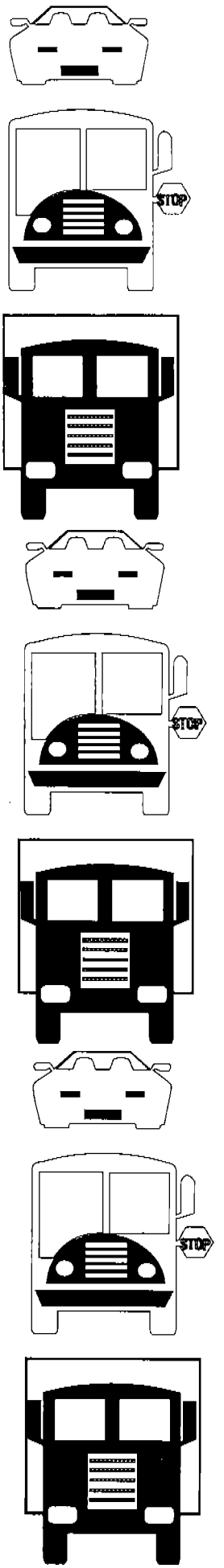

Biofuels
Project Summaries

Research Summaries
Fiscal Year 1992

Produced by the
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Golden, CO 80401-3393

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Contents

Preface

Domestic transportation fuels are almost exclusively derived from petroleum and account for about two-thirds of total U.S. petroleum consumption. In 1990, more than 40% of the petroleum used domestically was imported. Because the United States has only 5% of the world's petroleum reserves, and the countries of the Middle East have about 75%, U.S. imports are likely to continue to increase. With our heavy reliance on oil and without suitable substitutes for petroleum-based transportation fuels, the United States is extremely vulnerable, both strategically and economically, to fuel supply disruptions. In addition to strategic and economic affairs, the environmental impacts of our use of petroleum are becoming increasingly evident and must be addressed.

The U.S. Department of Energy's (DOE's) Office of Energy Efficiency and Renewable Energy (EE), through its Biofuels Systems Division (BSD), is addressing these issues. The BSD is aggressively pursuing research on biofuels—liquid and gaseous fuels produced from renewable domestic feedstocks such as forage grasses, oil seeds, short-rotation tree crops, agricultural and forestry residues, algae, and certain industrial and municipal waste streams. The research sponsored and funded by the BSD focuses on the domestic production, recovery, and conversion of these feedstocks to economically priced, environmentally beneficial fuels such as ethanol, methanol, and biodiesel. Producing ethers for oxygenated reformulated gasoline is also included in BSD's research.

The BSD is part of DOE's Office of Transportation Technology and is organizationally assigned to the Assistant Secretary for EE. Day-to-day research activities are managed by the National Renewable Energy Laboratory in Golden, Colorado, and Oak Ridge National Laboratory in Oak Ridge, Tennessee.

A strong DOE research role, in partnership with the nation's industrial and agricultural sectors, will be crucial in bringing competitively priced biofuels to the marketplace. The BSD goal is to develop technologies that are competitive with fossil fuels, in both cost and environmental performance, by the end of the decade. This goal is consistent with the foundation laid by the *National Energy Strategy* and the legislative mandates of the 1988 Alternative Motor Fuels Act and the Clean Air Act Amendments of 1990.

To accomplish the entry of these alternative fuels into the marketplace and promote their use, BSD has formulated a strategy that provides for

- The technology base for the private sector to increase sustainable supplies of biomass feedstocks suitable for economic conversion processes
- The technology base for the thermochemical and biochemical conversion of biomass feedstocks to liquid fuels
- The early transfer of technology to private industry by involving industry in cost-shared projects that will verify the technical feasibility and environmental benefits of biofuels technologies.

Since the inception of the program, the government and the private sector have worked together to develop an extensive biofuels knowledge base. This technical information has helped in identifying technical barriers to cost competitiveness, in determining priorities among the viable biofuels research options, and has brought us closer to entering commercial markets. Such technology transfer will continue to help build a strong U.S. biofuels industry, increase our economic competitiveness, reduce our dependence on foreign oil imports, and help maintain our environmental integrity.

The following is an account of the ongoing research sponsored by the DOE BSD. A summary sheet is presented for each project funded and/or in existence during Fiscal Year 1992 (October 1, 1991 through September 30, 1992). Each summary sheet contains an account of project funding, objectives, accomplishments and current status, and significant publications.

Ethanol

Purification of Cellulase, *M. bispora*

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
Rutgers, The State University
Piscataway, NJ 08855

Principal Investigator: D. Eveleigh

Telephone: (908) 932-9829

Contract Number: RN-1-11201

Contract Period: 01/92 – 01/93

Contract Funding (Source):
FY 1991: $89,979 (DOE)

Objective:

To evaluate and develop the expression of *M. bispora* endoglucanases and exoglucanases in rapidly growing bacteria. These enzymes will also be purified by classical methods in protein biochemistry for subsequent kinetic and biophysical characterization.

Approach/Background:

An immediate goal of the Biofuels Program is the expression of cellulase genes in efficient, rapidly growing bacterium. *Microbispora bispora* is a thermostable bacterium that produces endoglucanases of very high thermal stability. *M. bispora* does not appear to produce high-molecular-weight cellulase complexes (known as cellulosomes). Many bacteria produce these cellulosomes, and, as a result, are poor candidates for genetic engineering projects aimed at the production of improved cellulase enzymes. *M. bispora* should therefore be carefully explored as a potential source of cellulase-producing genes for NREL's genetic engineering work.

Status/Accomplishments:

The development of an efficient expression system for *M. bispora* enzymes is ongoing.

Major Project Reports:

Eveleigh, D., *"Purification of Cellulase, M. bispora."*

Summary Date: December 1992

Purification of Cellulase, *T. maritima*

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
Rutgers, The State University
Piscataway, NJ 08855

Principal Investigator: D. Eveleigh

Telephone: (908) 932-9829

Contract Number: RN-1-11201

Contract Period: 01/92 – 01/93

Contract Funding (Source):
FY 1991: $89,979 (DOE)

Objective:

To evaluate and develop the expression of *T. maritima* endoglucanases and exoglucanases in rapidly growing bacteria. These enzymes will also be purified by classical methods in protein biochemistry for subsequent kinetic and biophysical characterization.

Approach/Background:

An immediate goal of the Biofuels Program is the development of efficient cellulase enzyme expression systems. *T. maritima* is a thermostable bacterium that produces endoglucanases of very high thermal stability and high activity. This bacterium should therefore be carefully explored as a potential source of cellulase-producing genes for NREL's genetic engineering work.

Status/Accomplishments:

The development of efficient expression systems for *T. maritima* cellulases is ongoing.

Major Project Reports:

Eveleigh, D., "Purification of Cellulase, *T. maritima*."

Summary Date: December 1992

Biocatalyst Development: Enzymology

Directing Organization:
U.S. Department of Energy
1000 Independence Avenue, SW
Washington, DC 20585

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: M.E. Himmel

Telephone: (303) 231-1799

Contract Number: In-house

Contract Period: 10/91 – 09/92

Contract Funding (Source):
FY 1990: $400,000 (DOE)
FY 1991: $600,000 (DOE)
FY 1992: $600,000 (DOE)

Objective:

To identify the most effective cellulase system and component enzymes for the production of ethanol from biomass. The work objectives in FY 1992 were focused on the critical examination of the thermal stable cellulases produced by *Acidothermus cellulolyticus* to determine suitability. A secondary objective was the development of a fundamental level of understanding of the pathways of denaturation for a critical fungal cellulase enzyme, cellobiohydrolase I (CBH I).

Approach/Background:

Experimental criteria have been designed at NREL to permit the accurate comparison of cellulase enzymes from well-studied or unknown systems.

Cellulase degradation is accomplished through the action of (at least) three different enzymatic activities: β-1,4-endoglucanase, β-1,4-exoglucanase, and β-D-glucosidase. Our assessment of cellulases being collected from various sources will rely heavily on a careful analysis of the synergistic effect exhibited by combinations of purified native and recombinant enzymes.

Status/Accomplishments:

Following purification, approximately 1.5 mg of the endoglucanase (E1) from *A. cellulolyticus* was prepared and subjected to key biophysical analyses, including the determination of the N-terminal amino acid sequence, the amino acid sequence of two internal proteolytic fragments, the definitive molecular weight, the amino acid composition, and an activity profile. The description of E1 has been filed with the U.S. Patent Office as a Divisional Amendment to patent No. 5,110,735.

The denaturation of *Trichoderma reesei* cellobiohydrolase I (CBH), under conditions of both thermal and pH stress, has been monitored by measuring changes in the polarization of tryptophan fluorescence as the temperature is increased at selected pH values. An important observation was that the temperature of the midpoint of the fluorescence polarization transition tracks with the smaller (lower-temperature) differential scanning calorimetry (DSC) component peak obtained by mathematical deconvolution and originally observed in FY 1990. The existence of this smaller endothermic transition has, until now, been inferred only from the asymmetry of the overall DSC denaturation endotherm. This information provides documentation for the behavior of native (unaltered) CBH I during the denaturation process.

Major Project Reports:

Baker, J.O., K. Tatsumoto, K. Grohmann, J. Woodward, J.M. Wichert, S.P. Shoemaker, and M.E. Himmel, "Thermal Denaturation of *Trichoderma reesei* Cellulases Studied by Differential Scanning Calorimetry and Tryptophan Fluorescence."

Himmel, M.E., W.S. Adney, and J.O. Baker, "Cellulase Assays: A Review."

Himmel, M.E., W.S. Adney, D.J. Mitchell, and J.O. Baker, "Isolation and Characterization of Two Forms of β-D-Glucosidase from *Aspergillus niger*."

Tucker, M.P., K. Grohmann, A. Mohagheghi, and M.E. Himmel, "Thermostable Purified Endoglucanase from Thermophilic Bacterium *Acidothermus cellulolyticus*."

Summary Date: December 1992

Recombinant *T. fusca*

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
Cornell University
Division of Biological Sciences
Biotechnology Building
Ithaca, NY 14853

Principal Investigator: D.B. Wilson

Telephone: (607) 255-5085

Contract Number: XZ-2-17019

Contract Period: 11/91 – 11/92

Contract Funding (Source):
FY 1991: $94,878 (DOE)

Objective:

To evaluate and develop the expression of *T. fusca* endoglucanases and exoglucanases in rapidly growing bacteria. These enzymes will also be purified by classical methods in protein biochemistry for subsequent kinetic and biophysical characterization.

Approach/Background:

An immediate goal of the Biofuels Program is the expression of cellulase genes in efficient, rapidly growing bacterium. *Thermomonospora fusca* is a thermostable bacterium that produces endoglucanases of very high thermal stability. *T. fusca* does not appear to produce high-molecular-weight cellulase complexes (known as cellulosomes). Many bacteria produce these cellulosomes, and, as a result, are poor candidates for genetic engineering projects aimed at production of improved cellulase enzymes. *T. fusca* should therefore be carefully explored as a potential source of cellulase-producing genes for NREL's genetic engineering work. The subcontractor has already successfully demonstrated the expression of one of the *T. fusca* endoglucanases.

Status/Accomplishments:

The subcontractor has produced several transformed bacteria capable of expressing a number of the endoglucanase enzymes from *T. fusca*. Several of these recombinant enzymes have been delivered to NREL for testing and characterization.

Major Project Reports:

Wilson, D.B., "Recombinant *T. fusca*."

Summary Date: December 1992

Biocatalyst Development: Genetics

Directing Organization:
U.S. Department of Energy
1000 Independence Avenue, SW
Washington, DC 20585

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigators: S.R. Thomas and M.E. Himmel

Telephone: (303) 231-7627

Contract Number: In-house

Contract Period: 10/91 – 09/92

Contract Funding (Source):
FY 1990: $400,000 (DOE)
FY 1991: $600,000 (DOE)
FY 1992: $800,000 (DOE)

Objective:

To develop, through recombinant technology, cost-effective, highly active cellulase enzymes for the enzymatic hydrolysis of lignocellulosic biomass.

Approach/Background:

The cellulase enzymes currently used to hydrolyze lignocellulosic biomass are derived from mutants of slow-growing fungi. Cloning selected cellulase genes from other microorganisms into a very rapidly growing host (i.e., a bacterium) will permit the production of the most highly active cellulase systems known. The ability to select and overproduce these cellulases will significantly lower the cost of production of cellulases for use in the hydrolysis of lignocellulosic biomass and consequently reduce the cost of production of ethanol for fuels. In FY 1992, the goal of this subtask was to identify and characterize the genes encoding the thermal stable endoglucanases produced by *Acidothermus cellulolyticus* so that more efficient expression systems than that of the natural source may be developed. Gene cloning and characterization has been investigated using a combination of two different approaches: (1) *A. cellulolyticus* DNA fragments were ligated into lambda phage vectors and screened for endoglucanase activity and (2) the purified and characterized E1 endoglucanase (U.S. patent pending) and the N-terminal peptide have been used to produce antibodies and oligonucleotide probes, respectively, to help in characterization of the gene and gene product.

