SERI/TP-231-3029 UC Category: 61e DE86014505

Overview of the DOE/SERI Biochemical Conversion Program

John D. Wright

September 1986

Presented at the SERI Biochemical Conversion Program Review Meeting Golden, Colorado 22-23 July 1986

Prepared under Task No. 5250.10

# **Solar Energy Research Institute**

A Division of Midwest Research Institute

1617 Cole Boulevard Golden, Colorado 80401-3393

Prepared for the
U.S. Department of Energy
Contract No. DE-AC02-83CH10093

### NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, expressed or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

Printed in the United States of America Available from: National Technical Information Service U.S. Department of Commerce 5285 Port Royal Road Springfield, VA 22161

> Price: Microfiche A01 Printed Copy A02

Codes are used for pricing all publications. The code is determined by the number of pages in the publication. Information pertaining to the pricing codes can be found in the current issue of the following publications which are generally available in most libraries: Energy Research Abstracts, (ERA): Government Reports Announcements and Index (GRA and I): Scientific and Technical Abstract Reports (STAR): and publication, NTIS-PR-360 available from NTIS at the above address.

## OVERVIEW OF THE DOE/SERI

## BIOCHEMICAL CONVERSION PROGRAM

John D. Wright
Solar Energy Research Institute
1617 Cole Boulevard
Golden, Colorado 80401

#### INTRODUCTION

The Solar Energy Research Institute (SERI) manages a program of research and development on the biochemical conversion of renewable lignocellulosic materials to liquid fuels for the Department of Energy's Biofuels and Municipal Waste Technology Division. SERI was assigned management responsibility for this effort in 1978, and has developed a coordinated in-house and subcontracted effort. The Biochemical Conversion Program is mission oriented, and therefore, effort is concentrated on technologies which appear to have the greatest potential for being adopted by the private sector to economically convert lignocellulosic materials such as wood and agricultural residues into high value liquid transportation fuels such as ethanol.

### GOAL AND OBJECTIVES

The goal of the program is to provide the technology base for the conversion of renewable resources into high value liquid transportation fuels (primarily ethanol). The program is structured to supply the technology for such fuels to compete economically first as an octane booster or fuel additive, and, with additional improvements, as a neat fuel.

In order to achieve this goal, the objectives of the program are to:

- Define processes for the production of liquid transportation fuels that are competitive with conventional and emerging options in the open market,
- Improve the pretreatment, hydrolysis, and bioconversion processes through research so that ethanol can be produced economically from cellulosic biomass,
- Develop technologies for the conversion of underutilized portions of the lignocellulosic feedstock (xylan and lignin) to high value liquid transportation fuels,
- Evaluate the performance of the emerging process technologies in the smallest possible scale integrated experiment to assess their readiness for commercialization, and to better define research needs,
- Transfer the technology to the private sector for rapid commercialization by involving industry in the research process at the earliest possible time.

### PROGRAM SCOPE

The overall process for converting lignocellulosic materials to ethanol is shown in Figure 1. The raw biomass is collected and reduced in size, then sent to a hydrolysis process. In the hydrolysis section, the carbohydrate portions of the biomass (cellulose and hemicellulose) are broken down into simple sugars. This hydrolysis can be carried out by either acid or enzymatic means. The acid processes use relatively well developed and understood processes and offer the potential for relatively near term commercialization. The central problems are low yields caused by the simultaneous formation and degradation of the sugars (dilute acid processes), or large expenses due to acid consumption or recovery (concentrated acid processes). The enzymatic processes are less developed, but have the potential for better long-term economics. They first use a pretreatment step to open up the cellulosic matrix and render the substrate more accessible to the cellulose enzymes. Enzymes produced by either bacteria or fungi are used to hydrolyze the cellulosic fraction to glucose with no sugar degradation.

