will be a completely integrated plant with the following processing sections:

- Feedstock preparation
- Dilute acid prehydrolysis
- Continuous enzyme hydrolysis
- Fed-batch enzyme production
- Neutralization
- Continuous fermentation
- Ethanol purification
- Carbon dioxide recovery
- Furfural recovery
- Steam generation from wood and wood wastes
- Waste treatment.

The offsites to be included in the design are storage facilities, a cooling water system, an electric power substation, process water and boiler feedwater pretreatment, buildings, piping, pollution control, general utilities, and site development. A brief process description follows.

Wood chips are transferred from the storage area via a conveyor to a disk refiner where particle size is reduced sufficiently for compatibility with prehydrolysis and hydrolysis equipment. The wood must be softened with steam before it is refined into fibers or particles. Steaming the wood also serves to pre-heat it for prehydrolysis. Thus, the refiner effluent is fed directly to the prehydrolysis reactor without intermediate pumping. The required acid and water solution is mixed with this solids stream at the entrance to the plug-flow prehydrolysis reactor. Within this reactor the hemicellulose fractions and amorphous cellulose are converted to their respective sugars. The reactor product is flashed to remove some water/furfural vapor and then proceeds to the first centrifuge. Solids from this centrifuge are repulped and centrifuged a second time. Hot liquids from the first centrifuge are sent to a polishing filter to remove the remaining cellulose. The liquid is then neutralized while hot and the precipitated calcium sulfate is filtered out. The cooler liquids

from the second centrifuge are sent to another polishing filter for final cellulose removal; the liquids are then combined with the liquids from the first neutralization, neutralized again, and filtered a final time. The solids from both centrifuges and both polishing filters are sent to enzyme hydrolysis, while the liquid stream proceeds to fermentation.

In enzyme hydrolysis, the solids are diluted with water and the pH is adjusted prior to the hydrolysis. Enzymes from enzyme production are added. The resultant sugar solution contains some unconverted cellulose, which is filtered and washed to remove the bulk of the unconverted cellulose, which is sent to heat generation. The filtrate is pumped to fermentation.

A portion of the prehydrolyzed wood is diverted to the enzyme production section where it is used as the carbon source. Two enzymes are produced in parallel trains--the RUT C30 cellulase enzyme (Trichoderma reesei) and QM329 cellobiase enzyme (Aspergillus phoenicius). The quantity of RUT C30 produced is approximately ten times the QM329 on a volume basis. Both are produced in fed-batch cycles that take approximately 12 days. In addition to cellulose, air and nutrients are introduced to the enzyme production tanks. The product from these tanks is sent to a cell centrifuge to remove most of the mycelium, which is then repulped, filtered, and washed. Part of the filter cake is recycled to enzyme production to maintain the initial cell concentration in the production tank. The remainder of the filter cake is recovered as single cell protein by-product or sent to heat generation. The centrifuge overflow and filtrate are sent to enzyme hydrolysis.

The pH levels of the sugar solution from prehydrolysis and the solubles from enzyme hydrolysis were adjusted and sent directly to continuous cascade fermentation. The continuous fermentation step includes yeast separation and recycle with a purge to by-product recovery or heat generation. Carbon dioxide produced during fermentation is also recovered for sale. The ethanol product from fermentation is sent to purification, which consists of a

beer still and azeotropic dehydration. The ethanol is concentrated to the water-ethanol azeotropic composition in the beer still, then dehydrated by distillation with an azeotroping agent to produce the anhydrous ethanol product. Aqueous stillage from alcohol purification is sent to steam generation or waste treatment.

In the furfural recovery section, the condensed flash vapors from prehydrolysis are concentrated to the furfural-water azeotropic composition in the azeotrope column and then dehydrated to produce an anhydrous furfural product in the dehydration column. A lights column is utilized to remove low boiling material from the decanted aqueous layer originating from the azeotrope column overheads.

Hydrolysis solids purge and possibly, stillage, waste yeast, and waste enzymes are neutralized with caustic and sent to a multieffect evaporator prior to being used as fuel to produce 600-psia steam for the steam boiler. Additional wood chips are fed to this steam boiler to bring the plant steam requirements into balance. Ash from the boiler is combined with the filter cake from neutralization and sent to waste disposal.

The condensate from various process concentration steps is combined with dilute aqueous waste stream and sent to a waste treatment pond. Waste treatment of stillage, if not handled in the steam boiler, may include anaerobic digestion. The yeast purge from fermentation and the cell purge from enzyme production may be collected for sale as by-product SCP.

### III. Process Optimization

A number of processing steps within the enzyme hydrolysis facility are being evaluated to improve overall plant efficiency and reliability. Among these are steam stripping of residual furfural from the prehydrolysis product, fermentation feed conditioning, make or buy options on the cellulase enzyme, alternatives for stillage utilization to generate steam, and alternative alcohol purification schemes.

For ethanol purification, three distillation schemes have been considered. The first is conventional cascade distillation that consists of a beer still and dehydration and stripping columns. This system includes extensive heat integration where the beer still overheads drive the two downstream column reboilers. Also, the dilute ethanol feed stream is preheated against the dehydration column condensers and the beer still bottoms. For a 2% ethanol feed stream, the overall energy requirement is estimated at 46,000 Btu/gal of anhydrous ethanol.

