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Develop and Demonstrate the Cellulose to Ethanol Process

September 17, 1980 - March 17, 1982

Subcontract No. [redacted]

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NOTICE

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EXECUTIVE SUMMARY

The Biomass Research Center at the University of Arkansas was contracted by the Solar Energy Research Institute to "Develop and Demonstrate the Cellulose to Ethanol Process". The purpose of the contract was to accelerate site selection, site specific engineering, and research and development leading to the determination of the feasibility of economically operating a cellulose to ethanol commercial scale plant.

The areas of work were divided into sections (Tasks) for reporting purposes, but it should be kept in mind that the sections overlap considerably and are, therefore, cross-referenced frequently. The following summary is a condensation of the significant findings and conclusions of the work accomplished in satisfaction of this contract.

TASK I: Summary of One Ton Per Day Pilot Plant

Interest in cellulose as a renewable energy resource has increased as the price of petroleum products continues to rise. As a result of this increased interest, the biochemical conversion of cellulose to ethanol was scaled up from a laboratory model to a pilot facility capable of processing one ton of cellulosic feedstock per day. During the 3½ years of operation, the feasibility of production of cellulolytic enzymes from the organims *Trichoderma reesei* was demonstrated on a substantially larger scale than has been previously reported. The subsequent use of cellulases in simultaneous saccharification fermentation (SSF) was accomplished using purified forms of cellulose as well as potential industrial feedstocks (example: pulp mill wastes and municipal solid waste). Enzyme production and SSF were studied in batch, semi-continuous, and continuous modes. The unfiltered product after SSF was fed into a baffle tray stripping column where ethanol recovery was accomplished.

After successful pilot scale production of ethanol from cellulosic material, it was concluded that technical feasibility had been proven and further scale-up of the process to a 50 TPD facility is the next logical step in the commercial development of this technology.

TASK II: Assessment of Feedstocks

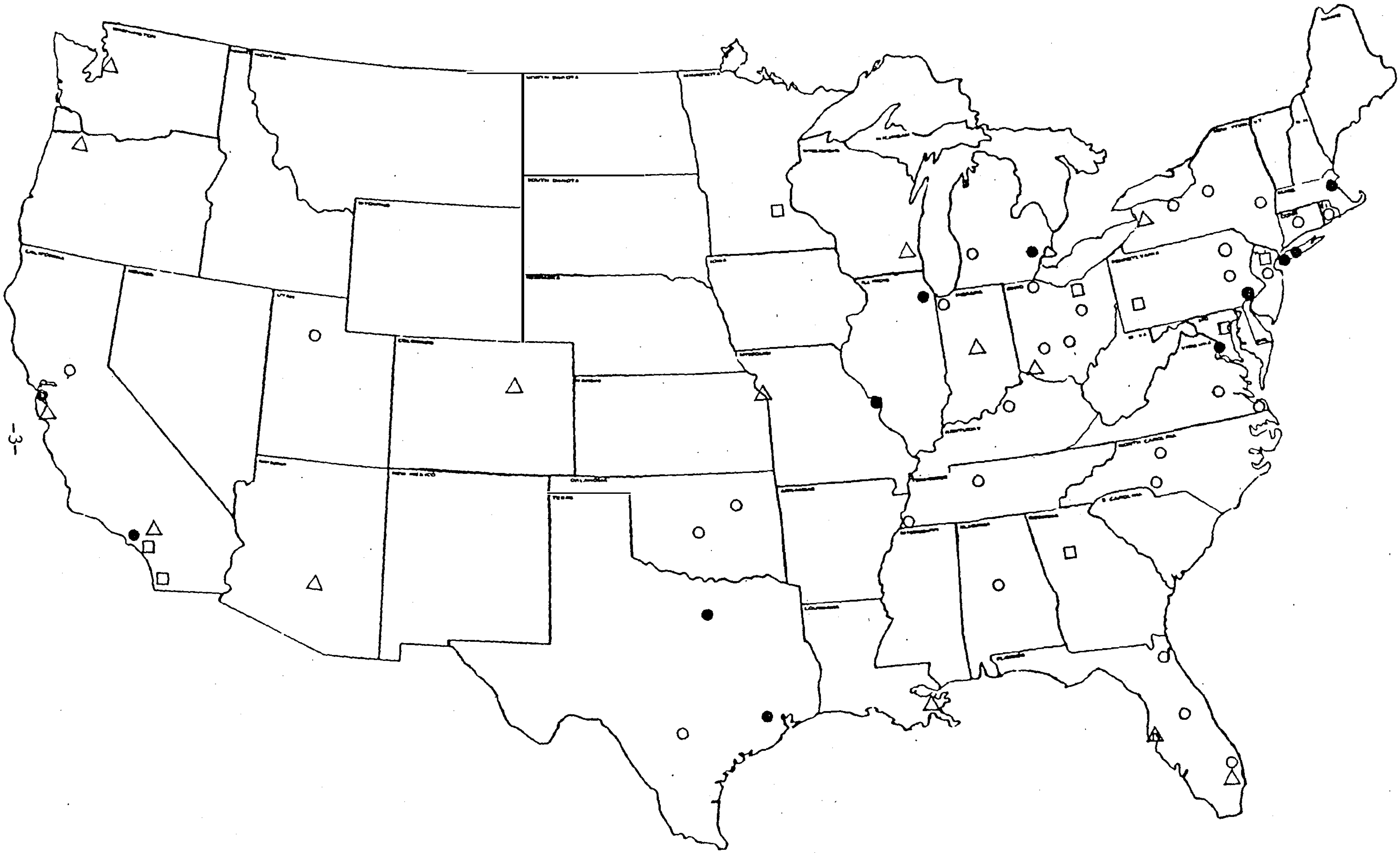
Four major categories of waste lignocellulosics have been considered as potential process feedstocks: municipal solid wastes (MSW), pulp mill wastes (PMW), agricultural wastes (AW), and saw mill wastes (SMW). In the U.S., MSW is produced at a rate of 3.5 pounds/person/day resulting in large quantities available in a relatively small geographic area. Following classification to refuse derived fuel (RDF), the cellulose content may be expected to range from 40-60% of dry weight. The MSW performs exceptionally well in cellulase and ethanol production with little pretreatment.

The PMW is located in areas of pulp production and is independent of population density. The cellulose content may be expected to range from 50-75% of dry weight in this substrate. The PMW is unacceptable as a cellulase production substrate, but performs excellently in ethanol production.

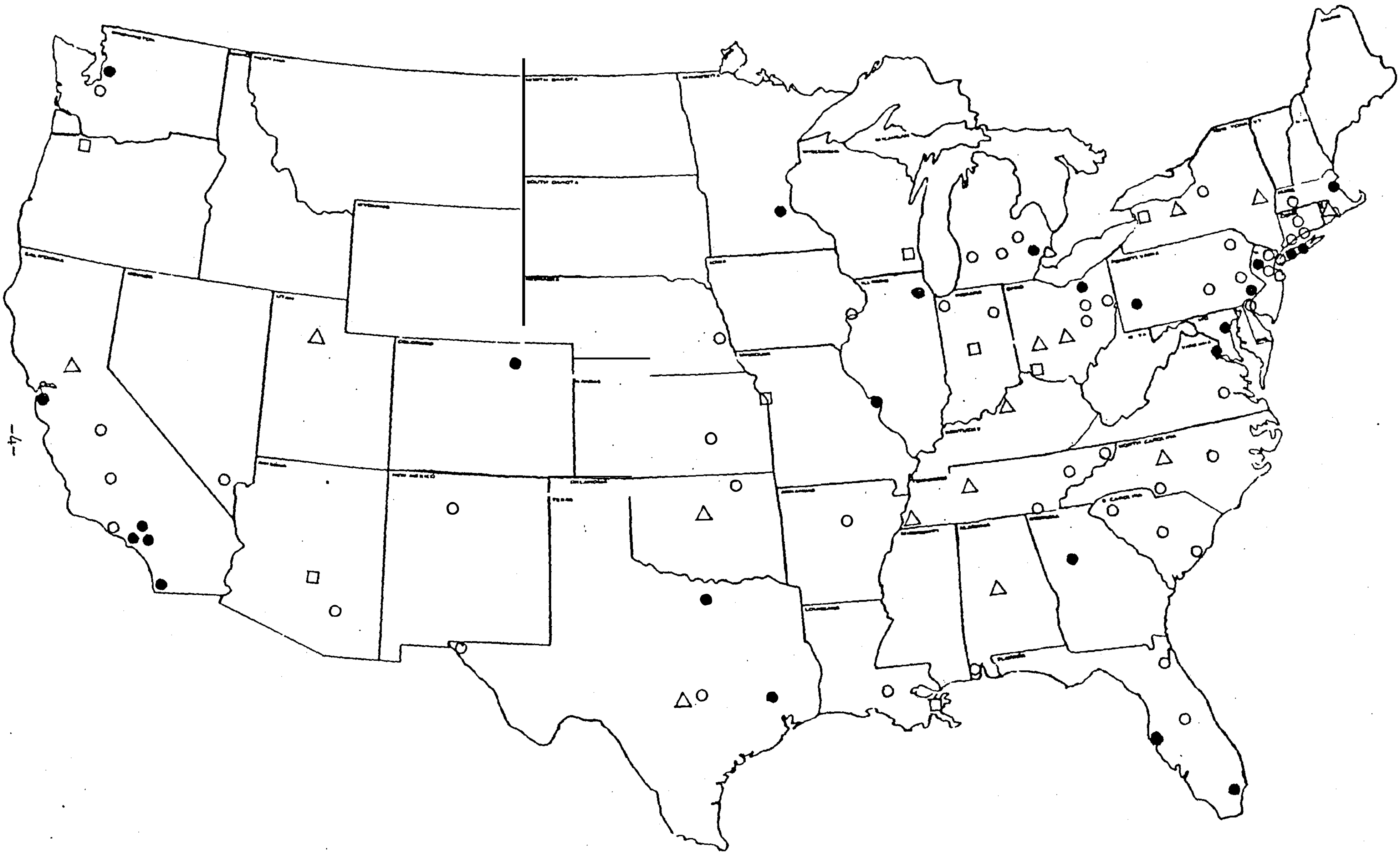
The AW are produced seasonally and are realtively independent of population density. The cellulose content may be expected to range from 35-45% of dry weight. This category contains lignocellulosics which vary widely in composition. Cellulase and ethanol production performance range from very good to very poor.

The SMW are located in areas of timber production and are independent of population density. Cellulose content may be expected to range from

Availability of MSW at 50% RDF produced 3.5 pounds/person/day (o = 500 - 1000 T/D, △ = 1000 - 1500 T/D, □ = 1500 - 2000 T/D, ● = 2000+ T/D).



Availability of MSW at 75% RDF produced 3.5 pounds/person/day (o = 500 - 1000 T/D, Δ = 1000 - 1500 T/D, □ = 1500 - 2000 T/D, ● = 2000+ T/D).



Availability of PMW estimated at 10% of total pulp capacity (o = 50 - 200 T/D, △ = 200 - 350 T/D, □ = 350 - 500 T/D, ● = 500+ T/D).