Status/Accomplishments:

In FY 1992, two additional lambda phage isolates were identified at NREL for their ability to express *A. cellulolyticus* endoglucanase activity, for a total of six such clones. Both restriction mapping and DNA hybridization analysis demonstrated distinct differences among the six clones.

Major Project Reports:

Laymon, R.A., S.R. Thomas, M.P. Tucker, T. Vinzant, and M.E. Himmel, "Codon Usage in *Acidothermus cellulolyticus*: Preliminary Comparisons."

Summary Date: December 1992

Biochemistry of Cellulase/Substrate Interactions

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
Oak Ridge National Laboratory
Oak Ridge, TN 37381

Principal Investigator: J. Woodward

Telephone: (615) 574-6826

Contract Number: DG-1-11138-1

Contract Period: 09/91 – 12/92

Contract Funding (Source):
FY 1990: $99,800 (DOE)

Objective:

To purify the major components of the fungal cellulase enzyme complex, including their core and cellulose-binding domains (CBDs), from *Trichoderma reesei* by using traditional biochemical methods. The purified CBDs will be used to conduct classical, competitive protein-binding assays on cellulose surfaces. Various chemical agents will be used to elucidate the chemical nature of the CBD binding site.

Approach/Background:

The nature of the chemistry at the site of interaction between the binding regions of cellulases and the substrate is currently unknown. Some theories contend that the stereochemistry of the cellobiohydrolase (CBH) binding site created by endoglucanase (EG) action necessitates the complexity observed in fungal CBH and EG enzymes. The elucidation of the roles played by cellulase component enzymes would significantly aid the development of "super" EG and CBH enzymes through enzyme engineering. The immediate goal of this project is to improve the level of understanding of the function of the cellulase enzyme components in relation to their structure, which includes a catalytic core and a CBD.

Status/Accomplishments:

Purification techniques have been developed for CBH I and II and for EG1. Furthermore, protocols have been developed for purification of the CBH core proteins. Samples of the purified core proteins have been sent to NREL for evaluation and for N-terminal peptide sequencing. Preliminary biochemical characterization of the core proteins has been completed.

Major Project Reports: None

Summary Date: December 1992

Characterization of Endo-1,4-β-glucanase Activity by Viscometry

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
University of California at Davis
258 Cruess Hall
Davis, CA 95616

Principal Investigator: S. Shoemaker

Telephone: (916) 752-2922

Contract Number: XA-1-11225

Contract Period: 10/91 – 11/92

Contract Funding (Source):
FY 1991: $116,790 (DOE)

Objective:

To develop a standard protocol for viscometric endoglucanase assay using modern rheological methods. These methods will be used to study the properties of selected, purified endoglucanases so that cellulase assessment activities will be better able to define ideal cellulase systems for process application.

Approach/Background:

Assays used to measure the activity of cellulases have always been somewhat problematic. Yet such measurements are critical to the development of new cellulase enzymes. For some years, the use of viscometric measurements (i.e., measuring changes in viscosity as cellulases breakdown cellulose polymers) has been shown to be highly sensitive. Some problems still exist with this type of assay. This study addresses some of these issues as part of an effort to develop a practical, sensitive, and accurate methodology for the analysis of endoglucanase (a type of cellulase) activity through viscometry.

Status/Accomplishments:

A thorough literature review on the state of the art for viscometric assay measurements has been completed. Preliminary development work on a viscometric assay has been completed. Protocols have been transmitted to and tested at NREL. NREL testing has confirmed the high sensitivity of the assay.

Major Project Reports:

Shoemaker, S., "Characterization of Endo-1,4-β-Glucanases by Viscosity."

Summary Date: December 1992

Development of Ethanologenic *E. coli*

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
University of Florida–IFAS
2029 McCarty Hall
Gainesville, FL 32611

Principal Investigator: L. O'Neal Ingram

Telephone: (904) 392-1906

Contract Number: DE-ACO2-83CH10093

Contract Period: 09/90 – 11/91

Contract Funding (Source):
FY 1990: $20,000 (DOE)
FY 1991: $ 5,000 (DOE)

Objective:

To develop a transformed strain of *E. coli* that can rapidly ferment xylose to ethanol in high yields, at final concentrations of 3% to 4% by weight. This organism will be an improved version of a similar recombinant strain already covered by patents to L. Ingram.

Approach/Background:

Recently, a genetically engineered bacterium incorporating useful traits from *E. coli* and *Z. mobilis* has been produced that can efficiently ferment both six-carbon and five-carbon sugars to ethanol. Patents related to this portable pathway in recombinant organisms have been granted for this work. The ethanologenic pathway from *Z. mobilis* utilizes three isoenzymes: (1) pyruvate decarboxylase, (2) alcohol dehydrogenase I (ADH I), and (3) alcohol dehydrogenase II (ADH II). Although both ADH I and ADH II are of equal importance in ethanol production, only ADH II has been expressed in the current recombinant *E. coli* used for ethanol production. The goal of this work is to produce a recombinant strain that expresses all three enzymes from *Z. mobilis*.

Status/Accomplishments:

New strains have been successfully constructed that have reduced levels of acid production (an undesirable by-product of fermentative metabolism of sugars). These have been developed using three strategies: (1) increased expression of alcohol dehydrogenase (by including expression of both ADH I and ADH II), (2) addition of plasmid-borne copies of ADH II and pyruvate decarboxylase to increase expression levels, and (3) inactivation of competing pathways. Preliminary evaluations indicate that some of these strains may be superior for ethanol production from six- and five-carbon sugars.

Major Project Reports:

Ingram, L.O. and K.T. Shammugam, "Development of Ethanologenic *E. coli* Expressing the Complete Ethanol Pathway from *Z. mobilis*."

Summary Date: December 1992

Plasmid Stability and Enzyme Activity in Recombinant *E. coli*

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
Virginia Polytechnic Institute and State University
301 Burruss Hall
Blacksburg, VA 24061

Principal Investigator: M. Potts

Telephone: (703) 231-5745

Contract Number: XK-7-07031-3

Contract Period: 08/87 – 12/92

Contract Funding (Source):
FY 1987: $69,832 (DOE)
FY 1990: $25,392 (DOE)
FY 1992: $21,367 (DOE)

Objective:

To optimize the production of xylose isomerase from recombinant *E. coli.*

Approach/Background:

Several options for developing a process that can convert the hemicellulosic sugars (primarily xylose) to ethanol are being developed. One process is known as Simultaneous Fermentation Isomerization of Xylose (SFIX). SFIX uses the enzyme xylose isomerase in conjunction with conventional ethanol-producing yeast to convert xylose to ethanol. This subcontract involves the optimization of xylose isomerase production from recombinant bacteria.

Status/Accomplishments:

Optimization of growth of recombinant *E. coli* based on fermentation conditions and feeding protocols has been completed. Work is now focused on achieving good overexpression of the enzyme during growth.

Major Project Reports: None

Summary Date: December 1992

Modeling/Optimize Dilute Acid Pretreatment

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
Auburn University
202 Samford Hall
Auburn University, AL 36849

Principal Investigator: Y.Y. Lee

Telephone: (205) 844-2019

Contract Number: XD-1-11121-1

Contract Period: 07/91 – 10/93

Contract Funding (Source):
FY 1990: $ 87,000 (DOE)
FY 1992: $135,861 (DOE)

Objective:

To develop an understanding of the effects of operating variables on yields of hemicellulose-derived sugars in NREL's Two Stage Dilute Acid Process. Through experimental and computer-modeling studies, the inter-relationship between particle size of feedstock, temperature, residence time, concentration of acid, and the yield of hemicellulosic sugars will be determined.

Approach/Background

Pretreatment is one of the key elements in the biomass-to-ethanol conversion process. The primary purpose of this step is to make cellulose in the biomass more amenable to enzymatic breakdown to its component sugars (which are then fermented to ethanol). This step also releases hemicellulosic sugars. The intent of this project is to analyze kinetics and physical aspects of the pretreatment to achieve the best possible yield of hemicellulosic sugars and enzymatically accessible cellulose. In particular, the subcontractor is focusing on a multistage version of the conventional dilute acid pretreatment process developed at NREL.

Status/Accomplishments:

A kinetic model describing the release of hemicellulosic sugars during dilute acid pretreatment has been developed. The kinetic model, based on experimentally derived kinetic parameters and theoretical kinetic analysis, has demonstrated that the use of a multistage pretreatment scheme allows for variable temperature profiles that enhance the process performance.

Major Project Reports:

Lee, Y.Y., B.J. Kim, and R. Torget, "On Optimal Temperature Policy of Percolation Process as Applied to Dilute-Acid Hydrolysis of Biphasic Hemicellulose."

Summary Date: December 1992

Engineering Research—Pretreatment

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigators: D. Hsu and R. Torget

Telephone: (303) 231-1878

Contract Number: In-house

Contract Period: 01/92 – 01/93

Contract Funding (Source):
FY 1992: $375,000 (DOE)

Objective:

To develop efficient, cost-effective pretreatment methods for enzymatic hydrolysis of lignocellulosic biomass.

Approach/Background:

Cellulose and hemicellulose in cell walls of hardwoods and herbaceous plants are not very digestible by cellulase enzymes. The lignocellulosic biomass has to be pretreated by chemical and mechanical means to increase enzymatic digestibility of cellulose. Dilute acid pretreatment was previously shown to be an effective and economically viable pretreatment for aspen wood and wheat straw, several grasses, and some fast growing hardwoods.

Status/Accomplishments:

A bench-scale percolation reactor system was designed and fabricated. The reactor system is being used to study a two-temperature, reverse-flow, dilute-acid process for prehydrolysis of lignocellulosic biomass. The process involves subjecting the biomass to a lower temperature to hydrolyze the easily hydrolyzable xylan, and later to a higher temperature to hydrolyze the remaining xylan that is more recalcitrant. Initial hydrodynamic studies with the reactor indicated nonideal flow patterns compared with ideal plug flow. These flow patterns were characterized and quantified in terms of a residence-time distribution function. Preliminary prehydrolysis results with the percolation reactor indicate that nearly 85% of the theoretical xylan can be hydrolyzed to xylose or oligomers of xylose using a single temperature for prehydrolysis. It has been predicted, using a model being developed at Auburn University, that xylose yields of 90% or higher can be achieved; the initial estimates for the two temperatures were 140° and 170°C. Studies are currently under way to confirm these predictions. The prototype reactor system and the mathematical model provide, in combination, an elegant and powerful tool for optimizing the process.

Major Project Reports:

Torget, R., M. Himmel, and K. Grohmann, "Dilute Acid Pretreatment of Two Short Rotation Herbaceous Crops."

Kim, B.J., Y.Y. Lee, and R. Torget, "On Optimal Temperature Policy of Percolation Process as Applied to Dilute-Acid Hydrolysis of Biphasic Hemicellulose."

Summary Date: December 1992

Development of Cellulose Conversion

Directing Organization:
U.S. Department of Energy
1000 Independence Avenue, SW
Washington, DC 20585

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: G. Philippidis

Telephone: (303) 231-1256

Contract Number: DE-AC02-83CH10093

Contract Period: 01/92 – 01/93

Contract Funding (Source):
FY 1991: $230,000 (DOE)
FY 1992: $295,000 (DOE)

Objective:

To design and optimize the hydrolysis and fermentation of cellulose to ethanol for the development of biomass-to-ethanol conversion technology.

Approach/Background:

Cellulose, the most abundant renewable carbohydrate polymer on earth, is the major component of lignocellulosic biomass and can be converted efficiently to fuel ethanol using cellulase enzymes and fermentative organisms in a single step—the simultaneous saccharification and fermentation process (SSF). Improvement of the SSF performance is imperative to enhance the technical feasibility and economic viability of the biomass conversion technology. Hence, a preliminary mathematical model of the SSF process was developed last year to support further improvements in the current technology. The model was based on considerations of the characteristics of lignocellulose; the intrinsic kinetic properties of the enzymes; the inhibitory effects of cellobiose, glucose, and ethanol on enzyme activity; and the adsorptive losses of the enzymes to lignin.

Status/Accomplishments:

The preliminary model for the simultaneous saccharification and fermentation process was improved, and key parameters were determined through critical experimentation. The model was improved by taking into consideration the dependence of the rate of cellulose hydrolysis on the surface area and structure of the lignocellulosic substrate. All parameters regarding (1) the growth and ethanol production characteristics of the fermentative organism and (2) the kinetic behavior of the hydrolytic enzymes employed in SSF (namely, cellulase and β-glucosidase) were determined. The model was successful in depicting the progress of the enzymatic hydrolysis of cellulose to glucose and the fermentative conversion of glucose to ethanol. Although not fully calibrated yet, the model is already used in various applications, such as the design of a continuous SSF process and the evaluation of ethanol removal schemes during the SSF.