The second scheme is a multistage distillation process proposed by Hoechst, which includes three beer stills and two dehydration columns operating at different pressures. This multi-effect system is designed so that the condenser of the highest pressure column drives the next column reboiler in the series. The energy consumption for this system is estimated at 17,000 Btu/gal based on 2% ethanol in the feed. However, the capital investment is 11% higher than the conventional system since several high-pressure columns are required.

The third scheme, proposed by Dartmouth, is a combination distillation/extraction process. The process consists of a beer still using vapor recompression that produces a 61% ethanol stream overhead compared to 94% in conventional beer still overheads. This stream is fed to an extraction column that uses potassium acetate to break the ethanol-water azeotrope. The potassium acetate-water bottoms from the extraction column are sent to a spray dryer for recovery and recycle of the solids to the reflux ethanol stream. The overall energy requirement is 21,000 Btu/gal of anhydrous ethanol. Although the energy usage is higher than the Hoechst scheme, the capital investment is 6% lower than the conventional process.

We decided to retain the conventional cascade distillation within the base-case design but to develop the more speculative distillation/extraction scheme as a potential process improvement.

#### IV. Options under Consideration

Two alternatives for total xylose utilization have been considered for incorporation into the facility design:

- Intentional furfural production from stillage
- Xylose fermentation preceding the standard glucose fermentation step.

For furfural production, the stillage from the purification beer still can be utilized since it contains most of the unconverted xylose. Typically, it will contain approximately 1%-1.5% xylose. At reactor conditions of 220°C, 1% acid, and 75 s, yields of 0.49 grams of furfural per gram of xylose can be obtained. The cooled reactor effluent can be combined with condensed acid prehydrolysis flash vapors and concentrated in a furfural recovery system as previously described.

A process design was also developed to ferment the xylose stream obtained from enzyme hydrolysis to ethanol using the yeast Pachysolen tannophilus. The initial xylose sugar content of the feed is 1.1 wt % with a total sugar content of about 4.9 wt %. With a residence time of 24 hours at 37°C in a cascade fermentation system, the beer contains about 0.45 wt % ethanol based on an overall yield of 0.40 grams ethanol per gram of xylose consumed in the fermenter. The fermented beer leaving the fermentation system enters a yeast separation centrifuge where the yeast is concentrated to a stream containing about 20% yeast solids and the filtrate is sent to the glucose fermentation area where pH adjustments, nutrient additions, and solids filtration are completed prior to introduction into the glucose fermentation system. Ethanol produced from both xylose and glucose fermentation is concentrated in a single purification system following glucose fermentation, thus avoiding heating the dilute aqueous ethanol stream twice.

We simulated a base-case process design for a completely integrated ethanol facility based

on cellulosic feedstock using enzyme hydrolysis technology. Variations of this process design incorporating the two xylan utilization alternatives were also developed and sensitized to pertinent process parameters. For intentional furfural production, evaluations were performed at varying sugar concentration levels. For the xylose fermentation alternative, only one case was developed.

The resultant capital investment for the base case was \$79 million for 25 million gal/yr of ethanol, and the ethanol selling price was \$2.07/gal. With the furfural reactor included in the system, concentrating the sugar solution has a positive impact because heating requirements for this reactor feed are diminished. The best furfural production economics seem to occur at 10% glucose concentration. However, the required selling price to achieve a 10% DCF is \$2.35/gal, about 28¢/gal higher than the base case. Where xylose fermenta-

tion is employed, the ethanol capacity is increased by approximately 25%. This results in an ethanol selling price of \$1.89/gal, making xylose fermentation the most favorable alternative for xylose utilization in an enzyme hydrolysis cellulose-to-ethanol facility.

Thus, although xylose fermentation will not be included in the base-case facility design in this feasibility study because of its speculative nature, it will be included as a process option for future economic improvement.

## V. Conclusions

In the near term, our major effort will be on the enzyme production system design and the integrated evaluation of prehydrolysis and enzyme hydrolysis. The flow sheet will be finalized and equipment specification will begin.

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## NEW YORK STATE ENERGY RESEARCH AND DEVELOPMENT AUTHORITY AND ARTHUR D. LITTLE, INC.

### Economic Feasibility Study of an Enzymatic Hydrolysis-Based Ethanol Plant

#### I. Introduction

Our preliminary process (as discussed at the last contractors' meeting) was based on enzymatic saccharification of two feedstocks: selected debarked northern hardwoods and mixed paper scrap. The enzymes required to hydrolyze these materials were to be produced through submerged culturing of Trichoderma reesei using deproteinated cheese whey. The principal product from this process, fuel-grade ethanol, was to be produced using a conventional yeast fermentation of the hexose sugars. Depending on the outcome of our market evaluations, the process may incorporate the production of several by-products, including lig-

nin-rich hydrolysis residues as potential phenolic extenders, dried yeast cells and fungal mycelia as animal feed, or concentrated pentose-based syrups for use as molasses feed or conventional molasses extender.