Availability of AW in T/Y by state exclusive of cotton gin trash and bagasse.

45-55% dry weight. This category is unacceptable in both cellulase and ethanol production without extensive pretreatment.

Depending on location, the feedstock value will vary, but generally can be predicted by consideration of a combination of contributing factors including competition, type of waste, environmental impact, and transportation. Based on these factors, MSW has the greatest process value due to its relatively high cellulose content and high concentration in small geographic areas. It is produced on a constant basis and has excellent performance characteristics in both cellulase and ethanol production. Municipal solid waste is first in relative process value followed by PMW, AW, and SMW. While actual costs involve site specific analysis, advantages in substrate purchase have been explored with respect to cellulose:solids ratio.

While an ideal commercial site is dependent on feedstock cellulose concentration, feedstock geographic concentration, transportation systems, and cooperation of the general populace, many potential commercial sites have been identified in the continental U.S. based on projected availabilities of MSW and/or PMW with seasonal supplements of AW.

TASK III: 50 Ton Per Day Demonstration Plant

Bench-level research and operation of a 1 T/D pilot plant cellulose-to-ethanol facility has allowed preparation of a detailed process engineering design study for a 50 T/D cellulose-to-ethanol plant. The feedstock proposed for this motor fuel grade (MFG) alcohol facility would be 37.5 ODT/D of municipal solid waste (light fraction) and 12.5 ODT/D pulp mill waste. The facility would produce 2,360 gal/day of 199^{o} proof MFG alcohol. This assumes an overall cellulose-to-ethanol conversion of 45.8%, or 51%

conversion during simultaneous saccharification-fermentation. The theoretical overall conversion expected is 72.8%, or 81% conversion during SSF, in which case the demonstration facility can produce 3,750 gal/day of MFG alcohol.

Pine Bluff, Arkansas Site Study Review

The purpose of this report is to present the investment and operating costs estimate for the proposed 50 T/D cellulose alcohol plant to be located at Pine Bluff, Arkansas.

Raphael Katzen Associates International, Inc. (RKAII) was authorized by the Biomass Research Center to review and update the process design for a 50 ODT/D demonstration plant to produce Motor Fuel Grade (MFG) alcohol from a feedstock consisting of:

Municipal solid waste - light fraction (MSW)	37.5 ODT/D
Pulp mill waste (PMW)	12.5 ODT/D
Total	50.0 ODT/D

The demonstration plant, as outlined in Block Diagram WH 382-10, will produce 2,360 gallons/day (gpd) of 199° proof MFG alcohol. This assumes a 51% conversion of cellulose to alcohol during simultaneous saccharification and fermentation (SSF), or an overall conversion efficiency of 45.8%. If the theoretical conversion of 81% during SSF is achieved (72.8% overall efficiency), the demonstration facility will produce 3,750 gpd of MFG alcohol. The demonstration plant design is detailed in the WH 382 Report: Cellulose Alcohol Demonstration Plant Study.

As originally conceived, the demonstration plant was to be located next to an existing pulp/paper mill, and the mill would furnish PMW and utilities for the plant. The Pine Bluff site is located adjacent to International Paper Company's (IPCo) Pine Bluff Paper Mill, however the mill is only capable of providing PMW and waste treatment/disposal services; steam, process and cooling water, electric power, compressed air, and natural gas must be supplied by the demonstration plant.

Investment Estimate

The investment estimate for construction of the demonstration plant at the Pine Bluff site is $19,950,000. This estimate includes both battery limits facilities and the necessary utilities to operate the plant. The battery limits estimate was developed by RKAII and is detailed in the WH 382 report. The estimate for utilities and support facilities specifically required at the Pine Bluff site was developed by Riddick Engineering Corporation (REC) under contract to RKAII, and is detailed in the REC Report: Cellulose Alcohol Demonstration Plant Site Study. Capital costs, adjusted to July, 1982, dollars to coincide with the midpoint of the engineering and construction expense period (October, 1981, through March, 1983), are summarized below:

	Installed Investment July, 1982
Battery Limits - RKAII	$ 18,560,000
Utilities & Offsites - REC	1,390,000
Total Fixed Investment	$ 19,950,000
Estimated Potential Modifications	4,000,000
Total Actual & Potential Investment	$ 23,950,000

These costs are presented in more detail in Table 1-1 and in Section 4.0 of this report. The $4MM for estimated potential modifications is included to provide a developmental budget for any changes in process design or equipment which result from operating experience.

Operating Costs

The utilities required to sustain plant operation are summarized below:

	Normal	Peak
Steam, lb/hr	7,907	20,000
Cooling Water @ 85°F nominal, gpm	1,539	--
Process Water (including boiler feed), gpm	14	348
Electric Power, kw	3,737	--

A packaged steam boiler burning natural gas is required to provide 150 psig process steam. Firing with natural gas requires the installation of a gas supply line from the main transmission line or from the distribution line serving the IPCo mill. Cooling and process water will be supplied from a 175 foot well to be dug at the plant site. Electric power supply requires the installation of a 3,000 KVA substation and related switchgear.

In addition, the waste streams generated must be treated or disposed. IPCo has tentatively agreed to handle the process and sanitary sewer wastes as long as the flow and BOD load remain relatively constant, and to burn the heads draw from distillation in their waste boiler. IPCo has also agreed to handle the concentrated stillage solids and waste molasses from evaporation in their existing landfill operation, and have also expressed interest in conducting trials to incinerate these wates in their waste boiler. The demonstration plant waste streams are:

Material	Quantity/Day	Dispostition
Soluble Solids (liquid) @ 60% solids	19 tons wet	landfill
Insoluble Solids (cake) @ 30% solids	91 tons wet	landfill
Dilute Waste (BOD-5 = 2,000 ppm)	138 gallons	sewer
Heads Draw (86% ethanol, 10% acetaldehyde)	24 gallons	incinerate

The personnel requirements of the plant are estimated at having 69 individuals placed in 28 different positions, 19 of which involve rotating shift work for 24 hour, 7 day operation. This estimate also includes a maintenance group of 12 people.

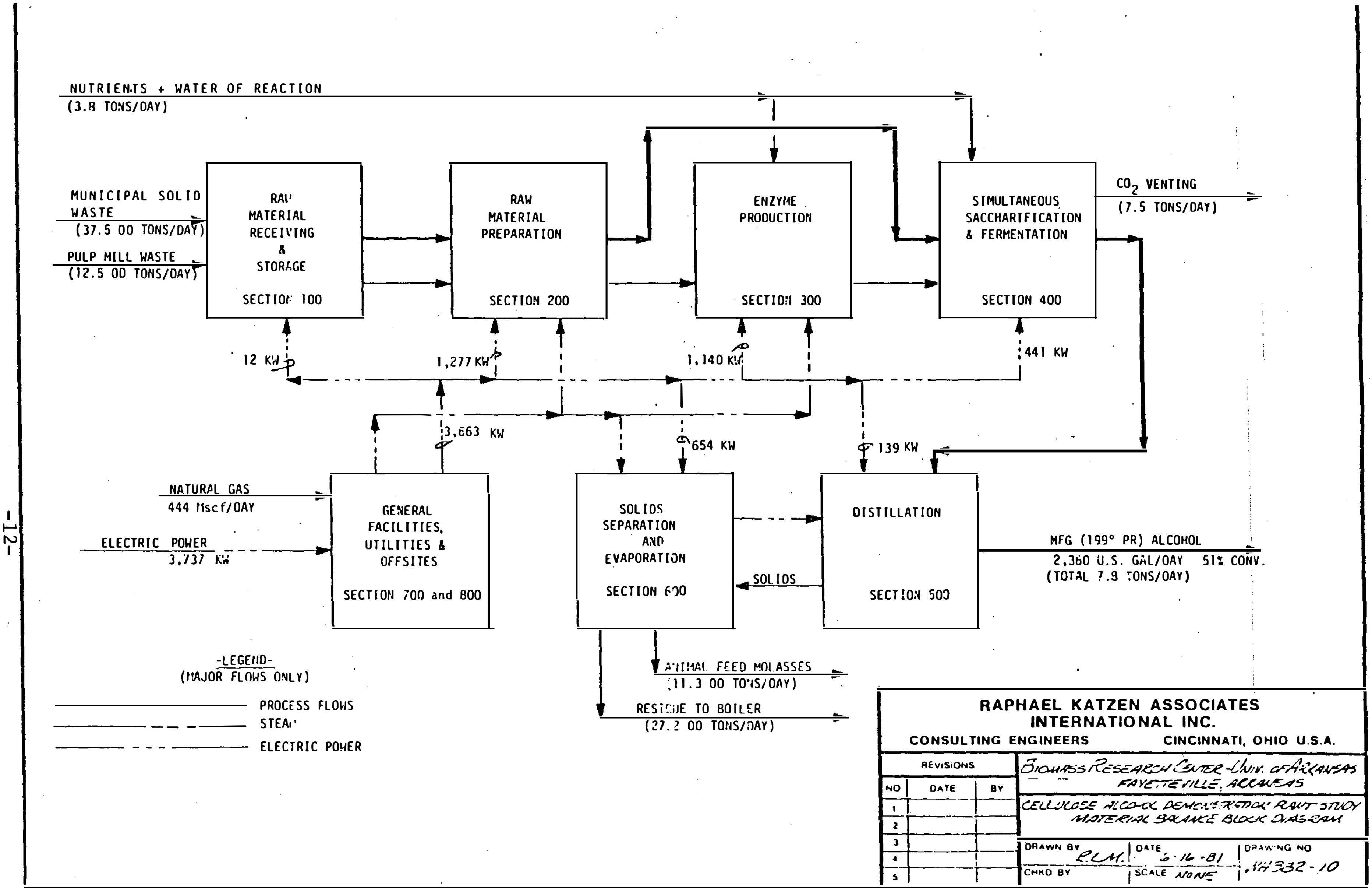
Operating costs for raw materials, chemicals, utilities, labor, maintenance, and miscellaneous expenses amount to approximately $5.6MM for the first year or operation, and to $15.1MM for the full 2 1/2 years of opearation. The 2 1/2 year costs include an inflation/escalation factor to account for wage and price increases during the period of operation. These costs are summarized in Table 1-2 and are presented in more detail in Section 4.0 of this report.

Total Costs

Total costs for construction and operation of the demonstration plant are summarized in the table below. Also included is the potential credit from the sale of MFG alcohol made during the plant's period of operation. The net budget required to build and operate the plant for 2 1/2 years is estimated at $36.3MM.