Major Project Reports:

Philippidis, G.P., "Conversion of Cellulosic Biomass to Ethanol."

Philippidis, G.P., T.K. Smith, and C.E. Wyman, "Study of the Enzymatic Hydrolysis of Cellulose during the Simultaneous Saccharification and Fermentation Process for Production of Fuel Ethanol."

Philippidis, G.P., D.D. Spindler, and C.E. Wyman, "Mathematical Modeling of Cellulose Conversion to Ethanol by the Simultaneous Saccharification and Fermentation Process."

Philippidis, G.P. and C.E. Wyman, "Production of Alternative Fuels: Modeling of Cellulosic Biomass Conversion to Ethanol."

Philippidis, G.P. and C. E. Wyman, "Technology for Fuel Ethanol Production from Cellulosic Biomass."

Schell, D.J., J.D. McMillan, G.P. Philippidis, N.D. Hinman, and C. Riley, "Ethanol from Lignocellulosic Biomass."

Summary Date: December 1992

Advanced Cellulose Conversion

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
Dartmouth College
P.O. Box 7
Hanover, NH 03755

Principal Investigator: L. Lynd

Telephone: (603) 646-2231

Contract Number: XD-1-11068-1

Contract Period: 05/91 – 09/92

Contract Funding (Source):
FY 1991: $119,662 (DOE)
FY 1992: $101,656 (DOE)

Objective:

To evaluate and compare the feasibility and applied potential of solids processing via the upflow solids retaining bioreactor (USRB) concept for both the direct microbial conversion (DMC) and simultaneous saccharification and fermentation (SSF) process configurations. Both the yeast/*T. reesei* cellulase system and the thermophilic DMC will be used, because of the applicability of the USRB to both DMC and SSF.

Approach/Background:

One of the major obstacles to widespread utilization of ethanol as a transportation fuel is the cost of the biologically mediated steps for conversion of lignocellulosic biomass to ethanol. Several process concepts have evolved that are intended to reduce this cost. These include DMC and SSF. Thermophilic bacteria are most frequently considered for use in the DMC process. Although less developed, the DMC process has the potential to be the lowest cost alternative. The primary goal of this work is to evaluate and compare the feasibility and potential of solids processing via the USRB concept for both process alternatives.

Status/Accomplishments:

Experiments have been conducted to determine optimum nutrient requirements for ethanol conversion. Some continuous fermentation studies have been conducted on wood flour (a highly milled wood). Efforts are now aimed at operating these continuous fermentations using more realistic feedstock—dilute acid hydrolyzed hybrid poplar. This material is being supplied to the subcontractor by NREL.

Major Project Reports:

The draft final report for the first year's work is now under review at NREL.

Summary Date: December 1992

Engineering Research—Hemicellulose Conversion

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: J.D. McMillan

Telephone: (303) 231-1961

Contract Number: In-house

Contract Period: 10/90 – 09/91

Contract Funding (Source):
FY 1990: $260,000 (DOE)

Objective:

To identify and develop biocatalysts capable of high-yield conversion of xylose (and other pentose sugars) to ethanol. Xylose is not readily fermentable to ethanol by conventional biocatalysts. Because xylose represents almost one-third of the total sugars obtained by pretreatment, however, efficient conversion of xylose to ethanol would markedly increase overall ethanol yield.

Approach/Background:

The fact that direct xylose conversion is possible has prompted a reexamination of the two-step scheme originally conceived wherein xylose is enzymatically isomerized to xylulose and then fermented to ethanol by any of a variety of glucose-fermenting yeasts able to ferment xylulose. The approach taken in this project was to first conduct a thorough review of the xylose fermentation literature. Results of the literature survey will be used to direct future in-house and subcontracted research.

Status/Accomplishments:

Numerous microorganisms are now recognized to be capable of directly fermenting xylose to ethanol. Wildtype yeast and recombinant bacteria currently offer the best overall performance in terms of high yield, final ethanol concentration, and volumetric productivity. The best performing bacteria, yeast, and fungi can achieve yields greater than 0.4 g/g and final ethanol concentrations approaching 5% (w/v). Productivities remain low for most yeast and for fungi, in particular, but volumetric productivities exceeding 1.0 g/L-h have been reported for xylose-fermenting bacteria. In terms of wildtype microorganisms, strains of the yeast *P. stipitis* show the most promise in the short term for direct high-yield fermentation of xylose without by-product formation. Recent research at NREL has been directed at characterizing the xylose conversion performance of this yeast. The primary disadvantage with *P. stipitis* appears to be that very low levels of aeration are necessary to achieve optimal performance.

The construction of recombinant bacterial and yeast strains exhibiting improved xylose fermentation performance characteristics represents the most significant development in xylose conversion research in the past several years. Although it remains unclear to what extent regulatory obstacles will hinder the large-scale use of recombinant species, genetic engineering efforts to develop improved strains are continuing, and the development of recombinant *S. cerevisiae* strains capable of high-yield xylose fermentation appears imminent. Of the recombinant xylose-fermenting microorganisms actually developed to date, recombinant *E. coli* ATTC 11303 (pLOI297) and ATTC 11303 K011 exhibit the most favorable performance characteristics. The genetic stability of these organisms remains unproven in prolonged culture, however, and from a processing standpoint, there is a greater potential for contamination using *E. coli* relative to yeast, because *E. coli* must be cultivated at near neutral pH. It is also likely that recombinant *E. coli* strains also suffer from inferior ethanol tolerance relative to recombinant yeast.

Major Project Reports:

McMillan, J., "Xylose Fermentation to Ethanol: A Review."

Summary Date: December 1992

Development of Cellulase Production

Directing Organization:
U.S. Department of Energy
1000 Independence Avenue, SW
Washington, DC 20585

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: K. Kadam

Telephone: (303) 231-7826

Contract Number: DE-AC02-83CH10093

Contract Period: 01/92 – 01/93

Contract Funding (Source):
FY 1992: $310,000 (DOE)

Objective:

To design and optimize an enzyme production unit that will synthesize cellulolytic enzymes for the needs of the biomass-to-ethanol conversion process.

Approach/Background:

Production of cellulases, the cellulose-hydrolyzing enzymes, is an integral part of the biomass-to-ethanol conversion process via the simultaneous saccharification and fermentation (SSF) of the cellulose content of pretreated lignocellulosic biomass. The importance of the cellulase production unit stems from the fact that cellulase productivity affects the economics of this unit operation, and the cellulase quality is critical to the kinetics and economics of the SSF process. Consequently, identification of promising cellulase-producing organisms and improvement of their enzyme productivity will enhance the performance of the overall biomass conversion process.

Status/Accomplishments:

Work in this area is just beginning, and several approaches are being pursued to develop a base case cellulase fermentation—the parameters of interest are an efficient *T. reesei* mutant, a medium, and a feeding strategy that promote growth, enzyme production, and oxygen transfer. Several mutants of *T. reesei* were evaluated for cellulase production in shake flasks. No significant differences could be seen in cellulase activities measured as international filter paper units/ml. However, one of the mutants (MTC-A13) showed β-glucosidase activity that was twice as high as that for Rut-C30 (the benchmark mutant). The current studies use the L27 mutant, which is of industrial origin and is a high producer of β-glucosidase. Besides attempting to establish a reproducible fermentation, the research emphasis so far has been on novel inducers of the cellulases and evaluation of a pneumatic reactor that can provide oxygen transfer without excessive shear and without the need for oxygen supplementation. The results from these efforts are still preliminary.

Major Project Reports: None

Summary Date: December 1992

Lignin Utilization

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: D.K. Johnson

Telephone: (303) 231-7633

Contract Number: In-house

Contract Period: 01/92 – 01/93

Contract Funding (Source):
FY 1991: $175,000 (DOE)
FY 1992: $200,000 (DOE)

Objective:

The production of valuable coproducts from a lignin fraction that could be generated during the biochemical conversion of biomass to ethanol can improve the overall economics of biomass conversion to fuels and chemicals.

Approach/Background:

Pyrolysis is being explored as a method for the conversion of lignins into valuable low-molecular-weight products. Additionally, the presence of various additives and catalysts is being studied to determine their influence on lignin pyrolysis products. A very large body of data is available on the pyrolysis of whole biomass feedstocks on an analytical scale in the molecular beam mass spectrometer (MBMS) and on a production scale in the vortex reactor (and in the literature on other reactors). In these systems, the lignin component depolymerizes into volatile phenolics and hydrocarbons that can be used in making high-octane fuels or chemicals. Because of the nature of pyrolysis, products resulting from the pyrolysis of lignins in whole biomass materials should be very similar to those coming from the pyrolysis of isolated lignins. A lot of work has also been performed on modifying the product slate from the pyrolysis of whole biomass feedstocks, using zeolite catalysts.

For several years, the Biofuels Program has supported the characterization of lignins and the development of improved methods of lignin analysis. Lignins are amorphous polyphenolics containing a variety of functionalities and repeating unit linkages. Pretreatments pertinent to the biomass-to-ethanol process substantially alter the lignin component. Only after thorough characterization can lignin be most efficiently utilized.

Status/Accomplishments:

A rapid analytical technique for quantifying functional groups (such as methoxyl and phenolic hydroxyl) in lignins using partial least squares (PLS) regression of Fourier transform infrared (FTIR) spectra of lignins has been developed. This is part of a growing area in which various chemometrics techniques (e.g., PLS and multivariate analysis) are being applied to data-rich spectroscopic measurements, (e.g., FTIR, pyrolysis mass spectroscopy, and solid-state nuclear magnetic resonance spectroscopy) to give rapid methods of analyzing lignins.

The pyrolysis conditions for the model lignins were applied to a set of lignin residues from simultaneous saccharification and fermentation (SSF) runs. The optimal temperature for the production of low-molecular-weight phenolics was lower than that required for model compounds. The conversion of SSF lignin residues to low-molecular-weight phenolics was also higher than that for model compounds.

Major Project Reports:

Hames, B., S.K. Black, F. Agblevor, R. Evans, D.K. Johnson, and H.L. Chum, "Measurement of the Functional Group Contents of Lignins Using FTIR and Partial Least Squares Regression."

Milne, T.A., H.L. Chum, F. Agblevor, and D.K. Johnson, "Standardized Analytical Methods."

Summary Date: December 1992

The Preparation and Characterization of Catalysts for Use in Hydroxydeoxygenation of Lignin Type Feed

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
Colorado School of Mines
1500 Illinois Street
Golden, CO 80401

Principal Investigator: S.W. Cowley

Telephone: (303) 273-3638

Contract Number: XK-6-06111-1

Contract Period: 10/89 – 09/92

Contract Funding (Source):
FY 1987: $19,476 (DOE)
FY 1988: $24,930 (DOE)
FY 1989: $24,909 (DOE)
FY 1990: $31,462 (DOE)
FY 1991: $39,753 (DOE)

Objective:

The aim of this work is to develop (1) hydrodealkylation catalysts to be used in the conversion of lignin to volatile phenolics and (2) hydrocarbons that could be precursors to high-octane components with the correct boiling range for addition to gasoline or ethanol (or that could be used to make higher value products such as surfactants for enhanced oil recovery).

Approach/Background:

Lignins may be converted to volatile phenolics and hydrocarbons by catalytic hydrotreating. The catalysts previously used were those developed for coal and petroleum processing; however, lignin's low sulfur and nitrogen content permits the design of different catalysts more suited to lignin's chemical structure so that higher yields of phenolic and hydrocarbon products of the correct boiling range may be obtained. Acidic phosphoaluminate and silica-aluminate supported catalysts impregnated with active metals for hydrodeoxygenation and hydrocracking have been developed and tested with lignin model compounds. Relatively low-molecular-weight oligomeric lignin fractions are now being extracted using organic solvents so that the catalysts developed under this subcontract can be tested with more relevant feedstocks.

Status/Accomplishments:

Previous worked has focused on the hydrotreating of model compounds from lignin. Work this year focused on the evaluation of the hydrotreating of real lignin feedstocks. This work has included (1) isolation and characterization of low-molecular-weight fractions from lignin extracted from wood, using the "Organosolv" process; (2) fragmentation of the high-molecular-weight fraction of Organosolv-derived lignin via acid hydrolysis; (3) evaluation of the hydrotreating of low-molecular-weight lignin fractions using the most promising catalyst developed under this subcontract; and (4) determination of catalyst pore size effect on conversion efficiency. The subcontractor has provided NREL with catalyst samples for evaluation in-house. A final report is in preparation.