The dilute sugar solution from hydrolysis is fermented to produce an ethanol/water beer. The beer is distilled to purify the ethanol, the aqueous stillage stream cleaned up. The xylose fraction found in the hemicellulose of most and the water recycled. potential feedstocks is not fermentable with standard industrial yeasts and is either converted to methane by anaerobic digestion or concentrated and burned. Likewise, the lignin fraction is dried and burned. Technical and economic evaluations of the major acid and enzymatic hydrolysis methods were carried out on a consistent basis in a study earlier this year(1,2,3) and have lead to several major conclusions. The most important and least developed single process area is the hydrolysis section. The feedstock cost (the largest single component of the total selling price), is inversely proportional to the yield Yield also affects the overall plant size and capital of glucose from cellulose. investment. The size and capital cost, energy consumption, and operating costs of all the processes downstream of hydrolysis are inversely proportional to the concentration of the sugars leaving hydrolysis. Hydrolysis is the key to converting approximately two-thirds of the feedstock to fermentable simple sugars, and directly affects the design and cost of the balance of the plant. However, the technology is not well developed, and is often both expensive and inefficient. Development of low cost, high yield hydrolysis processes (and the associated pretreatment, and enzyme production steps) is therefore the first priority of the program.

An equally important realization is that the feedstock is poorly utilized. With present technology and markets, the xylan and lignin fractions are simply burned for their energy value, and the resulting steam is used to provide heat and electrical energy to run the process. At best, this results in only 50% of the feedstock being converted to sugars for production of the primary product, ethanol. In order for such processes to become economic, it will be necessary for one or both of these fractions to be converted to saleable commodities or fuels. Examples of this are the fermentation of xylose to ethanol (by alternate yeasts, bacteria, fungi, etc.) or higher alcohols, and the conversion of lignin to chemical intermediates or high octane fuels such as methyl aryl ethers. Therefore, research on utilization of the xylan and lignin fractions of the feedstock is an integral part of the program.

Other areas of the process, such as product separation, fermentation of the six carbon sugars, and waste treatment are minor cost items that use developed technology and do not require government funded research to prove their viability or to improve their operation.

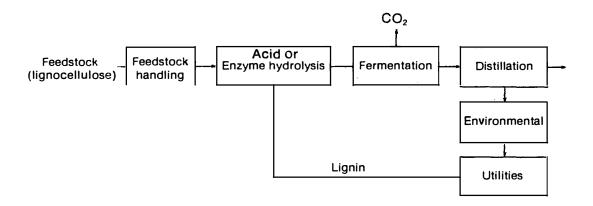


Figure 1. Block Diagram of Lignocellulose Hydrolysis Processes

### PROGRAM STRUCTURE

The Biochemical Conversion Program is structured around the processes we have identified as the most promising means of converting lignocellulosic materials to liquid fuels. By basing the program on a set of defined processes we are able to:

- Focus our effort on the most promising research areas so that we can make best use of our limited resources,
- Show and measure our technical progress,
- Set up a mechanism for the investigation, evaluation, and incorporation of new ideas into the program, and
- Foster better coordination and cooperation among the various subcontracted and inhouse research efforts.

A second result of the process orientation and the steady decrease in funding as the urgency of the energy "crisis" has receded, is that SERI will have prime responsibility for integrating the various research efforts, while the necessary research will be conducted in a coordinated fashion both at SERI and the subcontractors.

The four main research areas in the program are:

- Acid hydrolysis (plug flow dilute acid hydrolysis reactor (PFR), progressing batch dilute acid hydrolysis reactor (PBR), and concentrated sulfuric acid hydrolysis),
- Fungal Enzyme Hydrolysis (dilute acid pretreatment, enzyme production from <u>Trichoderma</u> mutants, simultaneous saccharification and fermentation, enzyme composition improvement),
- Xylan and Lignin Utilization (fermentation of xylose to ethanol, characterization and conversion of lignins to liquid fuel)
- Exploratory Research on processes which have the potential to substantially alter our basic process designs (anaerobic cellulase production, alternate fermentations, novel pretreatments, direct microbial conversion, solid state enzyme production).

The following sections describe our research efforts in each of these areas, the rationale for their inclusion in the program, and how they fit together with the other projects.

# Acid Hydrolysis

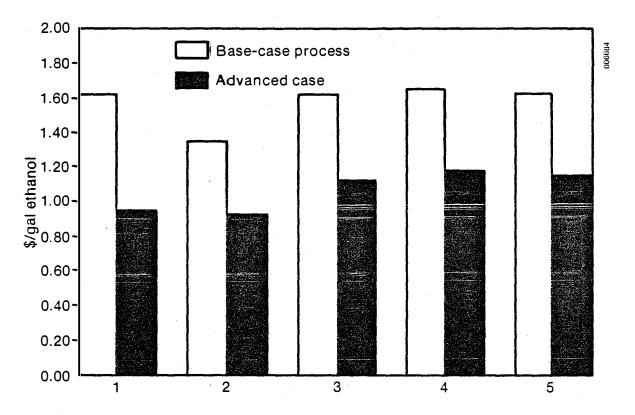
Acid hydrolysis technology holds the promise of economic production of ethanol, while providing a near-term option should oil prices once again spiral rapidly upwards. Acid hydrolysis processes are relatively well understood, and can largely make use of existing chemical process equipment. Most of these processes are now at the stage where integrated testing is required to establish their performance.