After briefly reviewing several areas in Minnesota, Vermont, Wisconsin, Pennsylvania, and New York, a site in Jefferson County, New York was selected for further study. The site is located in a major dairy shed and forested region in the upstate area. A brief review of the availability of raw materials indicated that there was sufficient raw whey and lignocellulosic feedstock to support a plant capable of producing 7.5-10 million gallons of alcohol per year.

During the first three months of this project, we examined the availability and cost of raw materials, markets for the by-products, general site characteristics, and various process alternatives for enzyme production, cellulose pretreatment and hydrolysis, and product and by-product production and recovery.

## II. Preliminary Evaluation

### A. Site Assessment

Jefferson County, New York, was chosen as the focus of this work because of its high concentration of dairy product producers, its proximity to substantial hardwood fiber resources, and its proximity to major gasoline markets throughout the Northeast. The county maintains a population of nearly 100,000 persons employed in a mixture of agricultural and manufacturing industries. The county is bisected north to south by Interstate Highway 81, which connects with the New York State Thruway (Interstate 90) at Syracuse to the south and with Canadian highways to the north. Major rail lines (Conrail) connecting Watertown to Syracuse and Montreal also pass through the county.

Jefferson County, due to its soils and land use patterns, has the lowest percentage of forestland in northern New York State (44% forest cover); however, it is surrounded by counties rich in forestland and particularly in the northern hardwood forest required by the proposed plant. The major sources of waste paper to the region would be the larger urban areas of Buffalo, Rochester, Syracuse, and Albany. These areas are all easily accessible from the Watertown area by either interstate truck route or direct one-line rail carrier.

### B. Feedstock Availability

#### 1. Lignocellulosics

Several feedstocks were evaluated as sources of raw material for the proposed facility. These were:

- Mixed-grade paper wastes
- Debarked hardwood chips
- Roundwood (hardwood only)
- Manufacturing residues (planer shavings, sawdust, and by-product chips).

Our current estimates indicate a feedstock requirement of approximately 400 oven dried tons (ODT) per day (limited by cheese whey availability, as discussed later).

The availability of the various feedstocks is estimated as follows:

- Mixed-grade paper wastes - 250 ODT/day
- Debarked hardwood chips - 50 ODT/day
- Roundwood - 400 ODT/day
- Manufacturing residues - 100 ODT/day.

We have concluded that the current weighted delivered cost of wood-fiber feedstock is approximately \$40.00/ODT (including debarking and chipping for the roundwood fraction). This can be expected to increase by about 5% when the plant first begins to purchase wood. Average real increases in delivered costs for sawdust and planer shavings will be 0.5%/yr between 1984 and 1990, and 0.75%/yr between 1990 and 2000. Increases for clean hardwood chips will be 0.75%/yr between 1984 and 1990 and 1.0%/yr between 1990 and 2000.

Although waste paper is available in quantities up to 250 ODT/day, the projected cost for cellulose from this source is greater than that from the various hardwood sources. Therefore, we intend to use only hardwood feedstocks in the saccharification process.

#### 2. Cheese Whey

The availability of cheese whey and whey permeate was evaluated for a 150-mile radius around the Watertown area (excluding Canada). Based on a survey of the current major cheese processors, we concluded that 36-40 million lb of lactose could be supplied to a site in Watertown per year. Based on current shipping costs and current and projected whey processing and concentrating at the major cheese plants, we estimated the cost of lactose to a plant in Watertown to be between \$0.01 and \$0.023/lb. Additional whey (containing approximately 60 million lb of lactose) from eastern Ontario might be made available

to the Watertown site at a slightly higher cost (\$0.04-\$0.05/lb).

## C. Product Assessments

### 1. Alcohol

Our partially completed market analysis indicates that the product fuel alcohol can be easily moved into several markets including the entire northeast corridor and the states of Ohio, Indiana, Michigan, and Illinois. The net plant return projected for this product (plant gate price) has not been determined but will depend on state and federal subsidies as well as projected energy (crude oil and gasoline) prices over the life of this plant.

### 2. Lignin

Our initial assessment of the market for lignin-rich by-products has been somewhat inconclusive, primarily because of a lack of experience with the potential by-product and a lack of product specification. The current U.S. demand for lignin chemicals is about 500,000 tons/yr with an average price of \$150/ton (\$0.075/lb). The 30,000 tons of pure lignin that could be produced annually from this facility would represent 6% of the total U.S. market. High-value lignin chemicals represent 150,000 tons of the total lignin market; therefore, the output from the proposed plant would be 20% of this market. Based on this information, we have decided to investigate the options of (1) recovering a lignin-rich product and (2) dewatering the hydrolyzate residue and burning the lignocellulosic material in the boiler. We will use the latter as our base case and evaluate the cost of recovery versus some projected net return for this material as one of our economic sensitivity analyses.

### 3. Pentose Molasses

Our assessment of the potential market for C<sub>5</sub> molasses in New York State has shown that the

product has value as a hexose molasses extender, but its bitter flavor will make it difficult, if not impossible, to sell. As a result, we have decided to incorporate an anaerobic digestion process to remove this material from the stillage stream leaving the plant. The methane generated in this process will be used as supplemental fuel for the boiler.