Major Project Reports: None

Summary Date: December 1992

Process Integration

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: J. Sheehan

Telephone: (303) 231-1932

Contract Number: In-house

Contract Period: 01/92 – 01/93

Contract Funding (Source):
FY 1992: $300,000 (DOE)

Objective:

To ensure that a fully integrated process operates reliably and economically when operated on economically realistic feedstocks. This work is based on the results of the laboratory research performed on individual elements of the overall biomass-to-ethanol process. It introduces the operational realities that will face a fully integrated commercial process.

Approach/Background:

Much of the research conducted over the last several years has been done at the bench scale using idealized feedstocks or substrates. Furthermore, the individual elements of the overall process were investigated in an isolated fashion rather than being integrated into complete feedstock-to-product process. This approach was taken to understand the scientific basis of each process step because of the lack of funds to do otherwise. The approach of the Process Integration task is based on the need and financial ability to obtain the engineering data required to design a commercial process and to transfer this information to industry.

The approach is three-pronged: (1) based on performance data from the research program, perform conceptual process design and economic analysis to define the most economic processing options for a commercial facility; (2) conduct experiments using realistic feedstocks in a fully integrated, bench-scale process that mimics the chemical interactions that will be found in a commercial process; and (3) design, construct, and operate a pilot plant to research and demonstrate a chemically and mechanically integrated process that produces ethanol from lignocellulosic feedstocks.

Status/Accomplishments:

In addition to bench-scale testing carried out by the integration group under cooperative research and development agreements in 1992, the integration group has been working on evaluating the effects of real feedstocks on the fermentation steps in the ethanol conversion process. Experiments have now successfully demonstrated xylose and simultaneous saccharification and fermentation (SSF) steps based on realistic hybrid poplar feedstock, pretreated using the dilute acid process. Xylose fermentation turned out to be far more difficult to achieve. Successful growth and fermentation was finally achieved through an extensive program of gradual adaptation to hydrolyzed wood. The SSF step was demonstrated using hydrolyzed wood chips from the Tennessee Valley Authority in several batch fermentations at the 150-L scale.

Major Project Reports: None

Summary Date: December 1992

Process Development Unit

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
John Brown Engineers and Constructors
300 South Riverside Plaza
Suite 1800
Chicago, IL 60606

Principal Investigators: P. Bergeron (NREL) and R. Sapko (John Brown)

Telephone: (303) 231-1932

Contract Number: YS-2-1161-1

Contract Period: 05/92 – 01/94

Contract Funding (Source):
FY 1990: $1,406,000 (DOE)
FY 1991: $ 31,000 (DOE)
FY 1992: $1,602,285 (DOE)

Objective:

To design, build, and operate a 1-ton-per-day biomass-to-ethanol facility that will be used to collect scale-up data for the design of a 40-ton-per-day biomass-to-ethanol engineering demonstration unit (EDU).

Approach/Background:

The biomass-to-ethanol process can be broadly classified into feedstock pretreatment, fermentation, and downstream processing. The process development unit (PDU) work involves both an integrated sequence of process steps and isolated process steps. NREL's integrated bench-scale research investigates chemical interactions among the process steps and thereby determines overall process performance for small-scale equipment. However, this equipment is not large enough to serve as an accurate model of a large commercial or near-commercial plant. This is especially true when the solids are being processed, as is the case for this technology. The size of the laboratory equipment presents physical limitations on the range of operating conditions that can be investigated. Also, certain physical phenomena that can greatly influence process performance change dramatically as the size of the equipment increases.

With the PDU, the individual process steps and overall process configuration can be developed using equipment large enough to investigate a full range of operating conditions and observe the various equipment-size dependent phenomena. The PDU must be designed with a high degree of flexibility and instrumentation. The next step is a full-scale commercial process.

Because the eventual goal is commercialization, the PDU will be a user facility where industrial partners can test new ethanol conversion technology.

Status/Accomplishments:

During FY 1992, proposals were evaluated, and John Brown Engineers & Constructors was selected. Negotiations were successfully completed, and the subcontract was initiated in May 1992.

Phase 1 work begins with testing critical pieces of equipment at vendor sites. This will be followed by a conceptual design that defines the scope of the PDU. A first estimate of the cost will also be done that will be accurate to approximately +30% and -15%. With this information, a preliminary design can be performed that will more closely define the process and the cost (+/-10%). Long lead time equipment will also be defined at this time and the purchasing process begun, as necessary, to maintain the schedule. During Phase 2, construction drawings will be made, the equipment will be fabricated and purchased, and the PDU will be installed in the Alternative Fuels Users Test Facility at NREL.

As of the end of FY 1992, vendor tests on milling, feedstock pretreatment, high solids mixing and pumping, and solid/liquid separation equipment were nearing completion. Phase 1 work was proceeding on schedule. Wood chips, wastepaper, and switchgrass were determined to be the primary feedstocks and were used in the these vendor tests.

Major Project Reports: None

Summary Date: December 1992

New Energy Cooperative Research and Development Agreement (CRADA)

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Industrial Partner:
New Energy Company of Indiana
3201 Calvert Street
South Bend, IN 46680-2289

Principal Investigators: R. Elander (NREL) and L. Russo (New Energy)

Telephone: (303) 231-1330

Agreement Number: 91-001

Agreement Period: 04/91 – 01/94

Agreement Funding (Source/Cost Sharing):

	DOE:	New Energy:
FY 1991:	$ 84,499	$ 6,909
FY 1992:	$101,009	$257,391
FY 1993:	$ 58,049	$605,520
FY 1994:	$ 14,512	$151,380

Objective:

To establish a collaborative effort between NREL and New Energy to conduct research and development on a process for converting the cellulosic fraction of New Energy's existing ethanol feedstock (to improve the overall yield from its facility).

Approach/Background:

This work will be performed in three phases during a period of approximately 4 years. At the conclusion of each phase, revised engineering analyses will be carried out on the process as defined at that point in the project.

Status/Accomplishments:

Phases 1 and 2 are complete, and work on Phase 3 has begun.

Major Project Reports: None

Summary Date: December 1992

Amoco Corporation Cooperative Research and Development Agreement (CRADA)

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Industrial Partner:
Amoco Corporation
Suite 600
305 East Shuman Boulevard
Naperville, IL 60563-8408

Principal Investigators: C. Riley (NREL) and
R. Lumpkin (Amoco)

Telephone: (303) 231-7638

Agreement Number: 91-003

Agreement Period: 10/91 – 10/97

Agreement Funding (Sources/Cost Sharing):

	DOE:	Amoco:
FY 1992:	$ 794,000	$ 749,000
FY 1993:	$1,226,500	$ 568,000
FY 1994:	$1,226,500	$ 568,000
FY 1995:	$ 281,000	$7,798,333
FY 1996:	$ 281,000	$7,798,333
FY 1997:	$ 281,000	$7,798,333

Objective:

To establish a collaborative effort between NREL and Amoco to conduct research and development on a biomass-to-ethanol conversion technology.

Approach/Background:

This work will be performed in four phases during a period of approximately 6 years. At the conclusion of each phase, revised analyses will be carried out on the process as defined at that point in the project.

Status/Accomplishments:

Phase 1 is complete, and Phase 2 work has begun.

Major Project Reports: None

Summary Date: December 1992

Chemical Analysis and Testing

Directing Organization:
U.S. Department of Energy
1000 Independence Avenue, SW
Washington, DC 20585

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: M.E. Himmel

Telephone: (303) 231-1799

Contract Number: In-house

Contract Period: 10/91 – 11/92

Contract Funding (Source):
FY 1992: $400,000 (DOE)

Objective:

To perform key activities that have been identified for the successful assurance of quality analytical functions within the Biofuels Program.

Approach/Background:

The Chemical Analysis and Testing Task will provide certification and validation services to both NREL and DOE subcontractors working for the program.

Status/Accomplishments:

A major project conducted by the task in 1992, and one representative of task activities, was the complete analysis of the fermentability of wood samples obtained from Appalachian region saw mills. The compositional analysis of 15 selected wood samples showed low levels of variance (ave = 46%; sd = 3.3%) in glucan (cellulose) contents; however, sycamore and buckeye were substantially above the average (53% and 52.6%, respectively). The hemicellulosic sugars proved more highly varied in content, with xylan average composition = 17.4% (sd = 2.9%), arabinan average = 1.9% (sd = 0.4%), and mannan average = 3.1% (sd = 2.6%). On the basis of glucan contents alone, sycamore, buckeye, and poplar should provide the highest conversions to ethanol on a dry-weight, native wood basis. However, this was not the case. The wood pulps were again subjected to compositional analysis following dilute sulfuric acid pretreatment (160°C, 30 min, 0.45% v/v H_2SO_4). After pretreatment, hickory, poplar, maple, and white oak showed the highest glucan contents. Simultaneous saccharification and fermentation (SSF) was based on *Saccharomyces cerevisiae* D_5A and Genencor Laminex cellulase (25 FPU and 25 βGU/gram cellulose content). SSF experiments were conducted in quadruplicate with Sigmacell 50 controls. Maple, sycamore, and red oak produced the highest theoretical yields of ethanol (88%, 85%, and 84%, respectively) in 4 days. Cherry, walnut, and poplar were also efficiently converted. Pine and hard maple showed low levels of ethanol production.

In the past year, the task has also processed approximately 60 work orders originating from program researchers. This work includes the compositional analysis of wood, paper waste, herbaceous crops, and fermentation samples, as well as several methods development projects.

Major Project Reports:

Ponfick, L.R., T. Vinzant, N.J. Nagle, T.I. Ehrman, K.M. Magill, J.B. Reynolds, and M.E. Himmel, "SSF Comparison of Selected Woods from Southern Saw Mills."

Ehrman, T. and M.E. Himmel, "Chemical Analysis & Testing Standard Procedures: Determination of Total Solids/Moisture in Biomass; Two Stage Sulfuric Acid Hydrolysis for Determination of Carbohydrates; Determination of Klason Lignin in Biomass; Determination of Acid Soluble Lignin in Biomass; Determination of Ash in Biomass; Measurement of Cellulase Activities; Dilute Acid Pretreatment Methods; SSF Experimental Protocols."

Summary Date: December 1992

Co-Products Markets Assessments

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
Rice Chemical Market Research
3 Chapel Road
Orono, ME 04473

Principal Investigator: P. Rice

Telephone: (207) 866-02397

Contract Number: 11158-1

Contract Period: 09/91 – 10/92

Contract Funding (Source):
FY 1991: $49,963 (DOE)

Objective:

To assess the markets, restrictions in degree of purity, competing markets, and evolving competitive routes for each of the co-products being studied. These co-products of ethanol production will include enzymes, lignins, glycerol, acetic acid, carbon dioxide, and others that will emerge from the reference case and improvements in the process.

Approach/Background:

Co-products derived from biomass-to-ethanol conversion would improve the yield and economic return on the biomass raw materials utilized. There are three fractions in biomass—cellulose, hemicellulose, and lignin. The lignin fraction is not utilized at all in the production of ethanol. The aim of this work is to identify potential co-products that can be made from the unused portion of biomass after ethanol production. The minimum criterion for such co-products is that they have a value greater than the value derived from burning the residual material left over after ethanol production.

Status/Accomplishments:

A final report has been completed that provides an assessment of markets for 11 types of co-products. These include lignin-based surfactants, glycerol, acetic acid, anthraquinone, enzymes, single-cell protein, hydroquinone, acrylate monomers, lactate, and others. The assessments include information on capacity, potential for growth in demand, prices, production processes, and applications for the co-products.

Major Project Reports:

Rice, P., "Co-Products Market Assessment."

Summary Date: December 1992

Analysis of Probable Cost of Ethanol from Lignocellulosic Material

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: N. Hinman

Telephone: (303) 231-1281

Contractor:
TDA Research, Inc.
12345 West 52nd Avenue
Wheatridge, CO 80033

Principal Investigator: J. Wright

Telephone: (303) 422-7819

Contract Number: AA-2-11286-1

Contract Period: 12/91 – 09/93

Contract Funding (Source):
FY 1990: $39,864 (DOE)

Objective:

To document the results of a series of analyses performed by J. Wright while he was employed at the Solar Energy Research Institute (SERI)—now NREL. Wright analyzed five design cases to determine the probable cost of ethanol from lignocellulosic material. For each case, information on the overall process design in terms of each major process step, a detailed description of each process step, a summary of capital and operating costs, and an explanation of the financial parameters used will be included.

Approach/Background

Five design cases representing changes in process technology for ethanol conversion have been analyzed. These analyses demonstrated the approach to reducing ethanol conversion costs using technology developed between 1979 and 1988. The purpose of this work is to provide detailed documentation of these analyses to provide a historical perspective on the development of ethanol technology.

Status/Accomplishments:

Although documentation of the five design cases is now under way, the subcontractor has not yet submitted any deliverables. The project will likely continue into FY 1993 because of delays in start-up of the work.