The economics of five major acid hydrolysis processes (at the current state of the technology) are shown in Table 1, while the relative economics of the current state of the art and processes incorporating most of the important potential process improvements, are shown in Fig. 2. In all cases, the single most important improvement is the incorporation of a xylose fermentation process. The progressing batch reactor has

Table 1. Cost of Production Summary for Lignocellulose to Ethanol Facilities with Various Hydrolysis Processes (25,000,000 gal/yr, mixed hardwood at \$42/ton)

	Cost of Production (@/gal Ethanol)						
	Plug Flow	Plug Flow*	Progressing Batch	Concentrated H <sub>2</sub> SO <sub>4</sub>	Liquid HCl	Liquid HF	
Raw Materials				·			
Wood	104.6	104.6	77.5	68.2	69.9	62.1	
Acid	6.5	6.5	6.5	17.4	11.9	39.9	
Lime	3.5	3.5	3.5	9.4	1.6	0.7	
Chemicals	3.5	3.5	2.6	2.3	2.3	2.1	
Utilities							
Water	0.9	0.9	1.1	1.3	0.9	0.5	
Steam	0.0	0.0	0.0	0.0	11.9	2.7	
Labor	7.2	7.1	6.0	7.6	7.4	5.9	
Overhead & Maintenance	34.0	33.7	28.6	35.9	35.3	27.9	
By-products							
Furfural	-70.5	0.0	-3.2	0.0	0.0	0.0	
Electricity	-35.7	-53.0	-40.7	<b>-37.3</b>	38.2	-30.2	
Annual Operating Cost	54.0	106.8	81.9	104.7	102.9	111.6	
Capital Charges (FCR=0.13)	60.0	59.4	50.3	63.2	62.1	49.2	
Ethanol Selling Price	114.0	166.2	132.2	167.94	165.0	160.8	

<sup>\*</sup>Furfural concentrated and burned for energy production.



- 1) Plug flow dilute sulfuric acid
- 2) Progressing batch dilute sulfuric acid
- 3) Low temperature concentrated sulfuric acid
- 4) Concentrated liquid HCI
- 5) Concentrated liquid HF

Basis: 25,000,000 gallons of ethanol per year Mixed hardwood feedstock at \$42/dry ton

Figure 2. Comparison of Acid Hydrolysis Processes for the Production of Ethanol and Electricity

the potential for producing ethanol for approximately 20% less than the other processes. The plug flow reactor has economics which are dominated by the value assigned to the furfural by-product. The concentrated acid processes all have high yields and low feedstock costs but suffer from the cost of acid consumption or acid recovery.

In dilute acid hydrolysis processes, the acid hydrolyzes cellulose to glucose while simultaneously degrading the product sugars. Thus sugar degradation and the consequent low yields are the principle problem. The progressing batch reactor system invented and under development at SERI uses several percolation reactors in series to achieve the benefits of counter current operation while retaining the simplicity and proven technology of the percolation reactor. This method quickly removes the sugars from the reaction zone, thus minimizing both sugar degradation and product dilution. This process has the potential to produce ethanol for 20% less than other acid hydrolysis technologies, but this potential must be proven experimentally.(4,5) The main tasks include determining how closely a real unit will be able to approach the results of the simplified theoretical models used to predict its performance and determining the clean-up processes and fermentation techniques necessary to efficiently ferment the hydrolyzate. This technology will be the main focus of the acid hydrolysis research task over the next few years.