### 4. Yeast and Fungal Protein

Our present plans are to incorporate provisions for recovery, mixing, and drying of the T. reesei residues from enzyme production and the excess yeast from fermentation. Our assessment of the animal feed protein market is based on a survey of the feed mills near Watertown and Jefferson County. The mills indicate a willingness to accept the small quantities (25-35 tons/day) of this material at a price equivalent to its protein content based on the prevailing market price for soybean meal. Current value for this material (FOB Buffalo) adjusted for a 35%-40% protein content is \$191.00/ton.

## III. Process Description

### A. Alternatives Considered

#### 1. Enzyme Production

Enzyme production is based on continuous fermentation of whey permeate using T. reesei MCG80. A 5% lactose concentration and 36-hour holding time will be used, producing an enzyme broth containing 6 IU/mL.

#### 2. Lignocellulosic Feedstock

As previously mentioned, we have concluded that only northern hardwoods (except oak) are acceptable feedstocks for enzyme conversion processes. Debarking is highly desirable and perhaps necessary, since the only available data for saccharification is based on debarked

material. We have, therefore, provided for roundwood receiving, debarking, and shipping prior to feedstock pretreatment. Manufacturing residues will be accepted as available. A sensitivity analysis will consider the use of whole tree chips, which are projected to be available at \$35/ODT.

### **3. Pretreatment**

Explosive decompression using steam (Iotech process) will be used to pretreat the wood. Based on meetings with Iotech personnel, we have established the material balance, energy requirements, and costs for this operation. The exploded wood will be washed to remove toxic by-products prior to hydrolysis.

### **4. Hydrolysis**

A batch hydrolysis using 20 IU of enzyme per gram of substrate and achieving 66%-70% total carbohydrate conversion in 48-72 hours will be used. Actual holding times will be finalized after additional economic evaluation. Fed-batch operation to attain a high solids-to-water ratio will be employed.

### **5. Water Removal**

Water removal to concentrate the hydrolyzate before fermentation will probably be incorporated. Multiple-effect, low-temperature evaporation, reverse osmosis, and one other technique will be compared to the alternative of processing low-concentration solutions. This concentration must take place without initializing adverse protein/sugar (Maillard) reactions, which have been shown to render the sugar solutions unfermentable by conventional yeasts.

### **6. Fermentation**

Continuous fermentation with yeast recycle will be used for production of alcohol.

### **7. Alcohol Recovery**

Conventional distillation will be used to recover the alcohol from the fermentation beer. A fuel-grade anhydrous product will be produced.

### **8. Lignin Residues**

Two options for lignin processing will be carried forward (see II.C.2). Final selection of the lignin-rich byproduct recovery process has not yet been made.

### **9. Stillage Handling**

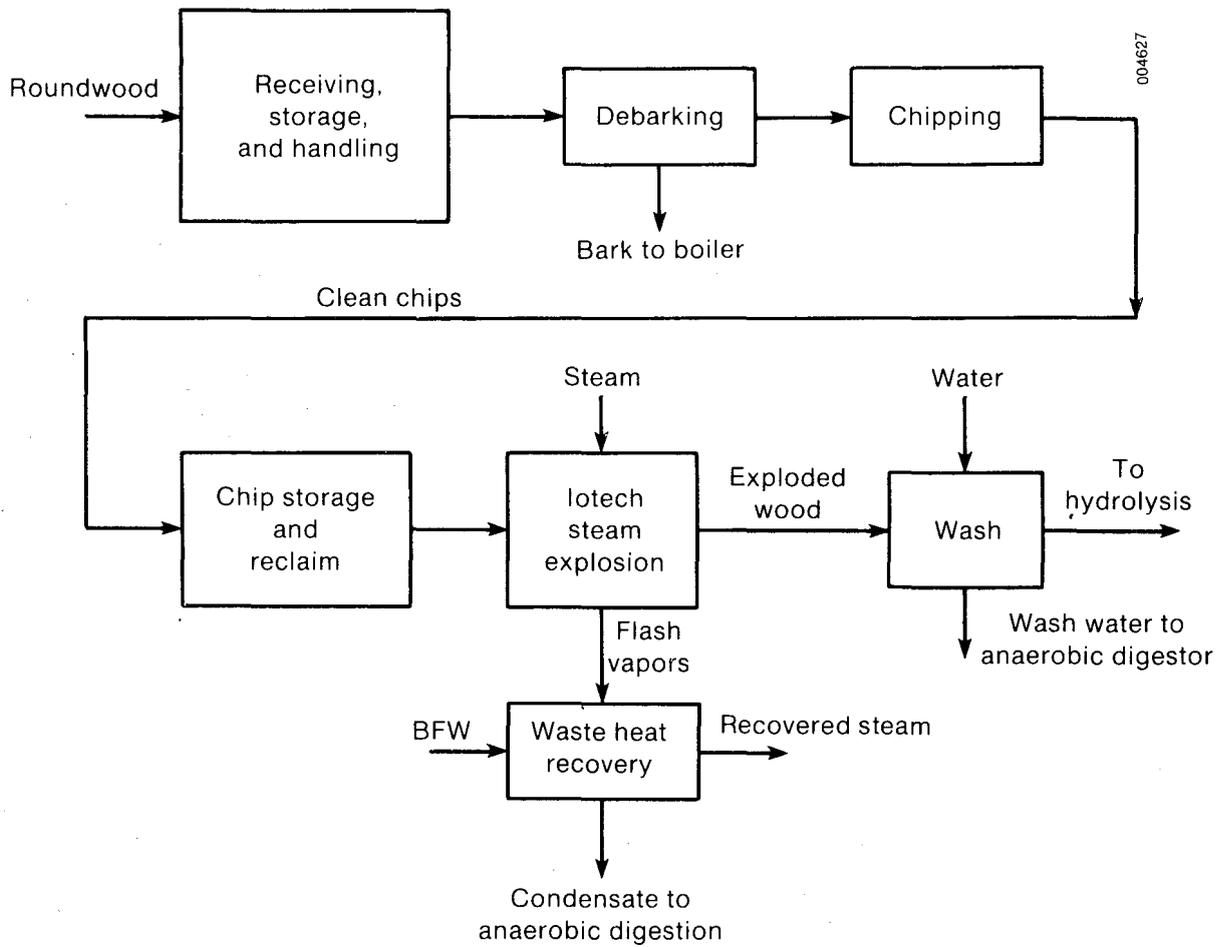
Stillage will be combined with the process wash waters and the condensate from the explosive decompression pretreatment and anaerobically digested. An Anamet system and a Paques system will be evaluated.