Major Project Reports: None

Summary Date: December 1992

Methanol
and
Higher Alcohols

Indirect Heated Biomass Gasifier

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C. Gregoire

Telephone: (303) 231-7019

Contractor:
Battelle Memorial Institute–Columbus Operations
505 King Avenue
Columbus, OH 43201-2693

Principal Investigator: M. Paisley

Telephone: (614) 424-4958

Contract Number: YM-2-11110-1

Contract Period: 12/91 – 05/93

Contract Funding (Source):
FY 1991: $1,072,451 (DOE)

Objective:

To aid in the development of a commercially viable methanol production process based on indirect biomass gasification. The purpose of the program is to provide additional gasifier operating data for an existing gasifier (using both woody and herbaceous feeds), to incorporate into gasifier operation a slip-stream hot-gas conditioning train, and to perform a detailed design study of gasifier modification for pressurized operation.

Approach/Background:

The thermochemical production of methanol from biomass involves the production of a medium or high Btu gas, rich in hydrogen or carbon monoxide, which is then catalytically converted into methanol. Production of the synthesis gas is accomplished by thermal gasification.

Indirect gasifiers produce a solid carbon-rich char that is typically reacted with air in a separate combustor to produce heat. This heat is transferred to the gasifier by the circulation of hot solids, or by indirect heat transfer through the walls of heat-exchange tubes. Indirect gasifiers typically produce a gas rich in carbon monoxide with low carbon dioxide levels.

Downstream synthesis gas modification is required to produce a methanol synthesis gas with a suitable hydrogen-to-carbon monoxide ratio, and a low level of inert light hydrocarbons such as methane. The raw gas exiting the gasifier contains tars and char that must be removed prior to downstream catalytic conversion operations. Typically, biomass gasification systems use scrubbers to remove tar and residual char. An alternative to scrubbing is hot-gas conditioning.

Status/Accomplishments:

Biomass gasification tests were performed to produce a medium Btu synthesis gas and to determine process yields as a function of temperature and steam rate. Samples of the char and scrubber condensate were provided to NREL for assessment.

A slip-stream catalytic hot-gas conditioning train was designed, constructed, and operated. Promising tar destruction results have been obtained using a proprietary catalyst.

A detailed design study on the feasibility of operating the indirect gasifier system at elevated pressures was performed. The study included the design of unit modifications, the time estimated for procurement and construction, and detailed cost estimates.

Major Project Reports:

Breault, R.W., "Design and Economics of Electricity Production from an Indirectly Heated Biomass Gasifier."

Paisley, M.A., R.W. Breault, and R.L. Bain, "Design and Economics of Electricity Production from an Indirectly Heated Biomass Gasifier."

Summary Date: December 1992

Methanol Syngas Conditioning

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C. Gregoire

Telephone: (303) 231-7019

Contractor:
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: S. Gebhard

Telephone: (303) 231-1349

Contract Number: In-house

Contract Period: 12/87 – 12/93

Contract Funding (Source):
FY 1992: $650,000 (DOE)

Objective:

To develop a method to condition biomass-derived syngas for the production of methanol. Replacement of catalytic steam reforming (to remove tars and methane) by less severe gas conditioning technology holds the promise of significant cost reductions in the production of methanol.

Approach/Background:

Raw synthesis gas from leading biomass gasifiers contains particulate matter, methane and other light hydrocarbons, and appreciable amounts of tar. Raw biomass syngas is unsuitable for direct use in methanol synthesis because of these impurities. In addition, the hydrogen-to-carbon monoxide ratio in raw syngas is not optimum. Improvements in these areas will significantly reduce the cost of the thermochemical biomass-to-methanol process.

Status/Accomplishments:

Direct syngas conditioning, in one step, replaces expensive quenching and scrubbing to remove tars with subsequent reheating and steam reforming to reduce excessive levels of methane and light hydrocarbons. Successful completion of this research would bring projected methanol costs down to $0.63/gal (1990$) for a 2000 dry tons of wood per day (tpd) facility and $0.47/gal (1990$) for a 10,000-tpd facility. In comparison, the cost of methanol from natural gas is currently about $0.40/gal.

Ongoing screening studies examined a wide variety of catalysts, with the subsequent identification of a few very promising catalysts. The most recent research was performed using a microscale plug flow catalytic reactor interfaced with the NREL molecular beam mass spectrometer (MBMS), using a synthetic raw biomass syngas. The capability of the MBMS to detect and quantify entire slates of gasifier products permits catalyst performance to be determined under conditions in which a large number of organic impurities are present (especially high-molecular-weight polycyclic aromatic hydrocarbons). The ability to examine whole product slates is important because tar composition can vary significantly, depending on the severity of thermal cracking that occurs during tar generation. Experimental design methods were used to develop tests that determine the effects of temperature, raw syngas flow rate, and raw syngas steam content on the performance of the most promising catalysts identified to date. Similar experiments were performed with the 10-tpd process research unit biomass gasifier at Battelle Columbus Laboratory. Results from this work suggest that syngas conditioning experiments with a slip-stream reactor on the 10 tpd gasifier can be reproduced on the microscale at NREL.

Major Project Reports:

Bain, R.L., S. Gebhard, R.P. Overend, and D. Wang, "Evaluation of a Catalyst Screening Protocol for Syngas Conditioning."

Gebhard, S., "Evaluation and Modeling of Catalyst for Methanol Syngas Conditioning."

Gebhard, S., "Design, Construction, and Testing of Fluid Bed Catalytic Reactor."

Gebhard, S. and D. Wang, "Catalytic Reaction Modeling for Methanol Syngas Conditioning."

Summary Date: December 1992

Higher Alcohols from Synthesis Gas

Directing Organization:
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Project Manager: C. Gregoire

Telephone: (303) 231-7019

Contractor:
G. Alex Mills
1004 Dixon Drive
Newark, DE 19711

Principal Investigator: G. Alex Mills

Telephone: (303) 231-7019

Contract Number: HZ-1-11208-1

Contract Period: 09/03/91 – 08/31/92

Contract Funding (Source):
FY 1991: $30,930 (DOE)

Summary Date: December 1992

Objective:

To develop commercial processes for converting biomass-derived synthesis gas to higher alcohols.

Approach/Background:

The first priority is to carry out a state-of-the-art review of processes for higher alcohol production from syngas. Following the completion of this, NREL will perform an independent review of the technoeconomics of probable processes based on the systems analysis models already developed for methanol production from syngas.

Status/Accomplishments:

The final report has been received from G. Mills.

Major Project Reports:

Mills, G.A., "Status and Future Opportunities for Conversion of Synthesis Gas to Liquid Energy Fuels."

Technoeconomic Analysis

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
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Contractor:
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Principal Investigator: C. Gregoire

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Contract Number: In-house

Contract Period: 10/91 – 09/92

Contract Funding (Source):
FY 1992: $110,000 (DOE)

Objective:

To determine the economically optimum combination of unit operations that will make the production of methanol cost-competitive with, or better than, traditional processes used with conventional feedstocks.

Approach/Background:

The Syngas Program includes research elements and milestones necessary to advance technologies toward the ultimate goal of developing cost-competitive processes. To evaluate the impact of these research elements and to ensure that feasible processes are developed, a number of technical and economic issues must continue to be addressed and updated. Technoeconomic analyses must be updated as new information and experimental results become available. The implications of thermochemical fuels and end-use requirements will be analyzed. This work will address such technical and economic issues.

The approach is to develop simulation modules for the various gasifier and gas-conditioning treatments for use in a commercial simulation package, such as ASPEN/SP or PRO/II.

Status/Accomplishments:

NREL began a compilation of existing technoeconomic analyses on thermochemical fuels in FY 1989. In FY 1990–FY 1991, the effort was expanded to include detailed process analysis using a commercial process simulator, ASPEN/SP. In FY 1992, an additional simulation tool, PRO/II, was obtained to provide additional features to the simulation effort. Technoeconomic analysis, as an integral part of the research effort, will help direct the research toward processes giving the highest economic benefit.

Major Project Reports:

Gregoire, C.E., "The Application of Coal Gasification Technology to Biomass Gasification."

Summary Date: December 1992

Ether Additives

Thermochemical Conversion of Biomass to Ethers

Directing Organization:
National Renewable Energy Laboratory (NREL)
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Project Manager: C. Gregoire

Telephone: (303) 231-7019

Contractor:
National Renewable Energy Laboratory
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Principal Investigator: J. Diebold

Telephone: (303) 231-7250

Contract Number: DE-ACO4-83CH10093

Contract Period: 03/92 – 05/93

Contract Funding (Source):
FY 1990: $114,000 (DOE)
FY 1991: $500,000 (DOE)
FY 1992: $700,000 (DOE)

Objective:

To utilize the fast pyrolysis of biomass or refuse-derived fuel to maximize the production of oxygenated biocrude oil vapors; these vapors are then promptly cracked to olefinic intermediates for catalytic combination with methanol or ethanol to produce oxygenated ethers (i.e., methyl tertiary butyl ether or ethyl tertiary butyl ether) as additives for reformulated gasolines.

Approach/Background:

Investigations of optimal catalyst properties are being followed at a very small scale (1 gram of catalyst) on the NREL molecular beam mass spectrometer (MBMS) system. The more promising catalysts have been tested for batch-to-batch reproducibility and are now being scaled up to a larger batch size.

Experimentation is also conducted at a larger reactor size of 100 grams of catalyst, using a slipstream of vapors from the NREL vortex pyrolysis reactor. An even larger 10,000-gram reactor is fed the full output of the vortex reactor, using zeolite catalysts made commercially, i.e., supplied by Mobil.

Status/Accomplishments:

The research with the MBMS catalyst test system has continued, primarily looking at the batch-to-batch and scale-up reproducibility of the four most promising zeolite catalysts identified last year. These catalysts were made under the direction of F. Hanson of the University of Utah. Early results are promising with respect to catalyst reproducibility.

The fabrication of a riser-cracker reactor to match the full output of the NREL vortex pyrolysis reactor is in progress. Although there are many similarities between this riser-cracker and those commonly used in petroleum refineries, there are some important differences to take advantage of the characteristics of the biocrude feedstock. It is planned to completely demonstrate the conversion of biomass to ethers.

The initial technoeconomic assessment of this process to make oxygenated reformulated gasoline additives was done by hand. During this reporting period, the process is being modeled using the ASPEN process-simulation computer program in conjunction with additional process-simulation software. This modeling will facilitate the evaluation of process variables on costs.

Major Project Reports:

Bain, R., J.P. Diebold, R.P. Overend, A.J. Power, and B. Rejai, "The Production of Reformulated Gasoline Components from Biomass and RDF."

Diebold, J.P., A.V. Bridgwater, D. Beckman, D.C. Elliott, and Y. Solantausta, "IEA Technoeconomic Analysis of the Thermochemical Conversion of Biomass to Gasoline by the NREL Process."

Rejai, B., F.A. Agblevor, R.J. Evans, and D. Wang, "Catalytic and Feedstock Effects in the Thermoconversion of Biomass to Liquid Transportation Fuels."

Summary Date: December 1992

Biodiesel

Lipid Biochemistry

Directing Organization:
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Project Manager: L.M. Brown

Telephone: (303) 231-1321

Contractor:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: P.G. Roessler

Telephone: (303) 231-1321

Contract Number: DE-AC04-83CH10093

Contract Period: 10/91 – 09/92

Contract Funding (Source):
FY 1990: $ 25,000 (DOE)
FY 1991: -0- (DOE)
FY 1992: $190,000 (DOE)

Objective:

To conduct research on microalgal lipids and the mechanisms of lipid accumulation to develop methods for controlling their production for the efficient generation of liquid fuels.

Approach/Background:

Genetic improvement, with the goal of developing a microalgal strain in which the oil content is controllable, requires a knowledge of the pathways and enzymes involved in lipid accumulation. Once these mechanisms are understood, it should be possible to manipulate them to produce lipids of improved quantity and quality and in response to desired conditions, thus improving the overall economics of generating liquid fuels through microalgal mass culture. The technology has the potential to provide a considerable portion of the nation's liquid fuel needs and, in so doing, help to reduce the excess accumulation of carbon dioxide in the atmosphere (global climate change).

Status/Accomplishments:

Work continued on attempts to improve lipid yield from microalgal mass culture through cloning a key lipid gene, acetyl-CoA carboxylase (ACC). This gene was cloned from the diatom *Cyclotella cryptica* last year, representing a significant advance in the Biodiesel Project. This work followed lipid biochemistry studies in this organism that indicated that ACC is likely to be a key enzyme in controlling lipid production. During FY 1992, detailed study of the ACC gene was completed. The sequence of DNA bases in the gene has now been completely determined, and the presence and location of two intervening sequences (introns) were elucidated. Such analyses have revealed the likely start site for the protein product encoded by this gene. This information will facilitate the production of an expressible form of the ACC gene for use in the genetic engineering of microalgae for enhanced lipid production. In the long term, future modification work on this gene may lead to significant commercial opportunities in both microalgae and oilseeds crops.