The high temperature dilute acid plug flow reactor (originally developed at Dartmouth College, and now being scaled up at SERI) has low yields (55% of the crystalline cellulose is converted to glucose) but is capable of simultaneously converting the xylan fraction to furfural, a valuable chemical intermediate. The value assigned to the furfural greatly influences the process economics. Furfural production may provide a significant near-term advantage and highlights the importance of making beneficial use of all components of the feedstock. This task is focused on demonstrating the ability of this reactor system to process a feedstock with a 20% solids concentration, and demonstrating the technology at the smallest possible scale which allows the use of readily available processing equipment. Operation at such a solids concentration should be possible by carrying out a prehydrolysis step before the main hydrolysis, thus solubilizing the approximately 30% of the feedstock and rendering it more easily pumpable.(6) This concept was originated by Badger Engineers, Inc. (under contract to SERI), who are actively involved in this project, and in finding partners for the commercialization of the technology. This project is expected to be completed early in 1987.

The plug flow task is supported by research being carried out at Drexel University. This research looks at dilute sulfuric acid hydrolysis from a more fundamental basis than the largely empirical studies found in the literature. By incorporating information about the absorption of acid on the biomass, it is found that at low liquid to solid ratios, the liquid phase is depleted in acid, which has the effect of reducing the sugar degradation rate. This contract is designed to investigate this phenomena and determine whether it can be usefully incorporated into the design of dilute acid hydrolysis processes.(7)

In the concentrated sulfuric acid hydrolysis process, the concentrated acid breaks down the crystalline structure of the cellulose, rendering it amorphous and readily hydrolyzable at conditions where sugar degradation is minimal and yields approach 100%. The major barrier to economic success is the high cost of sulfuric acid consumption. Bench scale research is currently being conducted to identify methods for recycling the acid. Promising acid recycle technologies and other process improvements will be incorporated into a pilot scale facility constructed at the TVA facility in Muscle Shoals.(8) TVA and DOE are cost sharing this research as well as research in the areas of hydrolyzate and xylose fermentation. Acid recycle research is being carried out at Purdue University.

# Fungal Enzyme Hydrolysis

In the past years, SERI has funded research on a variety of enzymatic hydrolysis approaches, including separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and direct microbial conversion (DMC). Pretreatments studied have included steam explosion, dilute acid, organosolv, wet oxidation, and mechanical pretreatments. Many different enzyme preparations have been used as more advanced cellulases have been developed and different enzyme production options have been devised. As a result of this research and process evaluations recently carried out, the bulk of the research has been focused on a specific process using fungal enzymes from mutants of Trichoderma reesei in a SSF, with a dilute acid hydrolysis pretreatment.

The effect of the major potential improvements on the economics of a SHF process are shown in Fig. 3. Major improvements include xylose fermentation, improved cellulase mixtures, reduced enzyme loading, improved rate of enzyme production, reduced agitation power in hydrolysis, and credit for the high value use of lignins. Cumulatively, these improvements have the potential to cut the predicted cost of ethanol from enzymatic hydrolysis by a factor of three.(2)

#### Pretreatment

The most important characteristic of a pretreatment for enzymatic hydrolysis is its ability to produce an easily digestible pulp. The dilute acid pretreatment under investigation at SERI is capable of producing an easily digestible cellulose-lignin pulp at low temperatures (140-160°C), with low energy consumption and relatively low capital costs. By using a dilute sulfuric acid catalysis, hydrolysis of the xylan fraction occurs at low temperatures with only minor degradation of the xylose sugars. This results in clean pulp and five carbon sugar solutions and simplifies downstream processing of these fractions. This process produces a xylose solution which can be potentially fermented to ethanol, and a cellulose pulp for hydrolysis, and does not incur incremental costs for the recovery of lignin. The lignin produced after this process is more condensed than organosoly or steam explosion lignins, which may aid in enzyme recycle but decrease the value of the lignin as a separate product. Further research is necessary to clarify these issues.

Perhaps the most important outcome of the dilute acid pretreatment research is the realization that enzymatic digestibility is correlated with xylan removal (not lignin removal or degree of crystallinity). This realization helps to bring order to what has been something of a black art and may lead to rapid improvement in other pretreatment technologies as well as dilute acid processing. (9,10)

## CADCO

A project that cuts across the line of pretreatment and SSF enzymatic hydrolysis is the CADCO project. This project has the goal of testing pretreatments for enhancing the digestibility of municipal solid waste. This effort is directed at testing commercial scale equipment for inclusion in an MSW to ethanol process.