ESTIMATED INVESTMENT AND OPERATING COSTS
2½ Years Operation

	$MM	
	First Year	2½ Years
Fixed Investment (1982)	19.950	19.950
Operating Expenses	5.567	15.112
Potential Additions & Modifications	4.000	4.000
Gross Total Budget	29.517	39.062
Credit for Alcohol	(1.116)	(2.793)
Net Total Budget	28.401	36.269


NUTRIENTS + WATER OF REACTION
(3.8 TONS/DAY)
MUNICIPAL SOLID WASTE
(37.5 OD TONS/DAY)
PULP MILL WASTE
(12.5 OD TONS/DAY)
RAW MATERIAL RECEIVING & STORAGE
SECTION 100
RAW MATERIAL PREPARATION
SECTION 200
ENZYME PRODUCTION
SECTION 300
SIMULTANEOUS SACCHARIFICATION & FERMENTATION
SECTION 400
CO2 VENTING
(7.5 TONS/DAY)
12 KW
1,277 KW
1,140 KW
441 KW
3,663 KW
654 KW
139 KW
NATURAL GAS
444 Mscf/DAY
ELECTRIC POWER
3,737 KW
GENERAL FACILITIES, UTILITIES & OFFSITES
SECTION 700 and 800
SOLIDS SEPARATION AND EVAPORATION
SECTION 600
DISTILLATION
SECTION 500
SOLIDS
MFG (199° PR) ALCOHOL
2,360 U.S. GAL/DAY 51% CONV.
(TOTAL 7.8 TONS/DAY)
ANIMAL FEED MOLASSES
(11.3 OD TONS/DAY)
RESIDUE TO BOILER
(27.2 OD TONS/DAY)
-LEGEND-
(MAJOR FLOWS ONLY)
PROCESS FLOWS
STEAM
ELECTRIC POWER
RAPHAEL KATZEN ASSOCIATES
INTERNATIONAL INC.
CONSULTING ENGINEERS
CINCINNATI, OHIO U.S.A.
REVISIONS
NO DATE BY
1
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BIOMASS RESEARCH CENTER - UNIV. OF ARKANSAS
FAYETTEVILLE, ARKANSAS
CELLULOSE ALCOHOL DEMONSTRATION PLANT STUDY
MATERIAL BALANCE BLOCK DIAGRAM
DRAWN BY R.L.M.
DATE 6-16-81
DRAWING NO
AH332-10
CHKD BY
SCALE NONE

Table 1-1

INVESTMENT ESTIMATE FOR PINE BLUFF SITE

Item	Installed Investment July, 1981	Item Cost July, 1983	Investment Basis July, 1982
I. RKAII - Battery Limits			
Section 100 - Raw Material Receiving & Storage	$ 466,700		
Section 200 - Raw Material Separation	4,160,900		
Section 300 - Enzyme Production	4,056,900		
Section 400 - Simultaneous Saccharification & Fermentation	2,493,700		
Section 500 - Distillation	1,491,400		
Section 600 - Solids Separation and Evaporation	1,414,100		
Section 700 - General Facilities	847,200		
			$16,872,000
II. REC - Utilities & Offsites[1]			
Boiler (20,700 lb/hr, 150 psig)		$ 126,000	
Deaerator		33,000	
BFW Treatment Unit		17,500	
Instrument Air Dryer		9,400	
Well and Pump		21,700	
Waste Storage Tank		112,900	
Electrical Substation		585,000	
Gas Line Tie-in		112,800	
Buildings (addition to RKAII Estimate)		115,400	
Dump Truck		77,300	
			$ 1,081,300
Total Equipment[1]			17,953,300
10% Contingency			1,796,400
Miscellaneous Fees, etc.			200,300
Total Fixed Investment (TFI)			$19,950,000
Estimated Potential Modifications			4,000,000
Total Actual and Potential Investment[1]			$23,950,000

NOTE - 1. These estimates do not include waste facilities for disposing of the demonstration plant effluents in emergency situations.

Table 1-2

CELLULOSE ALCOHOL DEMONSTRATION PLANT STUDY

OPERATING COSTS*

	First Year 215 Days	2-1/2 Years 538 Days
Raw Materials (37.5 ODT/D x \$20/ODT) (12.5 ODT/D x \$20/ODT)	0.215	0.585
Chemicals, Nutrients, and Diesel Fuel	0.110	0.300
Electric Power (89,688 kwh/D x 4.5¢/kwh)	0.868	2.372
Natural Gas (444 Mscf/D x \$4.75/Mscf)	0.453	1.237
Cooling and Process Water	0.024	0.066
Waste Stream Handling	0.157	0.428
Labor (Technical, Clerical, and Operating)	1.544	4.175
Maintenance (Labor and Materials, 6% of TFI)	1.197	3.269
Taxes and Insurance (2% of TFI)	0.399	1.080
Vendor Charges and Other Outside Services	0.600	1.600
Operating Charges	5.567	15.112

*Note: Prices shown and costs for the first year of operation are projected for the year 1982. The 2-1/2 year operating costs are based on projected average values for the time period from 9/82 through 3/85.

FIGURE 1-1

REQUIRED ETHANOL SELLING PRICE FOR 15% ROIAT

VS

NUMBER OF ATTRITOR MILLS

Basis: 48.3 MM USGPY of MFG Alcohol

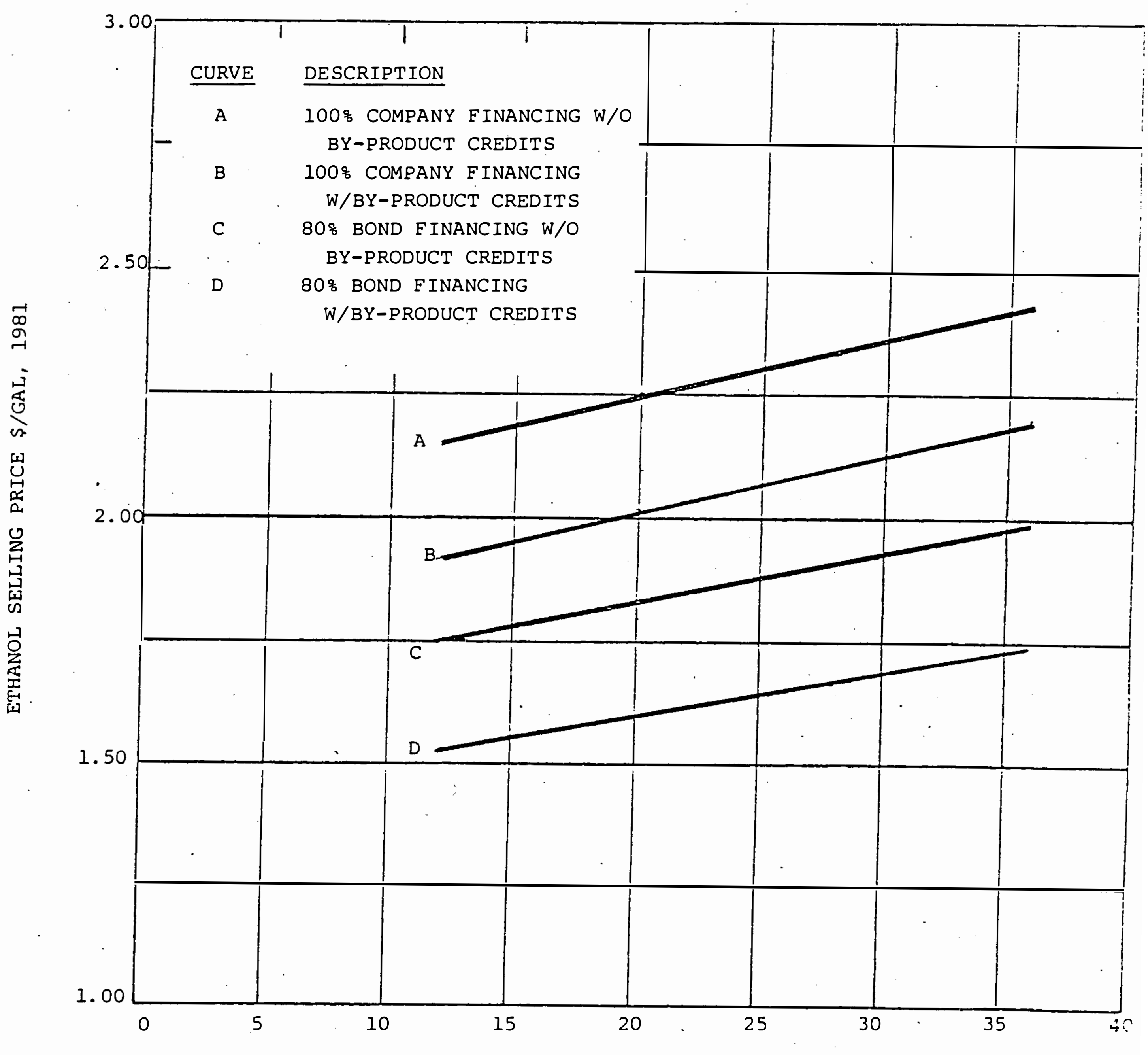
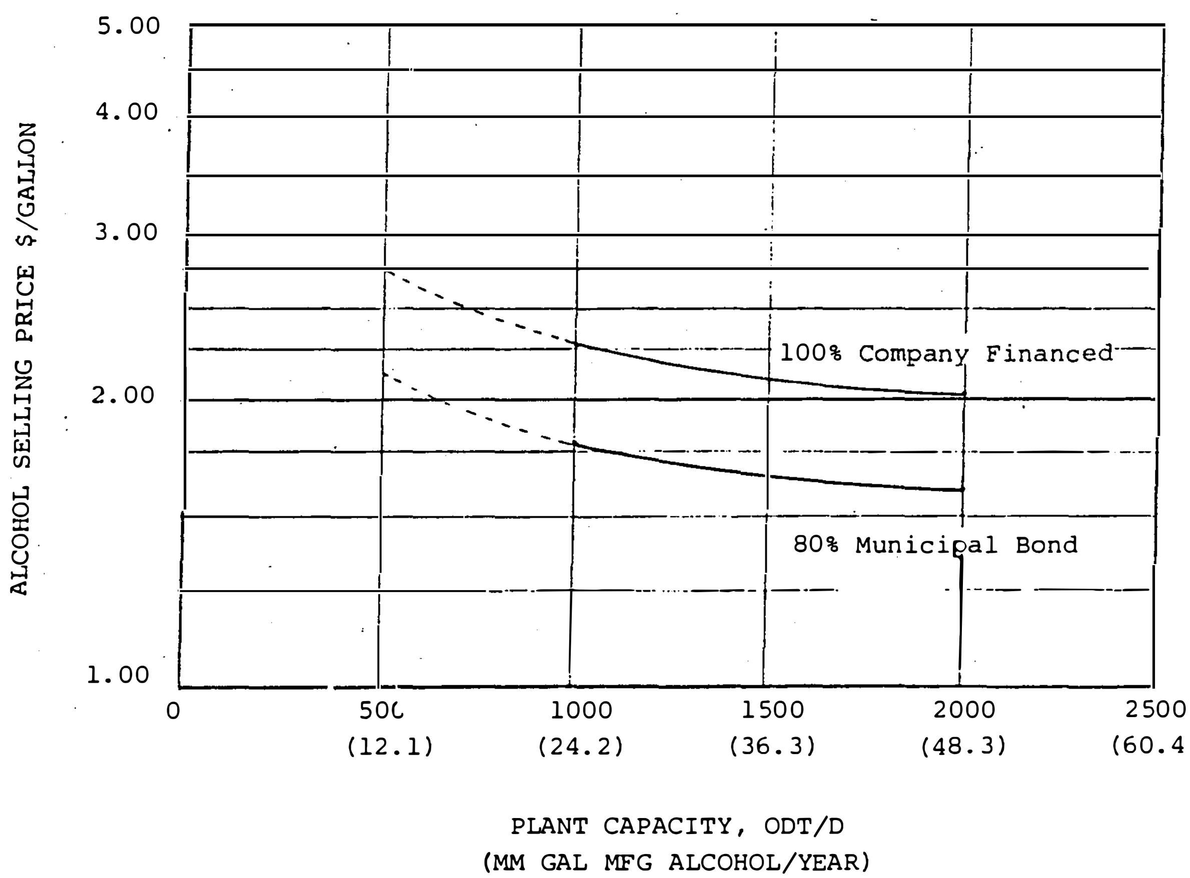