Major Project Reports:

Roessler, P.G., "Changes in the Activities of Various Lipid and Carbohydrate Biosynthetic Enzymes in the Diatom *Cyclotella cryptica* in Response to Silicon Deficiency."

Roessler, P.G., "Effects of Silicon Deficiency on Lipid Composition and Metabolism in the Diatom *Cyclotella cryptica*."

Roessler, P.G., "Environmental Control of Glycerolipid Metabolism in Microalgae: Commercial Implications and Future Research Directions."

Roessler, P.G., "Purification and Characterization of Acetyl-CoA Carboxylase from the Diatom *Cyclotella cryptica*."

Roessler, P.G. and J.B. Ohlrogge, "Cloning and Characterization of the Acetyl-CoA Carboxylase Gene from the Diatom *Cyclotella cryptica*."

Summary Date: December 1992

Genetic Engineering

Directing Organization:
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Principal Investigator: L.M. Brown

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Contract Number: DE-AC04-83CH10093

Contract Period: 10/91 – 09/92

Contract Funding (Source):
FY 1990: $285,000 (DOE)
FY 1991: $120,000 (DOE)
FY 1992: $110,000 (DOE)

Objective:

To conduct genetic engineering studies to improve microalgae for efficient production of liquid fuels.

Approach/Background:

To make the technology economically successful, genetic improvement is necessary to develop a strain in which the oil content is controllable. The technology has the potential to provide a considerable portion of the nation's liquid fuel needs and, in so doing, help to reduce the excess accumulation of carbon dioxide in the atmosphere (global climate change).

Status/Accomplishments:

Efforts in FY 1992 were aimed at creating a system for the stable expression of genes introduced into algal cells. Such genetic transformation methods are critical to efforts to genetically engineer microalgae for increased lipid production. Homologous selectable marker systems are being developed in which mutations are made in specific genes and the wild-type algal genes that can complement those mutations are cloned. Two genes were chosen for this work; the first is the gene for orotidine-5'-phosphate decarboxylase, and the second is the gene for the enzyme nitrate reductase. Mutations have been obtained in both these genes and will be used in future work.

A model system was used for the testing of a new transformation method that has been employed successfully in higher plants. In this technique, algae are agitated for various amounts of time in the presence of a foreign gene, chemical agents, and silicon carbide fibers. Experiments have demonstrated that the transformation of cells by this method is comparable in efficiency to a published method of agitating cells with glass beads.

Major Project Reports:

Brown., L.M., T.G. Dunahay, and E.E. Jarvis, "Strategies for Genetic Improvement of Microalgae with Ability to Grow in Outdoor Mass Culture."

Brown, L.M., T.G. Dunahay, and E.E. Jarvis, "Applications of Genetics to Microalgae Production."

Brown, L.M. and E.E. Jarvis, "Oil Production by Microalgae in Outdoor Mass Culture."

Dunahay, T.G., E.E. Jarvis, K.G. Zeiler, N. Nagle, L.M. Brown, P.G. Roessler, J.B. Ohlrogge, A.C. Cannons, and L.P. Solomonson, "Genetic Engineering of Microalgae for Fuel Production."

Dunahay, T.G., E.E. Jarvis, K.G. Zeiler, P.G. Roessler, and L.M. Brown, "Genetic Engineering of Microalgae for Fuel Production."

Jarvis, E.E. and L.M. Brown, "Transient Expression of Firefly Luciferase in Protoplasts of the Green Alga *Chlorella ellipsoidea*."

Jarvis, E.E., T.G. Dunahay, and L.M. Brown, "DNA Nucleoside Composition and Methylation in Several Species of Microalgae."

Jarvis, E.E., T.G. Dunahay, K.G. Zeiler, P.G. Roessler, P. Chelf, and L.M. Brown, "Aquatic Species Project: Annual Report."

Summary Date: December 1992

Culture Practice Development

Directing Organization:
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Project Manager: L.M. Brown

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Contractor:
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Principal Investigator: L.M. Brown

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Contract Number: DE-AC04-83CH10093

Contract Period: 10/91 – 09/92

Contract Funding (Source):
FY 1990: $40,000 (DOE)
FY 1991: $55,000 (DOE)
FY 1992: $50,000 (DOE)

Objective:

To study the effects of environmental parameters on the production of biomass and lipids by microalgae.

Approach/Background:

To develop a technology base to produce liquid fuels from microalgal biomass, it is essential that we understand the effects of environmental parameters on the production of biomass and lipids by microalgae. This is an important area that links efforts in genetic engineering to outdoor culture. The overall technology has the potential to provide a considerable portion of the nation's liquid fuel needs and, in so doing, help to reduce the excess accumulation of carbon dioxide in the atmosphere (global climate change).

Status/Accomplishments:

The maintenance of the microalgal culture collection is an ongoing activity, as it represents an important resource of material for the genetic engineering component of the program.

Methods continued to be refined in the areas of culture maintenance, with concentration on scaling down the amount of culture medium used, using fewer medium formulations, developing easier-to-prepare formulations, and introducing automation to culture transfer.

Major Project Reports:

Brown, L.M., "Culture Collection Status."

Brown, L.M. and S. Sprague (ed.), *Aquatic Species Project Report.*

Chelf, P. and L.M. Brown, "Microalgal Mass Culture and the Greenhouse Effect: Resources Update."

Chelf, P., L.M. Brown, and C.E. Wyman, "Aquatic Biomass Resources and Carbon Dioxide Trapping."

Chelf, P., "Environmental Control of Lipid and Biomass Production in Two Diatom Species."

Chelf, P., L.M. Brown, and C.E. Wyman, "Aquatic Biomass Resources and Carbon Dioxide Trapping."

Weissman, J.C. and D.M. Tillett, "Design and Operation of an Outdoor Microalgae Test Facility: Large-scale System Results."

Summary Date: December 1992

Biofuels Feedstock Development

Monoculture Viability Trial of Woody Crops for Energy Production

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Project Manager: J. Tuskan

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Contractor:
Amana Society
Amana, IA 52203

Principal Investigators: D.A. Shoup and L.G. Gnewikow

Telephone: (319) 622-3051

Contract Number: 19X-SB106C

Contract Period: 04/90 – 03/94

Contract Funding (Source):
FY 1990: $52,000 (DOE/ORNL)
FY 1991: $71,000 (DOE/ORNL)
FY 1992: -0- (DOE/ORNL)

Objective:

To generate accurate information on plantation cost and biomass yields, through a commercial-scale monoculture viability trial of silver maple (*Acer saccharinum* L.), and to identify the risk factors associated with growing a single tree species.

Approach/Background:

Accurate cost estimates of growing woody crops on a commercial scale for energy in short-rotation, intensive culture plantations are difficult to obtain under research conditions. Information is essential to ascertain the economic feasibility of future contributions of biomass energy to the U.S. energy supply.

Status/Accomplishments:

In April 1991, approximately 22,000 silver maple seedlings were graded and readied for planting. The seedlings (the majority was 2-0 stock [20,200] and the balance was 1-0 stock) were to be planted in Unit 2 (5.7 ha). Starting in early April and continuing into early July, the entire project site was flooded. Unlike the flood of 1990, however, this flooding was continuous from April. All planting, cultural, and research activities planned for the three units were altered or postponed as a result. We postponed planting in Unit 2 until the fall of 1991. By the end of FY 1991, Unit 2 was mowed. It will be treated with post- and pre-emergent herbicides prior to fall planting. Another 24,000 silver maple seedlings (2-0 stock) were ordered from the Iowa DNR State Forest Nursery for this fall planting.

In March 1992, applications of simazine and oryzalin were applied to Units 1 and 2.

Nearly 15,000 silver maple trees were planted in Unit 2, with the remaining 5,000 trees planted in Unit 1. E. Hansen's hybrid populus clones (USDA Forest Service North Central Forest Experiment Station) were planted on roughly 3/4 ha in Unit 1. Four hybrid populus clones used in other short-rotation woody crops research by Iowa State University researchers were planted on the remaining 1/4 ha. The clones are NC-5326 (Eugenei), NC-5328 (I-45/51), NC-5377 (Wis-5), and IS-31 (a pure cottonwood clone).

The integrated pest management survey system was applied for 2 weeks prior to the start of the flooding and for only a few weeks late in the growing season. No significant pest problem was found.

Major Project Reports:

Colletti, J., L. Gnewikow, J. Gan, C. Ball, A. Skadberg, D. Schulta, and J. Regula, "A Monoculture Viability Trial of Woody Crops for Energy Production."

Colletti, J., and L. Gnewikow, project poster "The Amana Project—A Monoculture Viability Trial of Woody Crops for Energy Production."

Gan, J., "An Economical Assessment of the Silver Maple Energy Plantation at Amana, Iowa."

Summary Date: December 1992

Development of Optimal Establishment and Cultural Practices for Switchgrass as an Energy Crop

Directing Organization:
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Contractor:
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Auburn, AL 36849-4109

Principle Investigator: D.I. Bransby

Telephone: (205) 844-3935

Contract Number: 19X-SL227C

Contract Period: 06/92 – 05/93

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: -0- (DOE/ORNL)
FY 1992: $72,250 (DOE/ORNL)

Objective:

To develop optimal establishment and cultural practices for switchgrass as an energy crop in the southeastern United States.

Approach/Background:

Switchgrass will be evaluated as an energy crop in small plot studies to determine optimal methods of weed control at establishment; evaluate effects of row spacing, subsoiling, fertilization, and legume companion crops for increasing yield; and identify and test alternative varieties of switchgrass across several locations.

Status/Accomplishments:

This project was initiated in June 1991. All plot studies were seeded, and good stands were obtained. Although yields will be obtained in this establishment year, they are not yet available.

Major Project Reports: None

Summary Date: December 1992

Selection and Improvement of Herbaceous Energy Crops for the Southeastern USA

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Project Manager: A. Turhollow

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Contractor:
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Auburn, AL 36849-4109

Principle Investigator: D.I. Bransby

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Contract Number: 19X-27409C

Contract Period: 03/85 – 07/92

Contract Funding (Source):
FY 1990: $27,000 (DOE/ORNL)
FY 1991: $27,000 (DOE/ORNL)
FY 1992: -0- (DOE/ORNL)

Objective:

To evaluate perennial herbaceous species for biomass production in the southeastern United States.

Approach/Background:

Perennial forage species were compared on five sites in Alabama representative of some 43 million acres of cropland in the southeastern United States. The trials examined productivity, nutrient requirements, planting and harvesting practices, and biomass composition.

Status/Accomplishments:

This project was terminated at the end of July 1992. It identified Alamo switchgrass as the most promising perennial herbaceous energy crop for the Southeast. Small plot yields of this variety were 17.4, 34.6, and 28.3 Mg/ha in 1989, 1990, and 1991, respectively. The plots had been seeded in July 1988. In fall 1991, napiergrass and energycane were harvested for the fifth consecutive year, providing the following yields: 25.6 Mg/ha (L79-1002 energycane); 11.8 Mg/ha (N-51 napiergrass); and 6.1 Mg/ha (PI 300086 napiergrass). The napiergrasses have winterkilled, but energycane continues to maintain a good stand.

Major Project Reports: None

Summary Date: December 1992

Variability for Biomass Production and Plant Composition in Sericea Lespedeza Germ Plasm

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Contractor:
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Auburn, AL 36849-5412

Principal Investigator: J.A. Mosjidis

Telephone: (205) 844-3976

Contract Number: 19X-SG301V

Contract Period: 09/90 – 12/91

Contract Funding (Source):
FY 1990: $11,000 (DOE/ORNL)
FY 1991: $23,000 (DOE/ORNL)
FY 1992: -0- (DOE/ORNL)

Objective:

To determine the variability for biomass production and plant composition among 81 genotypes of sericea lespedeza.

Approach/Background:

S. lespedeza is a deep-rooted legume that can be established successfully on eroded and depleted croplands. It is tolerant of drought, high levels of aluminum, and low soil fertility—environmental conditions found throughout the southeastern region of the United States. S. lespedeza is capable of improving soil by increasing its organic matter and nitrogen content rapidly. A field with a 4-year stand of S. lespedeza grown for soil conservation or biomass production may have more than 7 Mg/ha of residues on the surface. Once established, costs are relatively small compared with those of other plants. Although most herbaceous plants require nitrogen fertilization, S. lespedeza fixes its own, and it has relatively few diseases and insect problems. S. lespedeza appears to be one of the best herbaceous perennial plants that could be grown for the production of lignocellulosic biomass. This study was designed to test differences in biomass yield and composition among a large number of S. lespedeza genotypes (cultivars and experimental lines).

Status/Accomplishments:

Results indicated substantial differences for biomass yield among genotypes. However, dry matter content was the same. Genotype R194-79-290-9 had the highest mean biomass yield and consistently ranked among the top four during the years that this study was conducted. Other genotypes that also had a good performance over the 4 years are the cultivar Serala and the breeding line 75-2-3. Overall, it is not possible to decide which of the top 16 genotypes (top 20%) is the best, because this study was conducted in only one location. However, it is possible to select genotypes with a consistently high biomass yield.