## **IV. Integrated Process Concept**

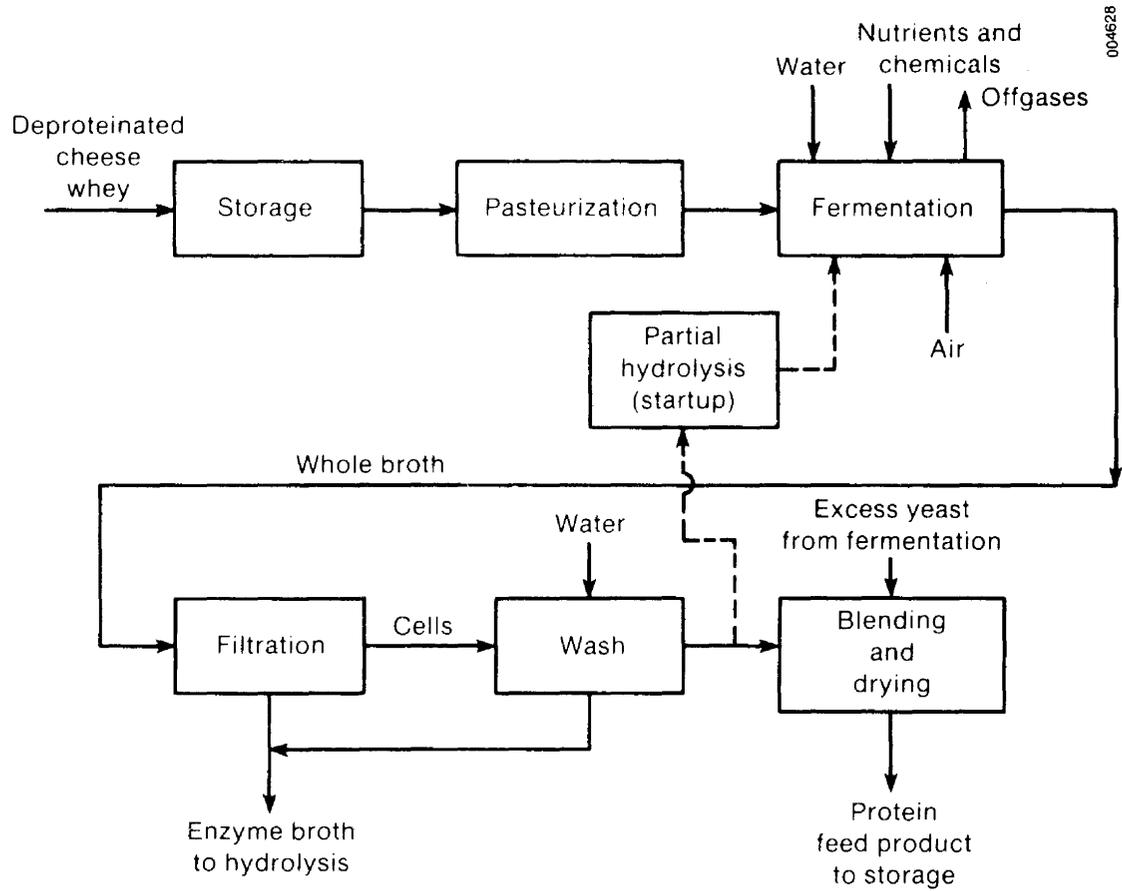
Figures 1-4 show the block flow diagrams for the current version of the integrated process. Table 1 summarizes the various inputs and outputs from the process as it is currently configured.