FIGURE 1-2

ALCOHOL SELLING PRICE REQUIRED FOR 15% ROIAT

VS

PLANT CAPACITY



TASK IV: Commercial Scale Plant

The University of Arkansas Biomass Research Center is developing a bioconversion process to use municipal wastes, forestry residues, and certain agricultural materials as feedstocks for production of ethyl alcohol. The process involves five major steps: 1) receipt, handling, and pretreatment of feedstock, 2) production of cellulase enzymes using a mutant strain of *Trichoderma reesei*, 3) production of a mutant yeast strain of *Candida brassicae*, 4) production of ethanol using a patented procedure called simultaneous saccharification fermentation (SSF), and 5) the separation and concentration of ethanol and by-products. Bench level research activities have resulted in the issuance of four process patents and development of considerable technological know-how. The process has been tested in a one ton of feedstock per day pilot plant (the first approximation of commercial design) whose operation for 3 1/2 years proved technical feasibility of the bioconversion of cellulosic feedstocks to ethanol.

The next developmental stage was to do the detailed engineering process design for a 50-fold scale-up to a demonstration size facility (the second approximation of the ultimate commercial design). The design is highly instrumented to provide for maximum acquisition of data in the areas of design uncertainties.

Projected commercial scale economics show constrution costs for a 2000 TPD plant to be $165.20MM. The alcohol selling price required to yield 15% after-tax return on equity under 80% bond financing is determined to be $1.59/gal.

UPDATE OF CELLULOSE ALCOHOL ECONOMICS
2000 ODT/D CELLULOSE ALCOHOL PLANT

General

The objective of this study is to review and revise the technology and economics of a 2000 oven dried tons/day (ODT/D) cellulose alcohol process. This is an updated version brought forth from an earlier study provided for the Gulf Oil Chemicals Company in 1978. In the newer version, the latest technology improvements are included and the economic conditions are revised reflecting the actual inflation rates that occurred during the period from 1978 to mid-1981. Table 1-1 details the section-by-section breakdown of the total fixed investment.

This project includes the production of motor fuel grade alcohol from cellulose, the evaporation of the stillage for use as an animal feed molasses, and the use of combustible insoluble residue as a source of fuel for the boiler.

The economic evaluation contains a budget estimate for grass-roots commercial plant investment, including working capital, a detailed analysis of annual operating costs using cellulosic feedstocks, and a financial analysis to establish a selling price which would yield to the investor a 15% return on equity after taxes (ROIAT). This has been calcualted for a 100% equity-financed case and an 80% municipal-bond-financed case. The operating cost is $1.06/gallon for the 100% equity-financed case and $1.40/gallon for the 80% bond-financed case (see Table 1-2). This includes an animal feed molasses credit of $120/ton, dry basis ($0.24/gallon of alcohol). To obtain a 15% ROIAT, the selling price would have to be $2.02/gallon and $1.59/gallon for the 100% equity-financed and 80% bond-financed cases, respectively. The present average selling price for Motor Fuel Grade (MFG) alcohol is $1.75/gallon (mid-1981 price).

Table 1-1

ERECTED COST OF THE ALCOHOL PLANT BY SECTIONS (MID-1981)

Section	Operation	Erected Cost ($MM)
100	Raw Material	6.97
200	Pretreatment	39.20
300	Enzyme Production	17.70
400	Fermentation	15.83
500	Distillation	12.86
600	By-Product Processing	20.85
700	Product Storage	3.11
800	Off-sites	27.13
Total		143.65
Contingency @ 15%		21.55
Total		$165.20 MM

Table 1-2

SUMMARY OF OPERATING COSTS AND SELLING PRICE
2000 ODT/D CELLULOSE ALCOHOL PLANT
Mid-1981 Dollars

Item	100% Equity Financing		80% Bond Financing	
	$MM/Yr	$/Gal	$MM/Yr	$/Gal
1. Total Production Costs				
Fixed Charges	18.6	.385	18.60	.385
Raw Materials and Chemicals	17.74	.367	17.74	.367
Utilities and Fuel	13.87	.287	13.87	.287
Labor	6.65	.138	6.65	.138
Interest	.68	.014	16.9	.350
Waste Disposal Fees	.77	.016	.77	.016
Royalties	4.47	.093	4.47	.093
Total	62.78	1.300	79.00	1.636
2. By-Product Credit	(11.35)	(.235)	(11.35)	(.235)
3. Net Operating Cost	51.43	1.065	67.65	1.401
Taxes (46% of gross profit)	21.11	.437	4.22	.027
Net Profit (assuming 15% ROIAT for company investment)	24.78	.513	4.96	.103
5. Required Annual Sales Revenue and Unit Selling Price	97.32	2.015	76.83	1.591

BASE CASE PRODUCTION OF ALCOHOL FROM 2000 ODT/D

Cellulosic Wastes

The plant is sized to produce 48.3MM GPY of denatured MFG alcohol. The overall alcohol product yield is 71.7 gallons (199^{o} proof) per oven dried ton of waste. In addition to the alcohol product, the plant produces 94,500 tons/year of animal feed molasses (dry basis).

This plant utilizes the latest technology developed by the Biomass Research Center-University of Arkansas Foundation (BRC-UAF). This conceptual design operates as a continuous process, including enzyme production; however, multiple trains of fermenters permit periodic sterilization of the equipment. The yeast required for fermentation is purchased. Alcohol recovery employs a pressure-cascaded distillation system, which improves steam economy in producing 199^{o} proof alcohol from a 3 mt % beer. The total plant steam usage is 58.2 lbs/gallon of alcohol produced. Of this total, the distillation system used 30.3 lbs/gallon of alcohol produced; the remainder is used for sterilization and process heat.

All of the utility requirements (see Table 1-3), with the exception of electric power and process water, are produced within the boundaries of the plant. The boiler burns combustible residue, retrieved from by-product processing. Waste water is processed in an activated sludge treatment facility. Cooling water is recycled from a cooling tower. Makeup water for cooling tower and boiler feedwater blowdown is purchased.

TABLE 1-3

ESTIMATED UTILITIES

ELECTRIC POWER (Operating):

Alcohol Plant

Section	Operating Horsepower	
100	25	
200	23,336	
300	4,462	
400	4,500	
500	1,400	
600	3,400	
700	14	
	37,167 hp	27,720 KW

Off-Sites	840 KW
Total Purchased Power	28,560 KW
STEAM, 600 psig - 750°F (internally generated)	347,500 lb/hr
WATER	2,500 gpm
NATURAL GAS	49.5 MM Btu/hr

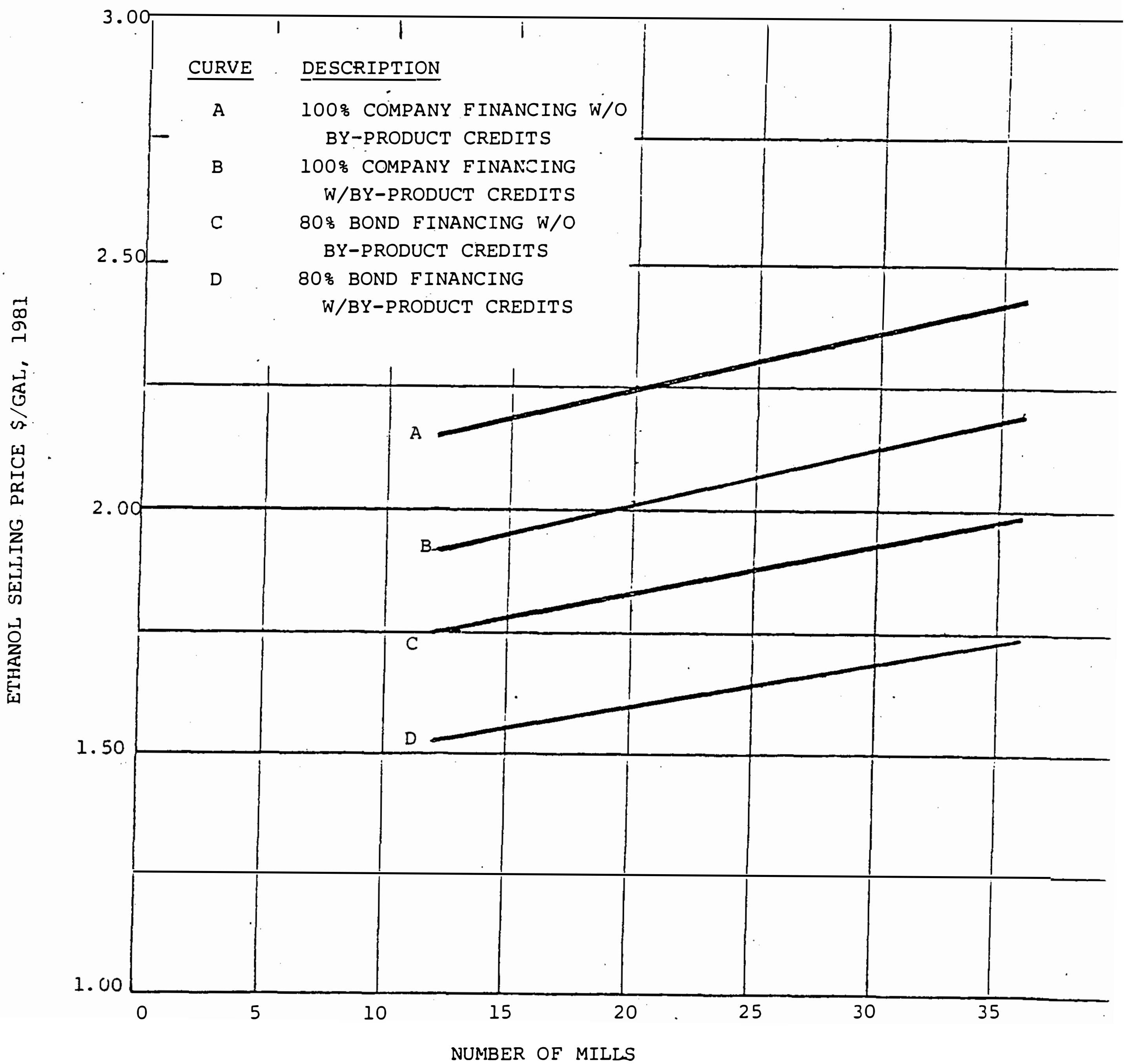
FIGURE 1-1

REQUIRED ETHANOL SELLING PRICE FOR 15% ROIAT

VS

NUMBER OF ATTRITOR MILLS

Basis: 48.3 MM USGPY of MFG Alcohol



SENSITIVITY ANALYSES

Number of Attritor Mills

A sensitivity analysis is provided to illustrate the effect the number of attritor mills, in Section 200 - Pretreatment, has on the selling price of alcohol (see Figure 1-1). Since there is some degree of uncertainty in this area, it was felt that a comparison of twelve attritors to thirty-six attritors is required to determine the effect this range would have on the cost of alcohol. For the base case study, twenty attritor mills are estimated to be sufficient.