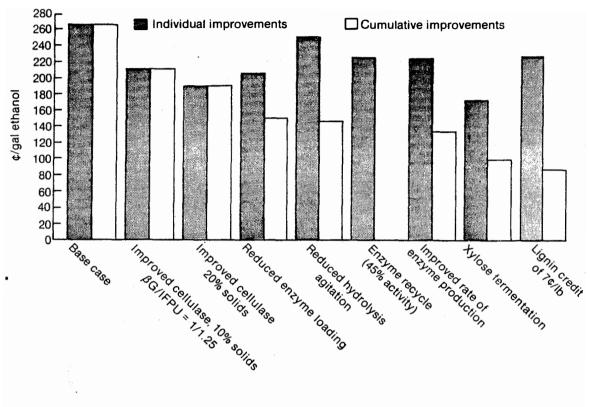


Figure 3. Effect of Potential Improvements, SHF Process (Steam Explosion)

# Enzyme Hydrolysis

The largest costs associated with enzyme hydrolysis are those of producing the enzyme. This results from the large amounts of catalyst consumed to overcome end product inhibition and the slow production rate of the processes currently defined. Enzyme consumption can be minimized by using enzyme mixtures which are less subject to end product inhibition, by continuously removing glucose and cellodextrins from the hydrolyzate (SSF), and by recycling enzyme. SSF processes are also seen as a means of improving the concentration of the product leaving the hydrolysis system. Other important issues include hydrolysis rate, yield, mixing power, and process design.

The composition of the enzyme mixture (relative proportion of endoglucanase, exoglucanase, and beta-glucosidase) has a dramatic effect on the rate and yield of enzymatic hydrolysis. Dekker (11) hydrolyzed steam exploded bagasse with equal enzyme loadings, but different enzyme preparations (QM9414, Rut-C30, Meicelase, Rut C30 plus supplemental beta glucosidose). Depending on the cellulose preparation used, the 48 hour yield ranged from 62 to 89%. Clearly, the development of better enzyme preparations has the potential for dramatic improvements in hydrolysis performance.

Investigations at several laboratories have shown a saturation enzyme loading of approximately 12 IFPU/gm cellulose. However, loadings much higher than this are commonly used in order to provide the beta-glucosidase necessary to overcome end product inhibition. Reduction in enzyme loading to the saturation value while retaining high hydrolysis rates and yields would have a large benefit. This can again be accomplished with improved cellulase preparations, but dramatic improvements can also be achieved by continuous removal of the end products via the SSF process. Recent SSF experiments at SERI have shown near theoretical yields of glucose with low enzyme loadings.

The product concentration from hydrolysis is also a major parameter which is improved by the SSF process. Recent experiments at SERI have achieved ethanol concentrations of greater than 4.5%, approximately twice the values generally reported in the literature for this process. Other issues being addressed are the recycle of the cellulase enzymes, and the effect of agitation on the hydrolysis process. (12)

## Enzyme Production

The high cost of enzyme production stems from the low volumetric productivity of the cellulase producing fungi and the consequent use of large and expensive fermenters. Secondary issues are power consumption for aeration, the removal of low level heat from agitation and metabolic processes, and the maintenance of the mono-culture during a long fermentation.

Enzyme production with <u>T. reesei</u> mutants is difficult because cellulase production is discontinued in the presence of easily metabolized substrates. Thus, most production work has been carried out with insoluble carbon sources such as steam exploded biomass or solka-floc. In such systems the rate of growth and cellulase production are limited because the fungi must secrete cellulase to carry out a slow enzymatic hydrolysis of the solid in order to obtain the necessary carbon. Efforts are underway at SERI and Lehigh University to increase productivity by decoupling the processes of growth and cellulase production, and by finding mutants which overproduce cellulase while growing on soluble substrates.

# Xylose and Lignin

Current state of the art designs for producing ethanol from lignocellulose do not make good use of the xylan found in the hemicellulose of many feedstocks or of the lignin. Therefore, a substantial portion of the Biochemical Conversion Program is focused on converting these fractions to fuels.

# **Xylose Fermentation Research**

Five carbon sugars (primarily xylose) make up 15-30% of the feedstock for hardwoods, agricultural residues, and herbaceous crops. Xylose is amorphous, readily hydrolyzed, and can easily be produced by virtually all processes. However, standard industrial yeasts such as Saccharomyces cerevisiae are not able to ferment xylose to ethanol. Although several bacteria are available to ferment xylose to higher alcohols, our research program is focused on producing ethanol from the xylose fraction. The five major approaches are: alternate yeasts, bacteria, fungi, extracellular xylose isomerase, and genetically altered industrial yeasts. Currently, none of these routes has the rate or ethanol tolerance of the standard six carbon sugar fermentation. However, progress in several areas has been rapid, and fermentation of xylose will greatly benefit the process even if it is not as good as the standard fermentation.