No significant differences were found in the percentage of crude protein content. Crude protein contents were found to be substantially lower (about 25%) than those previously reported. There were significant differences for neutral detergent fiber, hemicellulose, and holocellulose content. There were not significant differences for acid detergent fiber, lignin, and cellulose content. In short, there is variability for biomass yield and for some quality traits that are considered important for biofuel production.

Further testing of the best genotypes should be conducted at several locations to determine the genotype to be released for the specific purpose of biomass production. Screening of accessions from the Plant Introduction System should be conducted to determine their variability for biomass yield and for quality traits. If the results are positive, recombination of particular traits using genetic procedures could be carried out to obtain plants with higher levels of desirable traits.

Major Project Reports: None

Summary Date: December 1992

Optimizing Energy Yields in Black Locust through Genetic Selection

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Contractor:
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Athens, GA 30602

Principal Investigator: B.C. Bongarten

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Contract Number: 86X-95907C

Contract Period: 07/89 – 01/93

Contract Funding (Source):
FY 1990: $ 75,000 (DOE/ORNL)
FY 1991: $110,000 (DOE/ORNL)
FY 1992: $113,000 (DOE/ORNL)

Objective:

To determine efficient strategies for selecting and breeding black locust (*Robinia psuedoacacia*) for increased energy yield and to estimate the increase in energy yield resulting from the implementation of such programs.

Approach/Background:

Our results indicate that black locust is extremely genetically variable. Breeding efforts to increase biomass production are, therefore, expected to be very effective.

Status/Accomplishments:

Through 4 years of growth, biomass production of selected black locust families in the Piedmont of the southeastern United States is projected to range from 5 to 8 $Mg \cdot ha^{-1} \cdot yr^{-1}$, depending on the site and cultural conditions.

Techniques for vegetatively reproducing black locust via greenwood stem cuttings were refined in FY 1992. Success depends on using vigorous shoots (about 4 weeks of age), a porous, well-aerated rooting medium, and minimum misting. The control of insects and diseases on the stock plants is also essential. A 5-second basal dip in IBA (at 8000 to 16,000 ppm) dissolved in ethanol enhances rooting slightly. Using these practices, more than 90% of harvested cuttings can be rooted within 4 weeks in the majority of clones. To avoid plagiotrophism, stock plants should be cut back to the ground and the resulting stump sprouts used for cuttings.

Development of gene transfer technologies for black locust was also a primary focus in FY 1992. The immediate objective of this work is to introduce genes for resistance to two major insect pests of black locust—the stem borer and the leaf miner. Work we conducted in previous years revealed that some lines of black locust were capable of somatic embryogenesis, thus providing a means for propagating genetically transformed embryos. This year a search for additional lines capable of somatic embryogenesis was conducted. Six new cultures were discovered. Gene transfer was tested using the Biorad model PDS-1000/He particle delivery system to bombard plated cells and cell clumps with 1-μm-diameter gold particles coated with plasmid DNA. Plasmid DNA carried genes encoding neomycin phosphotransferase (NPT II) and β-glucuronidase (GUS) were used as markers of successful transformation. The promoter gene Ac2 was found to result in greater GUS expression than did the promoter gene CaMV35s. However, no plants showed definitive resistance to geneticin. Thus, the success of transformation efforts to date remains uncertain.

Major Project Reports:

Bongarten, B.C., D.A. Huber, and D.K. Apsley, "Environmental and Genetic Influences on Short-Rotation Biomass Production of Black Locust (*Robinia pseudoacacia* L.) in the Georgia Piedmont."

Bongarten, B.C., "Genetic Variation in Black Locust within its Native Range."

Johnsen, K.H. and B.C. Bongarten, "Effects of Nitrate Fixation and Growth of *Robinia pseudoacacia* Seedlings."

Summary Date: December 1992

Selection and Breeding of Pest-Resistant Clones of *Populus* for Biomass Energy Production in the North Central Region

Directing Organization:
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Contractor:
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Contract Number: 19X-43391C

Contract Period: 07/89 – 10/91

Contract Funding (Source):
FY 1990: $111,000 (DOE/ORNL)
FY 1991: $141,000 (DOE/ORNL)
FY 1992: $142,000 (DOE/ORNL)

Objective:

To develop new clones of *Populus* for use in the production of biomass energy in the North Central region of the United States.

Approach/Background:

Emphasis is being placed on selection and breeding for pest resistance, dry weight yield potential, and ease of propagation. Screening for pest resistance in hybrid *Populus* populations is a major component of this project.

Status/Accomplishments:

In 1992, we had 173 clones in their first cycle of selection, and 76 clones in the second-year nursery cycle. Survivors of this initial selection undergo a second screening cycle that is primarily for Septoria canker and ease of propagation. The clones that pass both levels of screening are increased and distributed within the region for field testing under local environmental and cultural conditions. Thirty-six clones were released to eight cooperators for field testing in 1992.

Given recent concern over new rust species and races in North America, we are shifting our emphasis to breeding and selecting for low levels of rust, with good leaf retention. We have discovered that a number of the early Marssonina infections appearing as red dots on selections and hybrids of *P. deltoides* do not develop into typical late symptoms. Although these trees have relatively high Marssonina ratings, there seems to be no effect on tree growth—indicating a type of field resistance. We have not been able to determine the characteristics or mechanisms of this resistance to date, but have determined that the trait is transmissible in crosses.

Supporting research is being done to establish correlations between laboratory, nursery, and field performance. In 1992, 27 clones were planted on three different types of sites for this test. An average 19% increase in survival of barbatels over that of unrooted cuttings is apparent at all three sites. A second year of study was initiated to determine the impact of defoliation on biomass production. Four new clones were planted for the study, and three series of defoliations were carried out in 1992; the 1991 study was defoliated four times. Growth and dry weight responses are now being determined for the 1991 plantation.

Major Project Reports:

Bingaman, C.R. and E.R. Hart, "Feeding and Oviposition Preferences of Adult Cottonwood Leaf Beetles (Coleoptera: Chrysomelidae) among *Populus* Clones and Leaf Age Classes."

Heuchelin, S.A., "Transformation of *Populus* × *euramericana* 'Ogy' with the *pin2* Gene."

Klopfenstein, N.W., R.W. Thornburg, H.S. McNabb, Jr., R.B. Hall, E.R. Hart, Y.W. Chun, A. Kernan, and N.Q. Shi, "Transformation of *Populus*—from System Development to Field Plantings."

Summary Date: December 1992

Biomass Production by Fescue and Switchgrass, Alone and in Mixed Swards with Legumes

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Contract Number: 19X-SC617C

Contract Period: 08/88 – 12/92

Contract Funding (Source):
FY 1990: $40,000 (DOE/ORNL)
FY 1991: $42,000 (DOE/ORNL)
FY 1992: -0- (DOE/ORNL)

Objective:

To (1) evaluate the biomass productivity and composition of tall fescue (*Festuca arundinacea* Schreb.) grown alone and with perennial legumes and (2) evaluate the biomass productivity and composition of switchgrass (*Panicum virgatum*) grown alone and with bigflower vetch (*Vicia grandiflora*).

Approach/Background:

Perennial and annual legumes meet their nitrogen needs and provide some nitrogen to associated grasses through symbiosis with Rhizobium bacteria, thus avoiding the need for exogenous nitrogen fertilization. Avoidance of this major input cost, and the potential for reduction or elimination of nitrogen fertilizer inputs with the associated environmental concerns, suggests the consideration of legumes as biomass fuel crops.

Status/Accomplishments:

During 1991, tall fescue yields increased linearly with an increasing nitrogen fertilization rate, up to an annual total of 225 kg/ha. Binomial mixtures with birdsfoot trefoil produced as much total biomass as the monoculture receiving 225 kg/ha of fertilizer nitrogen. Dry matter yields of nitrogen-fertilized tall fescue were as high as 10.9 Mg/ha, compared with 10.6 Mg/ha from the fescue-trefoil mixture. These results indicate that high yields of biomass can be produced from tall fescue-legume mixtures using nitrogen supplied symbiotically by perennial legumes.

Bigflower vetch, a winter annual legume that reseeds itself, was established into existing stands of four switchgrass cultivars during the autumn of 1988. During 1992, vetch shoot growth contained 73 kg/ha of nitrogen in early June, very similar to values found in 1989 and 1990. Although the quantity of nitrogen contained in vetch shoots is near that considered adequate for switchgrass production in every year, it appears that shading and early season competition restricts switchgrass shoot initiation and early growth. Switchgrass tiller density per unit area is reduced, and yields produced by switchgrass-vetch mixtures compare with those obtained from unfertilized pure-grass stands. During 1992, nitrogen-fertilized Kanlow switchgrass produced nearly 10 Mg/ha, less than yields obtained during 1989 and 1990. Reduced yields may have been caused by reduced solar radiation during the unusually wet growing season.

Major Project Reports: None

Summary Date: December 1992

Net Assimilation and Photosynthate Allocation of SRIC *Populus* Clones

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Principal Investigator: D.I. Dickmann

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Contract Number: 19X-95903C

Contract Period: 07/89 – 06/92

Contract Funding (Source):
FY 1990: $ 81,000 (DOE/ORNL)
FY 1991: $100,000 (DOE/ORNL)
FY 1992: $ 27,000 (DOE/ORNL)

Objective:

To provide a physiological basis for increasing short-rotation yields in *Populus*.

Approach/Background:

The physiology of two poplar clones (Tristis No. 1 and Eugenei) as affected by water, nitrogen, and coppicing is being investigated. Major emphasis has been placed on understanding root systems.

Status/Accomplishments:

Video images of fine roots of *Populus* clones Tristis and Eugenei growing along minirhizotrons in Experiment 1 have been analyzed. In general, Tristis produced more fine roots than Eugenei. Fine root number and length were seasonally fairly stable at 30–100 cm depth but variable at 0–30 cm—peaking in early spring for Tristis, but in midsummer for Eugenei. Irrigation tended to stimulate shallow fine root production in Eugenei, but the reverse was true in Tristis. At 30–100 cm, irrigation consistently depressed fine root production in both clones. Coppicing trees after 4 years' growth had no adverse effects on fine root production; in fact, both fine-root number and length increased substantially in the month following cutting. At the end of the third growing season, plots of clone Eugenei growing on the National Science Foundation Long Term Ecological Research site at Kellogg Biological Station were measured and analyzed. Weed competition significantly decreased tree diameter, height, leaf area, and aboveground biomass at low (2 × 3 m) and medium (1 × 2 m) planting densities, but not at high (0.5 × 1 m) planting densities. Although weed biomass decreased as the planting density of poplar increased, there were more weed species, and the equity of biomass distribution among these species increased at higher densities. Total aboveground community nitrogen content was not affected by weed competition at any planting density, regardless of the level of site occupancy. High-density, weed-free stands contained the highest amount of nitrogen.

Major Project Reports:

Dickmann, D.I., "Role of Physiology in Forest Tree Improvement."

Dickmann, D.I., Z. Liu, P.V. Nguyen, and K.S. Pregitzer, "Photosynthesis, Water Relations, and Growth of Two Hybrid Populus Genotypes During a Severe Drought."

Liu, Z. and D.I. Dickmann, "Abscisic Acid Accumulation in Leaves of Two Contrasting Hybrid Poplar Clones Affected by Nitrogen Fertilization Plus Cyclic Flooding and Soil Drying."

Summary Date: December 1992

Plant Biological Diversity in a Short-Rotation *Populus* Landscape

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Contract Number: 19X-95903C

Contract Period: 05/92 – 12/92

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: -0- (DOE/ORNL)
FY 1992: $22,000 (DOE/ORNL)

Objective:

The primary objective is to evaluate plant community richness of hybrid poplar plantations (weed diversity under three different planting densities) and to compare these ecosystems with other selected agricultural communities. The goal is to assess the effects of woody energy crop plantings on plant species diversity and to understand how the biological diversity of these management systems compares with the standard agricultural crops they might eventually displace in the landscape.

Approach/Background:

In 1989, a Long-Term Ecological Research (LTER) project funded by the National Science Foundation was established at the Kellogg Biological Station in southwestern Lower Michigan. This experiment was designed to better understand the ecological processes and mechanisms that drive sustainable production agricultural ecosystems. One of the treatments established as a part of this project was a short-rotation hybrid *Populus* plantation. These replicated *Populus* plantations were randomly established within a matrix of traditional high and low input row crop rotations, such as corn and soybeans. From the time of establishment in 1989 through the 1991 growing season, the entire weed community was sampled in several different agricultural treatments, including the *Populus* plantations. These data are rather unique and provided an opportunity to compare the impact of different agricultural practices on plant community diversity. This short-term research project was initiated to summarize the 3-year data set on plant diversity that existed at the LTER site. Tasks included computer data entry, data analysis, and data synthesis and reporting. The project was funded in July of 1992 and was designed to be complete by December of the same year.