TASK V: Economy of Scale

Plant Capacity

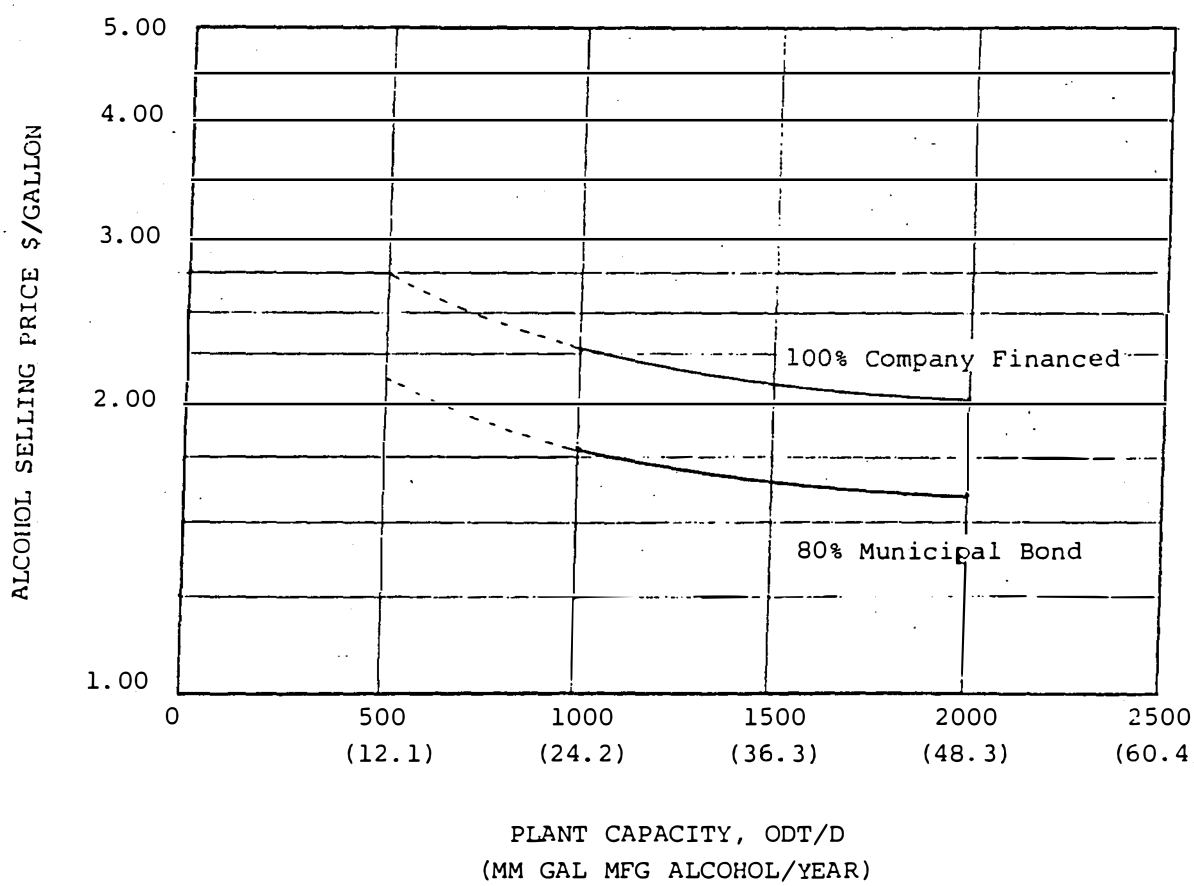
An economy of scale sensitivity analysis shows the effect of plant size on the selling price of alcohol (see Figure 1-2). In developing Figure 1-2, an economic evaluation was made for a 1000 ODT/D plant and this, along with the 2000 ODT/D information, was extrapolated to 500 ODT/D. Operating at a 2000 ODT/D capacity has definite economic advantage over the 1000 ODT/D facility (19¢/gal selling price differential). However, increasing feedstock capacity above 2000 ODT/D does not indicate significant economic merit.

FIGURE 1-2

ALCOHOL SELLING PRICE REQUIRED FOR 15% ROIAT

VS

PLANT CAPACITY



TASK VI: Research and Development

Laboratory research and development involved areas of the cellulose to ethanol process where improvements had the greatest chance of leading to cost reduction. Although all areas of research are intertwined, this task was sectioned into four major categories for reporting purposes and are summarized in the following pages.

PRETREATMENT OF LIGNOCELLULOSICS

Wet Vibratory Rod Milling

Wet vibratory rod milling of MSW does not produce a desirable process feedstock, at least under the conditions tested. Under standard SSF conditions, MSW could be expected to produce higher ethanol yields in an unpretreated condition than after vibratory rod milling.

Although results indicate no benefit was derived from VRM pretreatment of MSW, several features of the data are worth mentioning. The fact that the shorter milling time resulted in higher ethanol yield than the longer milling time could be because a point of diminishing returns was reached somewhere between the two run times. Therefore, longer milling times do not necessarily result in a more suitable feedstock.

The data also leads to the conclusion that increasing the solids content does not appreciably reduce the subsequent yields. That, along with the previously described phenomenon, could indicate that even shorter milling times could yield comparable ethanol levels.

Yet another conclusion is possible: that the low ethanol yields at 6% solids is the result of detrimental effects when 94% of the milling charge is water. The milling at such dilution may have broken up the more susceptible portions of MSW and as a result they were lost upon filtration.

Evidence from the twelve minute milling supports this in that even more yield is lost during periods of longer milling.

Rod diameter did not seem to play a significant role in any of the tests conducted. Apparently the difference between one inch and one and one-quarter inch rod diameter does not appreciably alter the shape or size of the impact surface.

Thermomechanical Pretreatments

Although agricultural wastes contribute a distinct feedstock category, they cannot be as easily lumped together when it comes to process performance. Bagasse showed marked enhancement in ethanol yield with increasing base or temperature conditions, even a possible synergism at mid temperature and low base conditions. The findings that elevated temperature will produce the same enhancement in ethanol yield is important when considering heating costs versus downstream waste clean-up problems as economic trade-offs.

The fact that rice and wheat straws exhibited such a marked difference in ethanol yields when exposed to identical pretreatment conditions points out the uncertainty in predicting process performance based on data from seemingly similar feedstocks. Such results emphasize the importance of evaluating specific feedstocks throughout the entire process to establish its economic potential.

Thermochemical treatment of agrigultural wastes is a technically feasible method of increasing ethanol yield in SSF. The increase in ethanol production may be attributed at least in part to a general alteration of the lignocellulosic structure. Relative cellulose concentration is increased at the expense of lignin and inorganic ash.

Cellulose Crystallinity

Preliminary data indicate that cellulose crystallinity is but one factor in controlling the enzymatic hydrolysis of purified cellulosics.

Reduced particle size and correlating reactive surface area are also of major importance. As a result, additional extensive experimentation is required to determine correlations and trends in the regulation of ligno-cellulose hydrolysis.

BOD and COD of Waste Streams

Biological chemical oxygen demand (BOD) and chemical oxygen demand (COD) analyses were performed on representative process waste streams. The data indicate that BOD levels expected from this cellulose ethanol process will be lower than previously projected. Estimates were for an initial 5714 mg/l BOD load and an effluent containing 1270 mg/l. However, a worst case analysis of BOD and COD from ethanol containing SSF beers showed an initial 1670 mg/l BOD which is 29% of the expected initial BOD.

Efficienct removal of ethanol from either SSF mash or SSF beer is critical in reducing potential BOD. When ethanol is reduced to 0.1-0.2% w/v, BOD levels were 605 mg/l and 450 mg/l, respectively. Both values are well below the projected discharge levels and therefore should require no waste treatment with respect to oxygen demand based on comparison with BOD discharge levels at corn alcohol plants.

Other potential contributing streams such as cellulose, ethanol, and post inoculum SSF beer were also analyzed for potential BOD contribution. All BOD levels were below the projected effluent release level except cellulase which was equal to the projected effluent release level. Therefore, waste treatment with respect to BOD may be necessary only in the unlikely event that an ethanol containing beer were to be an actual waste stream.

Pretreatments for Cellulase Production

Pretreatment of lignocellulosics for enzyme production substrates was evaluated. Forest product wastes, which require no pretreatment, produced unacceptable enzyme activities. Mechanical pretreatments enhanced substrate performance in subsequent enzyme production. The most effective of the mechanical pretreatments were ball milling, agitation bead milling, and dry vibratory rod milling with prior hammer milling. Of these, dry vibratory rod milling produced an acceptable substrate for enzyme production with the least cost incurred. Additionally, an apparent relationship exists between particle size and substrate enhancement, that being the smaller the particle size, the better the substrate for enzyme production.

ENZYME PRODUCTION

Continuous Cellulase Production

The three experiments designed to evaluate diauxic growth on either glucose or lactose and cellulose were inconclusive because of their short duration caused by mechanical failures. The CEP 15 experiment did not show any early repressive effects of glucose, which was metabolized in the first 24 hours. The CEP 16 experiment, which included 2% lactose initially, was interesting because of the appearances of a saccharification product in the enzyme broth at day four. The appearance of glucose may have caused a repression of enzyme synthesis or activity and resulted in a loss of culture maintenance in the first vessel. The duplicate diauxic lactose experiment, CEP 18, was typical in generating β-glucosidase and saccharification activity while net protein in the second vessel was negligible.

Continuous enzyme production 17 utilized the most recent batch of vibratory rod milled MSW. All recorded activities were low and culture maintenance was never estabilshed. The reason for this resides with this lot of MSW which also failed to elicit expected activities in batch enzyme production.

Continuous enzyme production 19 was a base case run designed to evaluate a new medium formulation, the low salt sulfate medium. After an atypical startup, the LSSM without $CaCl_2$ maintained activities. However, introduction of LSSM supplemented with 1.5 g/l $CaCl_2$ could not maintain prior activities. Enzyme activities were recovered by switching to the initial startup medium and by reducing the dilution rate. This run exceeded 1075 hours of aseptic operation.

Continuous enzyme production 20 was designed to evaluate a quick startup mode for commercial application. The design of this experiment failed to maintain the culture in the first vessel and normal continuous operational parameters were never met. Successful execution of this concept will require a continuous transfer of contents between the vessels during startup.

Medium Development

Medium formulations were devised to yield high cellulase activity at acceptable cost. Additionally, negative process interactions were eliminated by formulating media which can be heat sterilized, concentrated easily, have a reduced ash content, and post a negligible corrosion potential.

Evaluation of ammonia addition was inconclusive because of a lack of system refinement. The concept is presently practiced at the industrial scale and should be amenable to our process.