## **V. Future Plans**

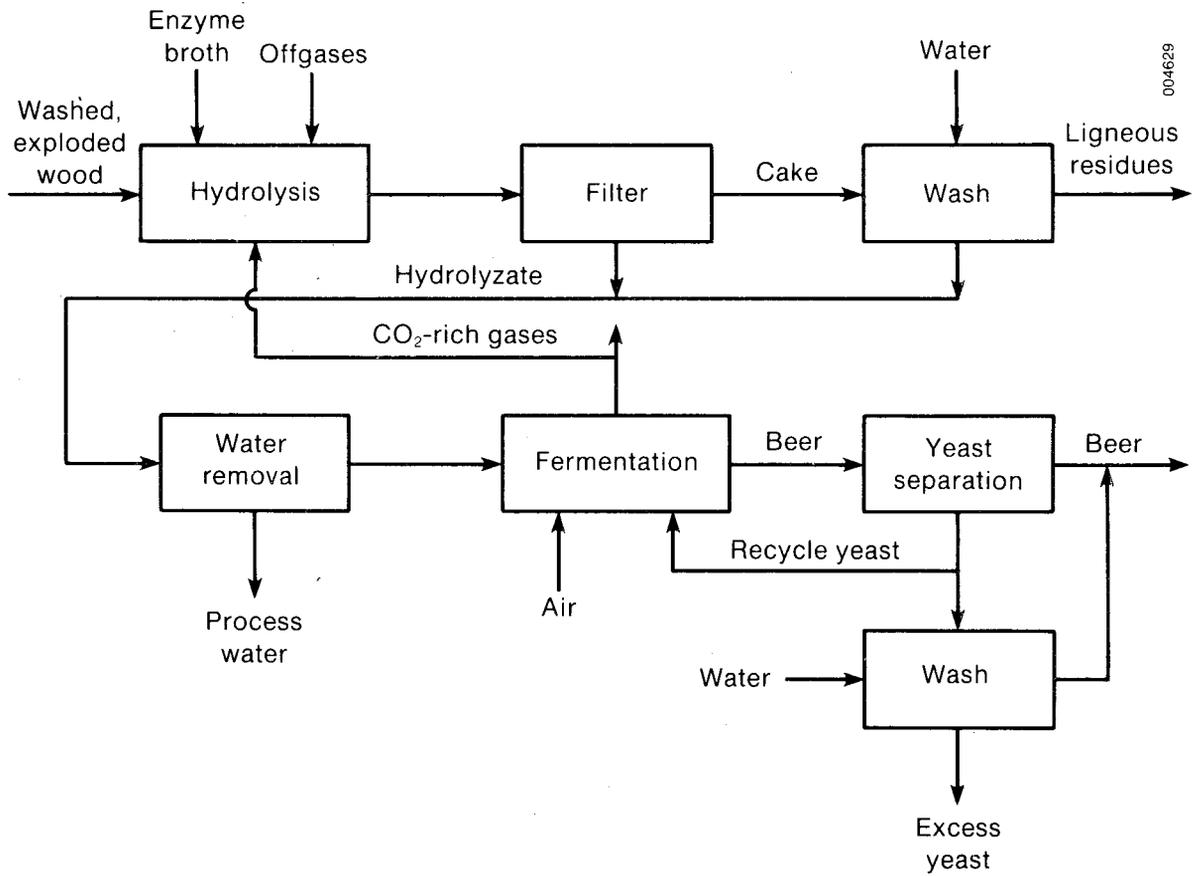
Our plan for the next three months is focused on process design, including finalization of process flow diagrams, equipment selection, and gathering cost data. We will also finish the alcohol market analysis and adapt our cash flow model for use in the economic analyses.