When the need for xylose fermentation became apparent, several groups began screening programs to search for xylose fermenting yeasts. Several such yeasts have been identified, the most notable being <u>Pachysolen tannophilus</u> and <u>Candida shehatae</u>. Optimization of culture conditions and other relatively simple steps have greatly improved the performance of these fermentations in the past few years. Work on five carbon yeast fermentations is currently being carried out at TVA.

Bacteria are capable of fermenting xylose to ethanol and other alcohols. Clostridium thermosaccharolyticum has been studied at MIT for over six years. Several of the mutants are capable of producing ethanol concentrations of 2%. One of the major drawbacks is the reduction in yield from the production of unwanted by-products such as lactic and acetic acid. Recent efforts have focused on identifying the pathways involved in by-product formation and removing the necessary enzymes from the organism.

Fungi, such as <u>Fusarium</u> which is being studied at Argonne National Laboratory and the strain NF1 which was isolated at SERI, also have the ability to ferment xylose. <u>Fusarium</u> fermentation yields are high at low ethanol concentrations, but the rates are low and yield is reduced when ethanol concentrations exceed 2-2.5%. NF1 has shown the ability to produce high (7.3%) concentrations of ethanol, but this fermentation is also very slow.

A fourth approach is to use cloning techniques to obtain overproduction of the enzyme xylose isomerase in <u>E. coli</u>. This enzyme catalyzes the isomerization of xylose to xylulose, another five carbon sugar, which can be fermented by standard yeasts. Overexpression has been achieved at SERI, and efforts are currently aimed at determining the optimal conditions and processes for its use. (13)

A final approach involves the cloning of the xylose isomerase gene from E. coli into a yeast. This work, carried out at Purdue, SERI and other laboratories proceeded much more slowly than anticipated and is no longer funded.

Progress in several of these areas has been rapid, and one or more approaches should be ready for commercialization within a few years. Still, there are more promising approaches than there are funds. To this end, a workshop on xylose fermentation has been scheduled on the day following this meeting to survey the state of the art and help prioritize our efforts in this area.

# Lignin Utilization Research

Lignin is a phenolic macromolecule, and as such cannot be fermented to ethanol. With present technology, the lignin is assumed to be burned to produce energy to run the conversion process. However, like xylose, conversion to a higher value fuel or chemical intermediate could greatly improve process economics. Current research is directed at understanding the effects of various pretreatment and lignin extraction techniques on the structure and potential uses of the lignin fraction, and at converting the lignin into a liquid transportation fuel/octane enhancer (methyl aryl ethers).

The potential uses and value of ligning from hydrolysis processes depend on the structure of the lignin after it is separated from the rest of the lignocellulose. In order to make efficient use of the lignin fraction, it is necessary to understand the relationship between the isolation method and the lignin properties, as well as the relationship between structure of the isolated lignins and the further processing required to upgrade it into a liquid fuel or chemical. Lignins from dilute acid prehydrolysis pretreatments will be compared with previously characterized lignins from steam explosion and organosolv pretreatments. Lignin characterization is made difficult because most of the previous methods were developed to analyze the higher molecular weight lignins typical of the softwood kraft pulping. Therefore, efforts on developing new analytical methods will continue. Methods used to date include (14) functional group analyses by chemical and spectroscopic methods, quantitative carbon-13 nuclear magnetic resonance, oxidative degradative analysis using liquid chromatography, gas chromatography, and mass spectro-The molecular weight distribution of lignin polymers is one of the main characteristics of the complex macromolecule. It changes markedly as a function of the pretreatment conditions. High performance size exclusion chromatography offers fast measures of these distributions. Problems do exist in finding suitable calibration polymers for a wide range of molecular weights. (15) Research addresses synthesis of model compounds for size exclusion chromatography that have structures similar to those present in lignins. A high field nuclear magnetic resonance method has been develoed to aid in interpretation of the fine structure of the macromolecule.(16)