Substrate Pretreatment

Scanning electron microscopy revealed that hammer milling of air classified MSW separated fibrils and moderately reduced fiber length. Vibratory rod milling of hammer milled MSW reduced fiber length considerably.

Hammer milled vibratory rod milled air-classified MSW has not produced ethanol yields in the standard 48 hour SSF at the levels previously observed. The VRM-MSW enzyme activities were lower but not to the extent observed with the SSF assay. No water soluble enzyme production inhibitor or SSF inhibitor was observed under the experimental protocol used.

Mutant Strain Comparison

Greater enzyme activities were realized when using the specific protocol for each organism tested. Of the *Trichoderma* strains evaluated, Rut-C30 gave the highest enzyme activity for all of the assays performed. Strain 9414G exhibited a lower rate of enzyme expression than Rut-C30, and lower assay values except for β-glucosidase activity. The MCG-77 organism gave the lowest assay values. When grown at pH $\geq$ 3.0, higher protein and FP values and lower β-glucosidase and SSF results were recorded. When grown at pH 4.75, the opposite was true. The higher pH gave greater β-glucosidase and SSF yields and lower protein and FP values. This is more than likely due to the inactivation of β-glucosidase activity at the lower pH and indicates the relative worth of the FP assay in predicting SSF yields.

A more thorough evaluation should be performed to validate the conclusions generated for the three strains tested. These results represent several comparison studies and may or may not reflect the average performance over extended runs, i.e. continuous operation where mutant stability and productivity can be evaluated.

Several practical observations can be made in addition to the experimental results. Comparing startup procedures, the BRC process requires two and one-half days from spores to cellulase production vessel. Both Rut-C30 and MCG-77 require eight to nine days and this does seem to be required since enzyme activities were considerably lower when the BRC startup procedure was used. Another difference noted is in the area of cellulase production medium. The BRC has optimized low cost, high yield media without expensive ingredients such as peptone or yeast extract. Also the cellulase concentration has been kept low, 3% vs. 5% w/v, because of the intended use of continuous cellulase production. If, for example, 5% w/v cellulose concentration was used and air-classified milled MSW was the substrate used for cellulase production, the solids loading to the cellulase production vessels would be in excess of 10% which would limit effective aerations and agitation in the vessels. Whether the Rut-C30 organism could be adapted to give high yields on lower cellulose concentrations at a reduced residence time has not been demonstrated. The MCG-77 strain has been evaluated in continuous operation on a soluble substrate but no outstanding yields were reported (Ryu). These observations reflect that laboratory results must be adaptable to potential process designs in an effective manner.

Cellulase Assay

The FP assay is able to crudely estimate SSF results for terminal batch cellulase productions and then only if adequate β-glucosidase expression is present. Continuous cellulase preparations are affected by high background sugars inhibiting the FP results. The FP assay is the least affected assay in comparisons of supernatant and whole culture activities and is relatively insensitive to β-glucosidase activity. The β-glucosidase assay should be performed on the whole culture as supernatant activity does not reflect the total activity present in a sample. Additionally, the SSF assay appears substrate limited since results level off at around 60-65% conversion despite enzyme activities.

The "Universal Method" appears to be a promising modification of the FP assay but will have to be further evaluated before it can be assumed to be accurate. This method was successful in predicting cellulase activity for any dilution based on a single dilution of an enzyme sample.

Chemical Sterilants

Only Cidex[tm] was successful in passing a screening procedure and *in situ* fermenter sterilization. This was not surprising since glutaraldehyde is one of a few agents with sporicidal activity. The contact time required to accomplish sterilization is lengthy, approximately 10-12 hours. Reduction of the time required for sterilization may be possible if the chemical agent could be used in conjunction with a physical agent such as temperature.

Product Analysis

Tremendous emphasis has been placed on the analysis of reducing sugars as glucose with the 2,4-dinitrosalicylic acid (DNS) assay to measure the efficiency of enzymatic saccharification of cellulose. However, while comparing several analytical methods using both known sugar solutions and saccharification supernatants of purified cellulosics as well as raw and pretreated waste lignocellulosics, only high performance liquid chromotography (HPLC) and glucose analyzer (YSI) data proved reliable. However, because a quick and easy wet chemical analysis is desirable for the analysis of large numbers of samples, a modified DNS assay was developed that is compatible with combined HPLC glucose and cellobiose when used judiciously. Glucose analysis should, however, be used only as an indicator of cellulase activity. The criterion for bioconversion efficiency should be the percent theoretical conversion of the initial substrate, i.e. cellulose, to the final product, i.e. ethanol, not an intermediate such as glucose.

CONTINUOUS SIMULTANEOUS SACCHARIFICATION FERMENTATION

The results of this experimentation have proven the technical feasibility of continuous simultaneous saccharification fermentation and has yielded some operation conditions that are valuable from a scale-up design standpoint regarding the startup of such a system and the expected product yield.

Cellulase Recycle

The results of eleven experiments in enzyme reutilization provide support for several key ideas regarding the most effective method of recycling cellulase activity. Not only does there not appear to be enough activity associated with the filtrate to produce sufficient ethanol levels, but the high content of ethanol in the SSF supernatant may be inhibiting to future ethanol production is used in a recycle design.

Clearly, a significant amount of activity exists in the SSF solids. Further, the results of the experiments in Section B and C suggest that this activity is associated with enzyme production solids. Our results seem to be consistent with recent work in a similar area (Kubicek, 1981). Washing of solids as performed does not prevent the remaining cellulases associated with the solids from producing a significant amount of ethanol. Recycling a fraction of active unwashed SSF solids performed significantly better than the manipulation of supernatant with fresh substrate (E.R. XI).

In all experiments, the results favor the recycling of a portion of SSF solids to enhance ethanol production and reutilize cellulase activity.

Yeast Recycle

The significance of the information gained in these experiments is applicable to yeast recycle. When a greater yeast population was maintained, SSFs could be run at a higher temperature, thus increasing ethanol yields. The glucose data obtained indicates that at the higher temperatures and lower yeast inoculum there is a buildup of glucose (Figure 2). This is indicative of lower ethanol productivity due to lower cell numbers. By increasing the yeast biomass (cell population) ethanol productivity surpasses the rate of saccharification and there is no glucose buildup. Although DADY would be easier to maintain, it does not have the fermentation ability that *C. brassicae* has. *C. brassicae* IFO 1664 has been the most efficient yeast used to date at the Biomass Research Center. Recycling this yeast could increase the yeast population, thus allowing the SSF to be run at higher temperatures enabling greater rates of hydrolysis to occur.

Ethanol Inhibition of Fermentation

Net ethanol production does decrease with increase of initial ethanol levels. Further, when high levels of ethanol are introduced at various times during fermentation, ethanol production rates drop to a greater degree upon early addition of high levels of ethanol.

The inhibition seems to stem from an affect on the yeast as well as the cellulase system; however, the results of our work point towards the yeast being effected to a greater extent. When additional glucose was added to fermentations in which approximately 4.5% w/v ethanol had already been produced, *C. brassicae* displated 37% inhibition in further fermentation; however, in another yeast, *S. carlsbergensis*, fermentation stopped almost completely. The amount of oxygen present initially in a batch fermentation does not produce any significant change in the observed effects on ethanol production and glucose utilization when performed in the presence of 4% initial ethanol (w/v). Therefore, possible ethanol utilization by *C. brassicae* does not appear to be due to the amount of oxygen present initially.

The present work reported on here is consistent with other work reported on in the literature indicating that growth in inhibited to a higher degree than fermentation capability by ethanol (Brown).

would be necessary to obtain a clearer picture of the effect of high ethanol levels in an SSF on further production as it appears to jointly affect yeast and cellulases.

Glucose Uptake

Ethanol appears to have an affect on glucose uptake by *Candida brassicae*. However, more data is needed to identify this effect with more certainty. The procedure outlined by Aldermann and Hofer for measuring glucose uptake may have to be refined for our system.

Enzyme Pretreatment

The use of agitated glass beads to release cellulase from the enzyme produced by *Trichoderma reesei* does not appear to create any significant change in enzyme activity.

Sonication of whole culture enzyme (including solids) and solids in buffer caused an increase in activity while this same treatment using enzyme filtrate caused a decrease. It appears as if sonication is able to break apart the enzyme solids making more enzyme available to combine and react with the substrate.

These experiments demonstrate that pretreatment of the solids portion of the enzyme might be beneficial to the saccharification step in a simultaneous saccharification fermentation process.

Immobilized Yeast

The method used for immobilization in these experiments did not interfere with the fermentation capability of *Candida brassicae* proving the feasibility of using this method in an SSF.

Mass Balance

Little variation occurred among the replicate flasks. Of all SSF components carefully weighed into each flask, approximately 96% of the beginning mass was accounted for at SSF termination. The data regarding percent solids, percent moisture, percent soluble solids, and percent ash assocaited with these SSFs using the MSW-PMW blend as substrate source will be used in sizing de-watering equipment for a future demonstration plant utilizing the University of Arkansas cellulose to ethanol technology.

ANIMAL FEED BY-PRODUCT

Chemical Characterization of MSW and SSF Residues

The IR contains some cellulose and could possibly be used in ruminant rations because of this.

Due to its ash content, the insoluble residue of the SSF process would have to be partially demineralized or added to a low ash feed if it is to be considered as a prospective animal feed.

Although silicon content of a feed is not a factor which influences cellulose digestibility in ruminants, the lignin content of a feed does. Therefore, the silicon content may not be a factor in evaluating the IR as a prospective feed, but the lignin content will and may represent a drawback to the use of the IR in ruminant rations.

The IR was found to have in *in vitro* digestibility of 90%. Although this technique is a standard, preliminary evaluation of a prospective feed, it provides no data concerning the nutritive aspects of such a feed.

The mineral content of the SSF residues shows there to be several metals which could pose potential problems if either residue were to be used. Lead, iron, copper, and cadmium show up in concentrations which could be detrimental to an animal if all these elements were present in forms which had a high bioavailablity.

Biological Characterization of MSW

Mortality Rates

Only the 40% MSW diet produced a mortality rate which was considerably greater than the mortality rates present in all other diets.

Growth Rate and Feed Efficiencies

Feeding Avicel resulted in significant body weight depression only at the 40% level.

Feeding MSW at levels of 10% of greater resulted in significant reductions of body weight.

At the levels tested, pelleting showed no advantage over not pelleting with respect to weight gain or feed efficiency.

Feed efficiencies decreased as dietary levels of Avicel or MSW increased.

Adjusted feed efficiency ratios did not differ significantly between the diets except the 40% MSW diet. Chicks fed 40% MSW had much lower adjusted feed efficiencies.

Feed efficiency, at the same level of addition, did not differ between Avicel and MSW except at the 40% level (MSW was lower).

Weights and feed efficiencies observed at days 7 and 14 were similar to that observed at day 20.

Dietary Densities

Diet density may have been a factor contributing to the poor performance of chicks fed 40% MSW, since chicks fed the same level of Avicel performed better.

Vitamin Analysis of Livers

The addition of ethoxyquin (an anti-oxidant) to the basal diet may have acted to protect the pro-vitamin A added to the basal diet.