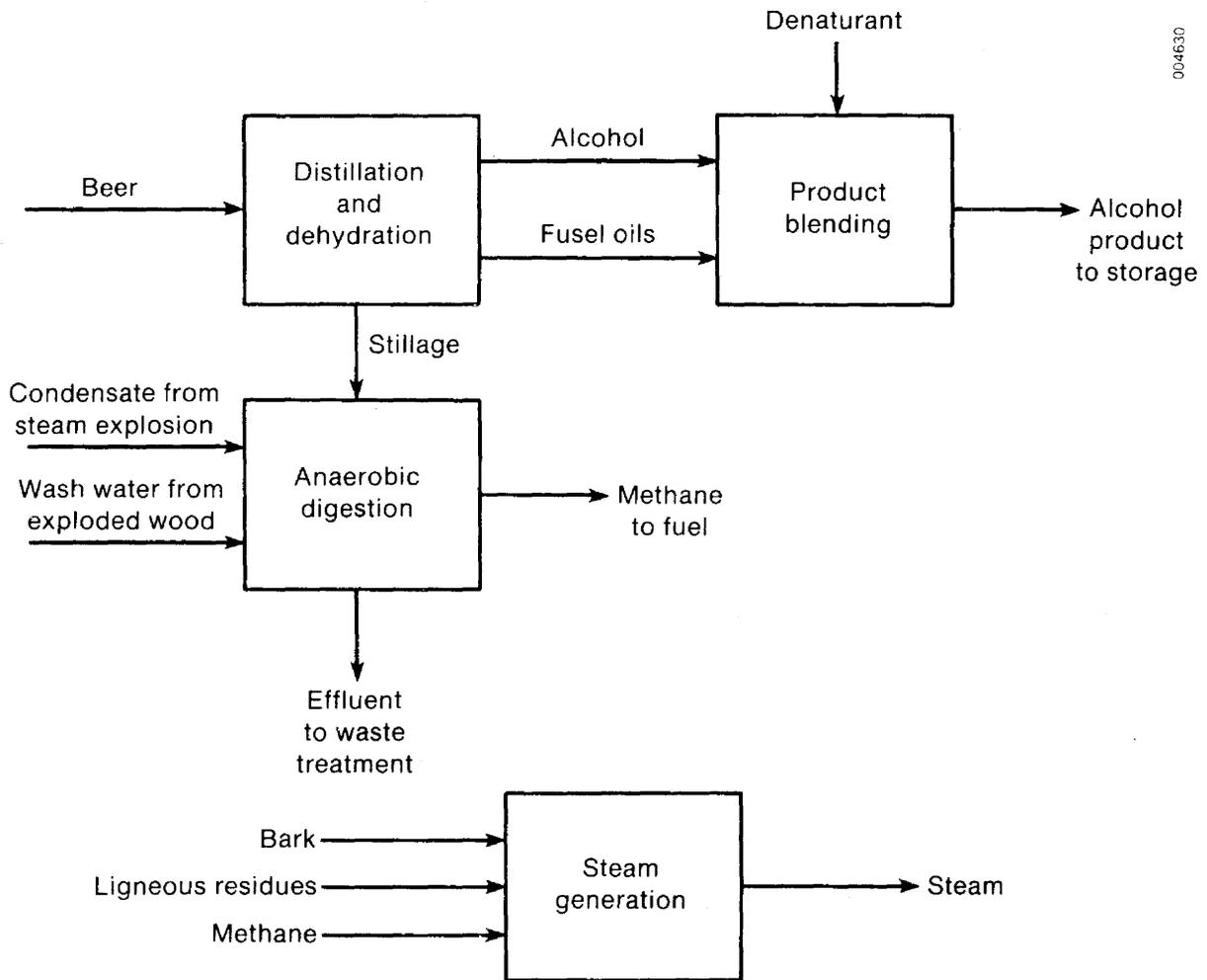
**Figure 1. Wood Receiving and Pretreatment**



**Figure 2. Enzyme Production**



**Figure 3. Hydrolysis and Fermentation**



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**Figure 4. Product Recovery/Steam Generation**

Table 1. Major Process Inputs and Outputs

	lb/h	tons/yr
<u>Onsites</u>		
Inputs		
Roundwood, dry basis	36,598	145,000
Deproteinized cheese whey, pure lactose basis	5,051	20,000
Denaturant	295	1,170
Total	41,944	
Output		
Bark, dry basis	3,660	14,500
Protein feed product, dry basis	2,507	9,900
Ligneous residues, dry basis	11,986	47,500
CO <sub>2</sub> from alcohol fermentation, pure basis	5,895	23,300
Denatured alcohol product	6,534	25,900
Stillage, dry basis (organic solids)	6,993	27,700
Total	37,575	
<u>Steam Generation</u>		
Inputs		
Bark, dry basis	3,660	
Ligneous residues, dry basis	11,986	
Methane, pure basis	1,680	
Output		
Steam, 600 psi	110,000	

# SERI Research Review

## THE SERI PROCESS DEVELOPMENT GROUP

The SERI process development and fermentation groups (within the Biotechnology Branch) support the DOE Alcohol Fuels Program. The fermentation group is performing research to improve the technology for simultaneous saccharification and fermentation, fermentation, and anaerobic digestion of five-carbon sugars, and pretreatment of cellulose for enzymatic hydrolysis. The process development group's activities include process design, process evaluation and testing, engineering research, and integrated process experiments. In FY 1984, the group's primary emphasis is acid hydrolysis. This report describes the process development group, their activities in the last year, and some future efforts.

The process development group consists of chemical and mechanical engineers and technicians. The group is housed in the SERI Field Test Laboratory Building (FTLB), and the process experiments will be carried out in the FTLB Annex, which is scheduled to be completed in October 1984. The Annex is a 4000-ft<sup>2</sup> high-bay building that is outfitted for chemical process experiments. It is designed to Class I, Division I standards so that high-temperature solvents may be used; it is supplied with high-pressure steam and other process utilities.

The group's efforts fit into four categories: process research, process evaluation and testing, engineering research, and integrated experiments. Process research includes the design of new processes and the evaluation of processes suggested internally or by current or potential SERI subcontractors. These evaluations are design studies in which a process flow sheet is developed, capital and production costs are estimated, and the resulting processes are evaluated to determine important research issues and economic potential.

Process evaluation and testing includes bench- and engineering-scale studies on processes identified by the process research task. The activities range from bench-scale testing to determine process parameters to design, construction, operation, and testing of integrated hydrolysis processes at the minimum scale needed to assess process viability. Engineering research involves the testing of commercially available equipment for use in an alcohol fuel process. Integrated experiments are a logical part of a successful research program, and involve running the process at the minimum scale required to assess its potential and determine research needed to make the process operational.