Research is also conducted at SERI on the conversion lignin into methyl aryl ethers. These compounds are excellent non-metallic gasoline blending agents and octane enhancers. The production process hydrotreats slurried lignin in the presence of a catalyst to produce a mixture of phenolic compounds and hydrocarbons. This mixture could be subsequently thermally dealkylated to produce phenol and benzene (Hydrocarbon Research, Inc. Lignol process). Although phenols are good octane enhancers, they are not suitable for addition to gasoline because of their corrosive nature. The conversion of the phenols into the corresponding methyl aryl ethers avoids the corrosion problems and also the need to add a thermal dealkylation step to strip alkyl groups from the aromatic rings. A process to convert lignins into mixtures of monomeric phenolic compounds and hydrocarbons, and the conversion of the phenolic portion into the methyl aryl ethers, is under investigation at SERI. Both the liquid hydrocarbons and the MAE are high value octane enhancers. MAE have been prepared from coal liquids and tested at Gulf Oil Co. They were found to be fully compatible gasoline octane enhancers. Efforts are currently focused on identifying and testing catalysts for converting the low molecular weight lignins to the mixture of phenols/hydrocarbon, and on assessing the relationship of the

lignin feedstock structure on the final product slate. The catalyst research is done in cooperation with researchers at the Colorado School of Mines. Experiments are also being conducted to upgrade the highly condensed lignins from dilute acid prehydrolysis processes.

# Exploratory Research

A totally focused program runs the risk of leading down a dead-end path or being bypassed by advancing technology. Therefore, a portion of the program is invested in "Exploratory Research," research which, if successful, could fundamentally change the design of the processes we are working on, but whose outcome is not certain. Such concepts include the anaerobic production of cellulases, alternate fungal cellulase producers, solid state cellulase production, new fermentative organisms, new pretreatments, and identification or creation of organisms for direct microbial conversion. The majority of the work in this area will consist of small subcontracts, which will be competitively bid in late summer or early fall.

One program in this area is the investigation of Acidothermus cellulolyticus, a thermophilic, anaerobic, cellulose degrading organism isolated by SERI researchers in a hot springs at Yellowstone National Park. Effort is directed towards purifying and characterizing the cellulases produced by this microorganism to determine whether it has the potential for use as an improved enzyme production system. (15) This project is scheduled to wind down at the end of the fiscal year. A second such project is the research at Rutgers University to clone the genes for cellulase production into the fermentative bacteria Zymomonis mobilis. (16)

#### PROGRAM FUNDING SUMMARY

A final way of showing the priorities of the SERI program is to present the summary of how the FY86 Biochemical Conversion Program budget is being spent. Table 2 breaks the FY86 budget down by major technology area and in-house vs. subcontracted research. Acid and enzymatic hydrolysis processes each receive approximately 25% of the total budget. Utilization of the lignin and xylan fractions totals 20%. Management and analysis come to 12 and 6% respectively, while exploratory research come to 12% as well.

Looking to the next year is difficult, as the FY87 budget is not yet finalized. However, the percentage of effort devoted to enzyme production and hydrolysis will increase moderately, while the fraction of funds spent on acid hydrolysis and pretreatment will be reduced.

Table 2. FY86 Funding Summary by Technology (in K\$)

	SERI	Subcontract
Acid Hydrolysis	500	50
Plug Flow Reactor	500	50
Progressing Batch Reactor	250	4.56
TVA Concentrated Sulfuric Acid		150
Total Acid Hydrolysis	750	200
Enzymatic Hydrolysis		
Dilute Acid Pretreatment	300	
Enzyme Production	100	120
SSF	200	102
Enzyme Recycle	150	80
Liizyine Recycle	100	
Total Enzymatic Hydrolysis	750	302
Xylose Fermentation and Lignin Utilization		
Overproduction of Xylose Isomerase	205	25
Xylose Fermentation with Yeast	200	100
Xylose Fermentation with Fungi		30
Lignin Characterization	175	30
Fuels from Lignin	225	25
		20
Flow Organosolv Experiment	30	
Total Xylose and Lignin	635	180
Management	495	
Analysis	220	40
Exploratory Research		
Acidothermus	150	
Contracts to be let	130	378
Contracts to be let	<del></del>	310
Total Exploratory	150	378
Total Biochemical Conversion	3000	1100