Birds fed 40% MSW exhibited a relatively high incidence of encephalomalcia - a manifestation of vitamin E deficiency. MSW, when fed at this level, probably interfered with the metabolism of vitamin E.

Pathology Report

Encephalomalacia, characterized by localized brain hemorrhages, had a high incidence in the 40% MSW-fed birds as compared to birds fed all other diets.

The 40% MSW diet caused feed impaction in the digestive system of chicks fed this diet, thereby greatly altering passage rates.

Refractory Organic Residue Analysis

The only ROR residue which was shown to be present in considerable quantities in raw MSW was chlordane. This substance also turned up in the feces, thigh muscle, skin, liver, and abdominal fat tissue of 40% MSW fed birds. If future sources of MSW continue to have such amounts of chlordane, then a potential problem arises when considering the use of any by-products which exit a process using this MSW.

Energy Value of Dietary Treatments

Diet dilution with 40% MSW and 40% Avicel diets caused the cellulose and lignin-corrected energy values of these rations to fall near 60% of controls. The low gains and feed efficiencies observed in the group fed 40% MSW could be a result of some physical or chemical property associated with the MSW. Otherwise, similar findings would have resulted from the feeding of 40% Avicel.

Mineral Analysis of Diet and Tissue Samples

No diet used in the experiment exceeded NRC maximum levels of zinc, copper, or chromium. High iron levels in MSW diets caused accumulation of this metal in livers taken from birds fed 10, 20, and 40% MSW. The iron concentration in the 40% MSW was higher than levels previously found to impair chick growth rates and the 40% MSW diet did, in fact, cause a significant reduction of growth and feed efficiencies in birds fed this diet.

Although manganese accumulation was observed in livers and kidneys taken from chicks fed all diets, the ease with which this mineral is excreted should counter any deleterious effects Mn accumulation could have.

Neither cadmium nor lead accumulated in livers taken from chicks fed the various diets. However, both these elements were found in liver, kidney and femur samples taken from 40% MSW-fed birds at levels significantly greater than other diets. The 40% MSW diet was highest in these metals and the concentration of cadmium and lead in liver, kidney, and femur samples taken from chicks fed this diet directly reflected this.

Dietary cadmium appeared to have no effect on bone ash content of femurs taken from birds on all dietary treatments.

Animal Feed Viscosity

Although dramatic increases in viscosity are indicated for the syrup with the 60% solids content, no handling problems are anticipated. The data for the 40% solids syrup show that viscosity can be substantially reduced by lowering the solids content if necessary.

A Review of MSW as Feedstocks

A new animal foodstuff(s), a by-product of production of ethanol from MSW, will probably be available in large quantities before the end of this decade. It is important to the animal industry, as well as to ethanol producers, that appropriate steps are taken to prepare for the suddenness of this expected event. At present only very small amounts of the new foodstuff(s) are available; animal feeding studies cannot begin until the demonstration plant begins production, hopefully before 1985. This review of research reports of appropriately related foodstuffs is part of the background that will be used to help guide the future research when that new foodstuff(s) suddenly becomes available in large quantities. Raw MSW fractions have received little research attention, but it appears to have some promise at low levels in some ruminant animal diets. Aerobically digested fractions of MSW have been mostly detrimental in animal diets and heavy metals and PCB's seemed a problem. Only a small smount of data is available on MSW products as an animal foodstuff. Over half of the combustible fraction of MSW are paper products with a wide range of lignin. Paper with less than 4% lignin is almost completely digestible; paper with high levels of lignin is nearly indigestible by ruminants. Non-ruminants digest some fiber, but more slowly or less completely than ruminants. Protein digestibility is reduced in swine when dietary fiber is increased. A hemicellulose extract of wood has provided unidentified growth factor (UGF) for chick growth, as have some fiber-related products from other plants. After examination of related literature, it is concluded that the new MSW by-product must be monitored for available or absorbable heavy metals and toxic organic materials. Because of the wide range of published results from the feeding of various fibers, it is not possible to accurately predict the efficacy of the new foodstuff(s). Overall, it is concluded that this new foodstuff(s) has some promise for providing usable energy, protein, and other nutrients in diets for food producing animals.

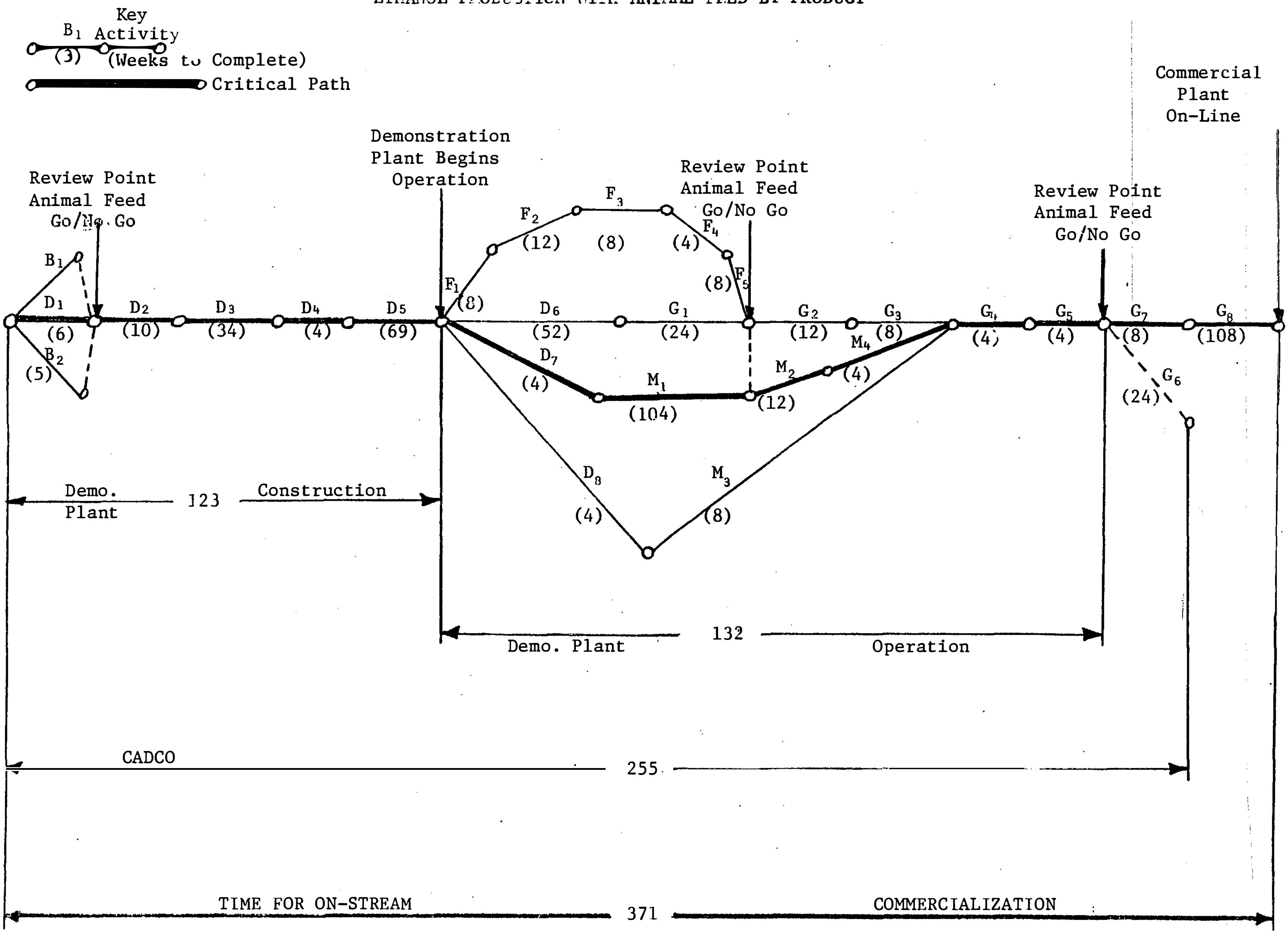
PROCESS COMMERCIALIZATION

The accompanying Program Evaluation and Review Technique (PERT) was constructed to identify and arrange in logical sequence the major activities necessary to scale-up the cellulose to ethanol process technology from bench level and demonstration plant through commercial plant operations. The critical path and decision points are denoted along the chart sequence.

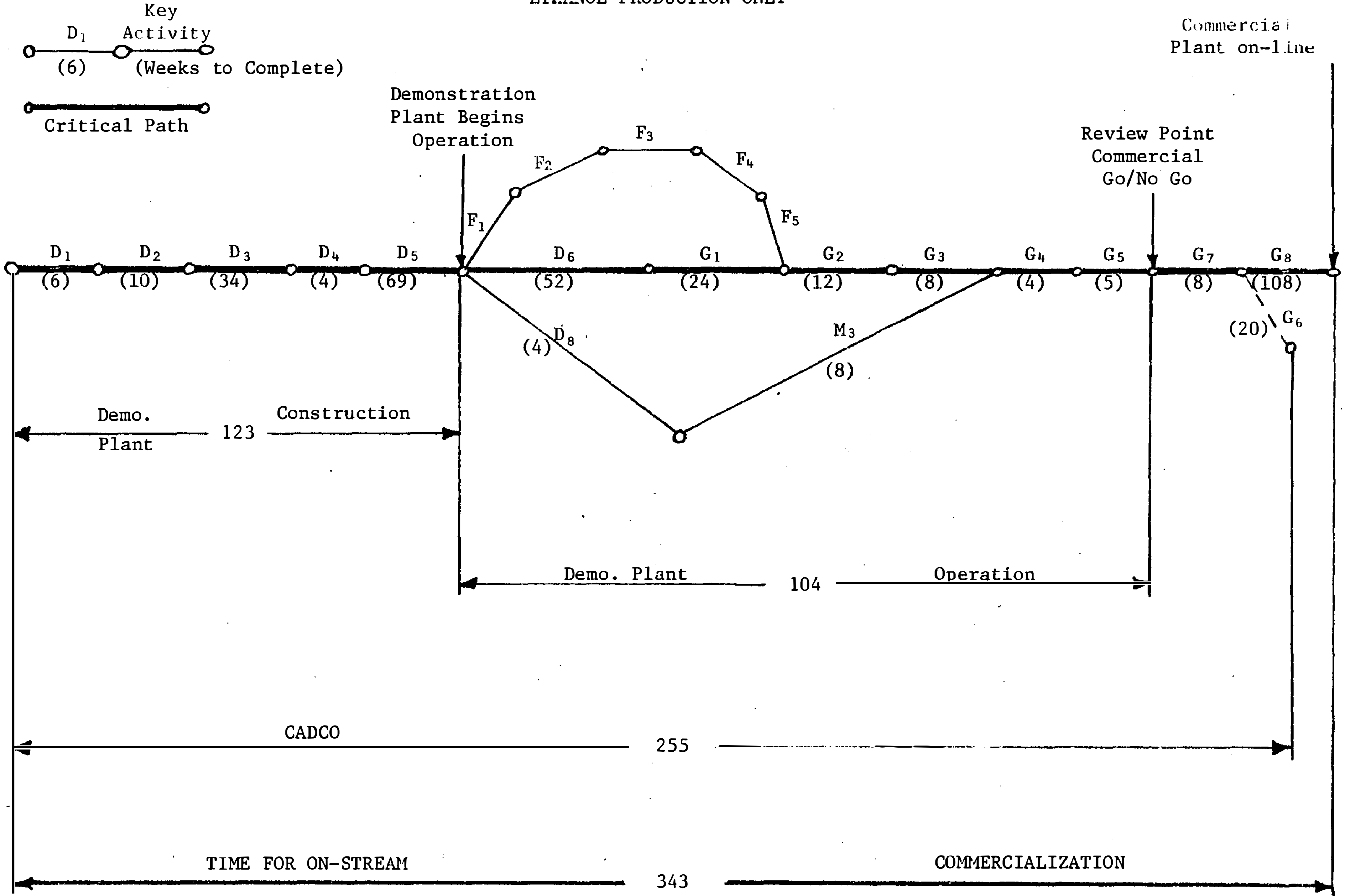
The Cellulose Alcohol Development Company (CADCO) activities were on target with preliminary approval in November 1980 for a $17.1 MM FmHA capital loan guarantee and a $10.5 MM operating fund grant from the U. S. Department of Energy. Shortly thereafter the Regan administration took office and the program approvals were suspended. In response to this action, CADCO began to pursue private financing for the construction of the demonstration plant. The effort began in February 1981 and as of April 1982 is not yet accomplished.