Engineering research to date has focused on the evaluation of the major acid hydrolysis processes and the design of new acid hydrolysis processes. The first study was a parametric analysis of the high-temperature dilute-acid plug-flow reactor. The second study evaluated the three major sulfuric acid hydrolysis processes: percolation, plug flow, and low-temperature concentrated acid. A recently completed study evaluated concentrated halogen acid processes (liquid- and gas-phase HCl processes and a liquid-phase HF system). Another recently completed study analyzed the cheese whey fermentation process developed by the SERI fermentation group. The progressing batch hydrolysis process, a new process that combines the mechanical simplicity of the percolation reactor with the yield and product concentration advantages of counter-current reactor was invented as a result of process research efforts.

In the next year several new studies will be conducted. One will address wood fractionation processes that produce a chemical-grade lignin as well as a sugar product. The other analyses will support work being carried out by the fermentation group: anaerobic digestion of five-carbon sugars, and an evaluation of acid pretreatments for enzymatic hydrolysis.

As a result of the analyses, two processes have been selected for evaluation and testing: the plug-flow reactor and the progressing batch reactor. The plug-flow reactor was originally studied by Dartmouth and NYU, and is currently the subject of feasibility studies by Badger and Stone & Webster. An engineering-scale reactor operating at a flow rate of 2000 lb of slurry per hour is being designed and fabricated at SERI. This reactor will use the smallest commercial-scale hardware available, use a realistic size feedstock (20-mesh sawdust), and demonstrate the engineering feasibility of this concept. Currently, experimentation with a batch hydrolysis reactor, which operates at very high solids loadings, supports the research. This experiment helps verify reaction conditions and operating parameters for the high solids operation of the plug-flow reactor.

Progressing batch hydrolysis, the second major experimental effort, will include several percolation-type reactors in series with counter-current flow of solids and liquids. This project has both experimental and analytical components. Its objective is to determine the yields and sugar concentrations that can be achieved by this approach.

Engineering research will identify suitable hardware for key sections of a hydrolysis plant. The main areas of interest, particularly in the plug-flow process, are high solids pumping, hydrolyzate recovery, and size reduction. The size and cost of the hydrolysis section and much of the downstream equipment are proportional to the amount of water used to slurry the reaction feed. The recovery of the product sugars from the residual solids is also an important and expensive step, especially when high solids concentrations are fed to the reactor. Reduction of wood chips to a sawdust-like material can be a very expensive process.

Currently, the fermentation group is monitoring the progress of the acid hydrolysis feasibility studies. If the results of these or other similar studies are promising, an integrated process experiment may be conducted.

In the next year, we expect that the two large acid hydrolysis experiments will be under way. In addition to the new studies envisioned, a closer experimental collaboration is expected with the fermentation group in the area of enzymatic hydrolysis.

## Supplemental Information

### Announcement of Biotechnology for Fuels and Chemicals Symposium

The seventh Oak Ridge National Laboratory (ORNL)/Department of Energy (DOE) symposium on "Biotechnology for Fuels and Chemicals," bringing together specialists concerned with the technical application of biological systems and processes to production of energy, fuels, and chemicals, will be held May 14-17, 1985, in Gatlinburg, Tenn.

ORNL is operated by Martin Marietta Energy Systems, Inc., for DOE.

Charles D. Scott, a research fellow in ORNL's Chemical Technology Division, is symposium chairman. Five half-day sessions, an evening poster session, and special-topic discussions groups will cover the following areas:

- Thermal and Chemical Processing--Advances in either preprocessing or conversion of biomass to other energy forms, fuels, or chemical feedstocks
- Applied Microbial Research--Fundamental work in recombinant DNA techniques, applied microbiology and applied biochemistry, with emphasis on emerging concepts that have a potentially significant impact on bioprocessing
- Innovative Bioconversion Systems--Advanced concepts, elucidation of new effects, definition of controlling phenomena, and optimization of bioconversion rates

- Bioprocess Engineering--Engineering and economic aspects including bioconversion of thermal/chemical processing of biomass; flowsheets that include a major bioprocessing step; and pilot-scale projects, demonstration systems, or new commercial processes
- Future Trends in Biotechnology--Experts in the field will give their views of exciting new areas of research and expected technological breakthroughs.

Besides DOE, symposium sponsors include E. I. duPont de Nemours & Company, Inc., and A. E. Staley Manufacturing Company.

Persons from educational institutions, industry, government agencies and laboratories who are involved in research and development in these areas are invited to participate.

Abstracts of 150 to 200 words should be submitted by December 15, 1984. Selection of papers will be made by January 15, 1985, and authors notified by February 1. Papers should be prepared in a form suitable for publication prior to the symposium. Proceedings will be published as a supplement to the journal Biotechnology and Bioengineering.

Abstracts and other correspondence should be addressed to Charles D. Scott, Chemical Technology Division, Oak Ridge National Laboratory, P. O. Box X, Oak Ridge, TN 37831, Telephone 615/574-6775, FTS 624-6775.

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