- 1) Wright, J. D.; Power, A. J., 1986 (April) "Comparative Technical Evaluation of Acid Hydrolysis Processes for Conversion of Cellulose to Alcohol in Energy from Biomass and Wastes X," Washington D.C., SERI/TP-232-2957.
- 2) Wright, J. D.; Power, A. J., Douglas, L. J., 1986 (May), "Design and Evaluation of an Enzymatic Hydrolysis Process (Separate Hydrolysis and Fermentation)," Biotech. Bioeng. Symp. 16 (in press).
- 3) Karpuk, J. D.; Gaines, L., 1986 (April) "Fermentation of Lignocellulosic Feedstocks: Product Markets and Values," in Energy from Biomass and Wastes X," Washington, D.C.
- 4) Wright, J. D.; P. Bergeron, P. J. Werdene, 1985 (Sept.), "The Progressing Batch Hydrolysis Reactor." Presented at the American Chemical Society National Meeting, Chicago, IL.
- 5) Bergeron, P.; J. D. Wright, P. J. Werdene, 1986 (May), "The Progressing Batch Hydrolysis Reactor Single Stage Experiment," Biotech. Bioeng. Symp. 16 (in press).
- Brennan, A.; W. Hoagland, D. Schell, 1986 (May), "High Temperature Acid Hydrolysis of Biomass Using an Engineering Scale Plug Flow Reactor," Biotech. Bioeng. Symp. 16 (in press).
- 7) Terry, K. F., R. Mutharasan, and E. D. Grossman, 1983, Biotech. Bioeng. 25, 19.
- 8) Barrier, J. W. and G. E. Farina, 1985 (June), "Ethanol from Cellulosic Agricultural Feedstocks," presented at Biochemical Conversion Program Semiannual Review Meeting, Golden, CO.
- 9) Grohmann, K.; R. Torget, M. Himmel, 1985, "Optimization of Dilute Acid Pretreatment of Biomass for Enhanced Enzymatic Digestibility of Biomass," Biotech. Bioeng. Symp., 15, 59-80.
- 10) Grohmann, K.; R. Torget, M. Himmel, "Dilute Acid Pretreatment of Biomass at High Solids Concentrations," Biotech. Bioeng. Symp. 16 (1986) in press.
- Dekker, R.F.H.; Walls, A.F.A, 1983, "Enzymatic Saccharification of Sugar Cane Bagasse Pretreated by Autohydrolysis-Steam Explosion," Biotech. Bioeng. 25, pp. 3027-3048.
- 12) Wyman, C. E.; D. D. Spindler, K. Grohmann, and S. M. Lastick, 1986 (May), "Simultaneous Saccharification and Fermentation of Cellulose with the Yeast Brettanomyces clausenii," Biotech. Bioeng. Symp., 16. (1986) in press.
- 13) Lastick, S. M.; M. Y. Tucker, V. Mackedonski, and K. Grohmann, 1986, Biotech. Let. 8, 16.

- (14) Chum, H. L., D. K. Johnson, M. Ratcliff, S. Black, H. A. Schroeder, and K. Wallace, 1985. "Comparison Between Lignins Produced by Steam Explosion and Organosolv Pretreatments." International Symposium on Wood and Pulping Chemistry, Vancouver, August 26-30, 1985. Proceedings of the Papers, pp. 223-227.
- 15) Chum, H. L.; D. Johnson, M. Tucker, M. Himmel, 1986 (Dec.), "Some Aspects of Lignin Characterization by High Performance Size Exclusion Chromatography Using Styrene Divinylbenzene Copolymer Gels," Holzforschung (in press).
- (16) Hawkes, G. E., C. Z. Smith, J. H. P. Utley, H. L. Chum, 1986. "Key Structural Features of Acetone-Soluble Phenol-pulping Lignins by <sup>1</sup>H and <sup>13</sup>C n.m.r. Spectroscopy," Holzforschung (Gorins Commemorative issue).
- 17) Mohagheghi, A.; K. Grohmann, M. Himmel, L. Leighton, and D. M. Updegraff, "Isolation and Characterization of a New Genus of Thermophilic, Acidophilic, Cellulolytic Bacteria, Acidothermus Cellulolyticus gen. nov.; sp nov.," Int. J. Syst. Bacteriol, 1986 (in press).
- (18) Shalita, Z. P., B. S. Yablonsky, M. M. Dooley, S. E. Buchholz, S. K. Kahrs, K. Murphy Holland, D. E. Eveleigh, "Genetic Engineering of Bacteria for Alcohol Fuel Production," in Energy from Biomass and Wastes X, Washington, D.C., 1986 (April).