The PERT chart indicates that 255 weeks will be necessary for construction and operation (132 weeks) of the demonstration plant. Upon successful operation of the demonstration plant, 116 weeks would be required to bring a commercial scale operation on-line. Two cases have been charted to accommodate ethanol production with and without an animal feed by-product.

ETHANOL PRODUCTION WITH ANIMAL FEED BY-PRODUCT



ETHANOL PRODUCTION ONLY



Ethanol from Cellulose
With and Without Animal Feed

Chart Code	Responsibility	Weeks to Complete	Demonstration Plant Activities (C)
D1	C	6	FmHA Application
D2	C	10	Partnership Information
D3	C&B	34	Design and Construction Plans
D4	C	4	Let Bids, Award Contracts
D5	C&B	69	Construction of Demonstration Plant
D6	C&B	52	Selection of Pretreatment(s)
F7	C	4	Production of Animal Feed
D8	C	4	Production of Ethanol for Market Studies
			Biomass Research Center Activities (B)
B1	B	3	Small Animal Feeding Trials
B2	B	5	Toxicity Tests on Representative MSW
			Feedstock Selection
F1	C&B	8	Select Optimal Feedstock(s)
F2	C	12	Complete Analysis of Feedstock Logistics
F3	C	8	Locate Commercial Plant Site (s)
F4	C	4	Prepare Feedstock Strategy
F5	C	8	Establish Feedstock Supplier Agreements

Ethanol from Cellulose
With and Without Animal Feed

Chart Code	Responsibility	Weeks to Complete	Market Testing and Strategy
M1	C&B	104	Complete Animal Feed Trials and FDA Approval
M2	C	12	Complete Market Plan for Animal Feed
M3	C	8	Complete Market Plan for Ethanol
M4	C&B	4	Complete Pricing/Optimum Mix for Animal Feed
			General Activities
G1	C&B	24	Prepare Preliminary Commercial Design
G2	C&B	12	Finalize Commercial Design
G3	C	8	Prepare Environmental Impact Statement
G4	C	4	Prepare Commercial Business Plan
G5	C	4	Present Business Plan to Partners
G6	C	24	Disposition of Demonstration Plant
G7	C	8	Prepare Partnership Agreement
G8	C&B	108	Construction of Commercial Plant

RECOMMENDED FUTURE RESEARCH

Pretreatment

Vendor testing will be carried out on various pieces of equipment including a horizontal agitation bead mill, disk refiner, and thermo-mechanical processes. Throughput rates, energy requirements, and equipment wear rates will be monitored during testing and evaluated with respect to commercial scale operation. Following each type of pretreatment, the lignocellulosic feedstock will be evaluated for its suitibility in ethanol production (SSF) or in cellulase production.

Lignocellulosics will be investigated with regard to the relationship between crystallinity, surface area, and particle size with respect to ethanol or cellulase production. Both individual and combined effects will be used to determine the attributes of a desirable lignocellulosic substrate. Crystallinity will be indexed by conventional powder X-ray diffraction. Particle size will be determined by Coulter Counter and Alpine sieves. Total surface area will be estimated with the use of a Coulter Counter and possibly, by gas adsorption. Reactive surface area may be evaluated through the use of a technique under development which uses purified cellulase system components that are either radiolabeled and/or reactive with specific antibodies.

Feedstock sterilization is imperative for cellulase production and desirable for ethanol production. Current process flow design employs distinct pretreatment and sterilization. A single simultaneous pretreatment/sterilization step is, however, desirable to reduce capital and operating expenses. The possibility of combining these two steps has been investigated previously in the cases of thermomechanical pulping and β-irradiation. Although sterilization of the lignocellulose was achieved in both cases, pretreatment was insufficient to

Enzyme Production

Determination of macromolecular response of *Trichoderma* to induction and repression substrates is important to the understanding of its metabolism. Quantification of DNA and RNA species, ribosomes, and protein change during the growth cycle will be included. Total carbon/nitrogen balances in response to induction and repression substrates will be examined. The effect and ultimate intracellular target of medium minerals will lead to a better understanding of their importance in the medium. Surfactants used in conjunction with antifoam agents often pose unknown problems in their reaction with the medium and hence, the culture. These combined effects will be examined in conjunction with enzyme production.

Fed batch enzyme production will be compared with continuous enzyme production based on productivity. Operational parameters will be subsequently modified to reduce complications which arise in this mode. Determination of residual sugar content as a function of time will be investigated. Evaluation of the efficiency of substrate utilization in regards to product formulation will lead to the development of an overall materials balance.

Productivity will be determined by varying the residence time and substrate concentration in continuous enzyme production. The effect of residual broth glucose on enzyme expression and activity in situ will help in modification of the parameters for continuous enzyme production. The information will be incorporated into a materials balance for the entire process. Cell recycle in enzyme production will be studied for its long range effects expecially in cultural senescence in extended continuous enzyme production.

The filter paper assay will continue under development so that it may be used in predicting the SSF results from enzyme produced in the produce the desired feedstock characteristics. Methods for achieving simultaneous pretreatment/sterilization which hold most promise would probably involve either steam and pressure or ionizing irradiation. Therefore, the cases of thermomechanical processes will be investigated.

Economic analyses indicate that increased SSF residence time has only a small effect on product cost. This factor may be exploited so that a more positive economic situation develops by eliminating expensive, energy intensive pretreatments by increased SSF residence time. Municipal solid waste, pulp mill waste, and agricultural waste will also be evaluated for the economics of ethanol production following relatively low cost pretreatments.

Simultaneous Saccharification Fermentation

Continuous SSF will be examined in order to determine the effects of employing enzyme and yeast recycle. Aspects such as aeration, temperature, and pH will be used to establish optimum residence time for the system. Investigation into enzyme recycle will be continued to determine the most efficient method of activity recovery. Approaches studied will include mechanical and chemical methods of enhancing soluble activity as well as the use of the solid residue as a vehicle for recycling cellulase activity. Increased SSF yields from yeast recycle incorporated with higher temperatures will be analyzed as to possible integration into the continuous SSF system. Evaluation of recovered yeast will also be related to increasing the value of the animal feed by-product.

The physiological and metabolic implications of ethanol inhibition of cellulose activity and decreased SSF yields due to yeast assimilation of ethanol will be addressed. Further work on microbial strain evaluation in SSF will be performed with emphasis on high temperature tolerant fermenting organisms. Investigation will involve utilization of hemicellulose for production of ethanol or other chemicals. The bioconversion of cellulosic material to other chemicals such as acetic acid, etc., will also be studied.

Treatment of whole culture enzyme inoculum in a manner that would solubilize more enzyme will include chemical and mechanical means of solubilization which will be integrated with the work in enzyme recycle. A materials balance of SSF will continue to be updated as changes occur which affect the process.

continuous mode and/or when MSW is the feedstock. New assays to cope with these problems will be developed with the aim of an overall correlation between enzyme assays.

Chemical sterilization is being evaluated to reduce the expense of steam sterilization. Identification of narrow spectrum sterilants will be integrated into other physical treatments to improve efficacy. The effect on various process streams will be followed from influent to effluent.

Medium development work will continue to lower costs and reduce adverse effects from corrosive elements. The fate of each species will be traced through the animal food by-product and consequent animal tissue fate. Accordingly, new and interesting microorganisms will be systematically evaluated as they arise from various sources.

By-Product Utilization

Further chemical analyses will be performed on the MSW to be used as a feedstock in the process. The components of any type of municipal waste vary from season to season. Samples of different batches of MSW will be taken over a given period of time. An analysis of the metal content and ROR content of these samples will be performed.

A similar protocol will be followed with MSW-grown enzyme. Several enzyme samples will be obtained from different enzyme production runs. These runs will utilize the MSW, analyzed separately, to see if any particular component in the MSW is affecting the enzyme production.

Chemical analyses of the fractions of the SSF slurry will be performed. In addition to repeating mineral and ROR analyses, CHN, protein, amino acid and lipid analysis of the residues will be performed. As with the raw materials, these tests will be performed on samples from different SSF runs to determine if wide variations exist from one run to another.

The rationale for using raw MSW in feeding studies was two-fold: 1) it was used in the absence of sufficient SSF residues for feeding trials and 2) any animal health or nutrition problem with the SSF residues would, in all likelihood, be attributed to the MSW substrate. The feeding study (already described) utilized raw MSW as part of chick diets. As a follow-up study, the MSW needs to be evaluated as a feedstuff for ruminant animals. Data for establishing diet formulation will come from preliminary *in vitro* and *in vivo* digestibility studies.

Approximately 8-10 successive SSF runs could generate sufficient SR to feed 8 chicks for 3-4 weeks. The SR will be fed at the level of 10-15% of the diet. This study could also incorporate the use of IR in the diets of additional birds. Since the SSF runs will yield much more IR than SR, the IR could be fed in the diet at several levels.

Since municipal waste is a feedstock for both the enzyme production and the SSF portion of the process, it is important to monitor what happens to its components during the process.

A simulated run through the entire process, keeping track of major substances of interest, will constitute a part of the materials balance study.

As previously mentioned, the IR also represents a potential feedstuff. Trials utilizing IR will be conducted in a manner similar to the trial using SR in diets. The IR (as well as SR) will be chemically characterized with respect to materials of interest or concern prior to the trial. Animal health and growth response would be observed during the experiment. Follow-up studies will include analysis of tissues for accumulation of substances such as refractory organics and selected minerals.

Alkali washes used in pretreatment of agricultural residues could be examined for the purpose of protein extraction. Incubation of various plant parts with caustic results in extraction of plant protein. Optimization of this protein extraction and coupling it with the cellulose to ethanol process in another area of research which is included in future plans.