Status/Accomplishments:

The 1989 data have been summarized, and we found that, as *Populus* plantation density increased, so did total plant community richness. This was related primarily to the fact that a few weed species dominated the community at low plantation densities. In terms of plant species diversity, the *Populus* plantations behaved more like the old-field successional treatment than the traditional row crop treatments. Species diversity was relatively high, and the proportion of annuals and perennial monocots and dicots was similar to the old-field treatment. The 1990 data are also now being processed.

Major Project Reports: None

Summary Date: December 1992

Bird and Mammal Usage of Hybrid Poplar Plantations

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Contract Number: 23-92-30

Contract Period: 04/92 – 04/93

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: -0- (DOE/ORNL)
FY 1992: $60,000 (DOE/ORNL)

Objective:

To assess and compare the abundance of birds and mammals in hybrid poplar plantations and nearby agricultural and wildland habitats in Minnesota, South Dakota, and Wisconsin; to determine whether there are overall positive or negative impacts of plantation establishment on these organisms; and to begin examination of relationships between plantations and nearby habitats in the context of understanding possible animal interchange between plantations and surrounding areas.

Approach/Background:

Standardized methods were used to sample birds and small mammals on plantations and in adjacent and nearby croplands, pastures, and wildlands.

Status/Accomplishments:

Birds and small mammals were studied on plantations and nearby habitats in Cloquet, Milaca, Granite Falls, and Fairmont, Minnesota; Sioux Falls, South Dakota; and Mondovi and Ashland, Wisconsin. Small mammals were sampled by snap-trapping in May–June and August–September 1992. Units of 50 traps each were placed simultaneously on plantations (typically a line in the center of the plantation and one along the long edge) and in nearby habitats, and trapping was conducted for two consecutive nights.

Preliminary analyses suggest that (1) species richness, total number of small mammals, number of rodents, and number of shrews were higher on wildlands than on plantations and agricultural lands, but most of these measures did not differ between the latter two land-use types, and (2) small mammals made greater use of portions of plantations bordering on wildlands than those bordering on agricultural lands. Additional studies on mammals will be conducted during the winter of 1992–1993.

Birds were sampled in the plantation and in all land use types bordering the plantation. We used a line transect method with transects bisecting the plantation and surrounding the perimeter of the plantation. Birds counts were done in the early morning on days with good weather during May, early June, late June, July, August, and September. Additional counts and nest searches will be done in October and November. Results of bird counts through August indicate that (1) the numbers of species and individuals were highest in adjacent forest and shrub communities and lowest in the plantations, but plantations and agricultural land types had similar values, and (2) the most common species within plantations were those typically associated with edges, second growth vegetation, and open woodlands—including the American robin, song sparrow, and common grackle.

Major Project Reports: None

Summary Date: December 1992

Early Selection Criteria and Clonal Propagation Methods for Increased Productivity of Sycamore in Short-Ration Energy Systems

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Contract Number: 19X-95902C

Contract Period: 04/91 – 06/93

Contract Funding (Source):
FY 1990: $40,975 (DOE/ORNL)
FY 1991: $77,000 (DOE/ORNL)
FY 1992: $85,000 (DOE/ORNL)

Objective:

To provide basic information on characteristics of American sycamore (*Platanus occidentalis* L.) reproductive materials (seeds, seedlings, rooted cuttings, and tissue culture plantlings); determine magnitudes of genetic and common environmental (maternal) effects; estimate sizes of juvenile-mature genetic correlations; develop "hedging" techniques for rooted cuttings; determine tissue culture techniques for plantlings; and determine if apomixis occurs in sycamore.

Approach/Background:

Seed and seedling characteristics should affect the performance of American sycamore in short-rotation plantations. The use of vegetative propagules rather than seedlings can allow greater utilization of genetic improvement in productivity.

Status/Accomplishments:

Preliminary calculations of genetic juvenile-mature correlations gave larger absolute values, but similar signs, to rank correlations. The mature traits in these correlations were (1) root growth potential of 11-month-old seedlings (January), (2) dry weights of 10-month-old seedlings in the nursery (December), and (3) tree size and limb characteristics of 14-month-old seedlings after planting in the field. The juvenile-mature correlations will be determined for seedling traits after 1 full year in the field.

Attempts to produce a new set of seeds from bagged, nonpollinated sycamore flowers in April 1992 were unsuccessful, because a late frost killed all flowers. Another attempt will be made in 1993 to produce these suspected apomictic seeds.

Significant variation among open-pollinated families has been detected for root growth potential, dry weight, percent of total dry weight allocated aboveground, and lateral root length of 10- to 11-month-old seedlings. Variation among control-pollinated crosses (combined average of reciprocal crosses), representing genetic differences, was found for lateral-root characteristics. Variation among reciprocals of the same cross, representing maternal effects on nonnuclear genetic differences, was detected for stem diameter and top root length.

Cuttings collected in February–April 1992 from 1-year-old sprouts on hedged sycamore grafts and planted over bottom heat in an irrigated outdoor nursery have shown (1) large clonal differences in rooting success (16%, 23%, and 25%) and (2) that cuttings collected in April (after bud break) did not root as well as cuttings collected in February and March (before bud break) (2% versus 23%–33%).

Open-pollinated, self-pollinated, and potential apomictic (nonpollinated) seeds from seven clones were germinated, grown in containers, and outplanted to a field site during 1992. Leaves from parent clones and from a sample of the seedlings will be collected and subjected to DNA analysis to confirm whether or not the suspected seedlings are apomictic.

Major Project Reports:

Land, S. B., Jr. and M. Cunningham, "Rooted Cutting Macropropagation of Hardwoods."

Tang, Z., "Genetic and Physiological Investigations of Seedling Development and Steckling Growth for American Sycamore (*Platanus occidentalis* L.)."

Summary Date: December 1992

Avian Species Diversity and Policy Issues in Large-Scale Short-Rotation Woody Energy Crops

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Contract Number: 1BX-SL237V

Contract Period: 06/92 – 05/93

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: -0- (DOE/ORNL)
FY 1992: $38,000 (DOE/ORNL)

Objective:

To evaluate short-rotation wood energy plantings in large plantations as a habitat for native birds, to assess the effects on local and regional bird species' diversity of concentrations on large plantations, and to develop policy options and alternatives that would provide greater environmental benefit from such plantings.

Approach/Background:

Studies of species diversity in short-rotation woody crop plantings are presently limited to small plots (10 acres or less) that may be heavily influenced by edge effects. The results of these studies are useful, but they are difficult to use in predicting the diversity of wildlife in large-scale plantations. Also, policies influencing the economic viability, placement, and characterization of energy crops could greatly influence the environmental effects of energy crop deployment. This project comprises two main efforts to address these concerns.

The first effort involves field investigations of bird diversity in large short-rotation woody crop monocultures where access to relatively large stands may be gained with industrial cooperators.

The second effort is an exploration of policy issues that may significantly influence the environmental costs and benefits associated with the deployment of energy crops on a large scale.

Status/Accomplishments:

We have assessed bird usage of hybrid poplar plantations in Ontario. Breeding birds were censused in three each young (2–3 years old), intermediate (5–7 years old) and mature (10–12 years old) first-growth stands, and two young coppiced stands.

Poplar plantations appear to be effective as buffers around tracts of more natural forest: forest interior species seem to use the forest right up to the edge of the poplars. In contrast, these species tend to avoid forest edges abutting on farmland or pasture. Some forest interior species, including Ruffed Grouse, Red-eyed Vireos, Ovenbirds, and American Redstarts, actually ranged into adjacent intermediate and mature poplar plantations. We have begun investigating opportunities for larger-scale projects.

Densities of birds in young plantations were substantially higher than in any of the adjacent cultivated farmland or hayfields. Young plantations were used extensively by old-field species, primarily Song Sparrows and Common Yellowthroats, Yellow Warblers, Indigo Buntings, and American Goldfinches.

Older plantation species diversity was higher, and the species composition seemed to be more dependent on the nature of the surrounding habitat. In particular, older plantations were used by more of the neotropical migrant species.

The retention of fence rows with mature trees greatly improved the poplar plantations' value as habitat for birds.

Major Project Reports: None

Summary Date: December 1992

Evaluation of Herbaceous Biomass Crops in the Northern Great Plains

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Contract Number: 19X-SB844C

Contract Period: 04/88 – 03/93

Contract Funding (Source):
FY 1990: $74,000 (DOE/ORNL)
FY 1991: $78,000 (DOE/ORNL)
FY 1992: $79,000 (DOE/ORNL)

Objective:

To evaluate the potential of several herbaceous species for biomass production on good and marginal cropland, and evaluate the economic feasibility of herbaceous energy crops with typical cropping alternatives in the northern Great Plains.

Approach/Background:

Data on herbaceous biomass crops managed for maximum yield were limited in the northern Great Plains (especially on marginal croplands), prior to this project.

Status/Accomplishments:

Biomass yields of four annuals and at least six perennial species were obtained at two good and two marginal sites in 1992. Maximum biomass yield was 20.3 Mg/ha from dryland forage sorghum (annual) grown on fallow land at Leonard, a marginal site, and 10.4 Mg/ha from switchgrass (perennial) in 1991. The 1992 perennial yields appear to be greater, with switchgrass ranging from 14.4 to 17.2 Mg/ha at Prosper and Leonard, respectively. Sorghum biomass at Glenfield good and marginal sites was substantially higher in 1991 than in previous years. Kochia yields in 1991 were low, with reliable yields at Hettinger only where it was the highest yielding species. Kochia results in 1992 appear promising again. Maximum 4-year biomass yield (16.9 Mg/ha) was obtained with irrigated forage sorghum; dryland forage sorghum at Prosper averaged 16.6 Mg/ha, however. Switchgrass has been the highest yielding perennial species at the two sites where adequate stands were established the last 2 years. Enterprise budgeting was used to make an initial comparison of net return of herbaceous biomass crops to popular conventional crops grown in North Dakota. Biomass yields were adjusted to county average yields, based on soil productivity classification of the sites. Yields were also adjusted for moisture content, harvest losses, and storage and transportation losses. Herbaceous biomass was valued at $39.2/Mg. One or more biomass crops in the three areas compared had a higher net return than the county's best conventional crop, indicating the potential for biomass cropping.

Major Project Reports:

Johnson, R.G., R.S. Sell, and D.W. Meyer, "Evaluation of Herbaceous Biomass Crops in the North Great Plains—Preliminary Economic Analysis."

Meyer, D.W., W.E. Norby, D.O. Erickson, and R.G. Johnson, "Evaluation of Herbaceous Biomass Crops in the Northern Great Plains—1991 Annual Report."

Summary Date: December 1992

Biochemical and Molecular Bases of Water Stress Tolerance of Poplar

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Contract Number: In-house

Contract Period: 10/90 – 09/92

Contract Funding (Source):
FY 1990: -0- (DOE)
FY 1991: $227,000 (DOE)
FY 1992: $227,000 (DOE)

Objective:

The long-term goal is to identify biochemical/ molecular traits that can be used to screen for water stress tolerance in traditional breeding or in a somaclonal selection via tissue culture. The primary objectives are (1) to determine genetic variability in and limits of water stress tolerance in existing poplar (*Populus*) clones, (2) to characterize the biochemical and molecular bases of water stress tolerance in responsive poplar clones, and (3) to develop biochemical and molecular screening procedures based on *in vivo* materials and *in vitro* materials that can be utilized to identify poplar clones that express maximum water stress tolerance.

Approach/Background:

Selected poplar clones are being screened in greenhouse and outdoor pot trials, using repeated water stress cycles. Relative growth rate (basal diameter squared times height [D^2H]) under stress, compared with well-watered plants, was used as an initial screening variable to determine which clones maintain their growth rate. Determination of the degree of osmotic adjustment and identification of the accumulating stress metabolites followed. Six clones in tissue and suspension cultures will undergo osmotic stress to determine whether the same stress metabolites accumulate in culture as in whole plants.

Status/Accomplishments:

Six clones (including four *P. deltoides* × *P. trichocarpa* hybrids 239 ♂, 240 ♀, 242 ♂, 246 ♀, and the two parents) were screened for water stress tolerance outdoors. In the first year of the study, repeated stress cycles induced some degree of osmotic adjustment in all clones except the ♀ parent. Stressed trees of the ♂ clones had the greatest degree of osmotic adjustment (0.25 MPa), compared with lesser adjustments (0.10 MPa) in the ♀ hybrids. By the final harvest, all three ♂ clones had higher main stem dry weight under stress conditions than did the ♀ clones. The growth advantage of ♂ clones was attributed to both osmotic adjustment and maintenance of a strong central tendency. In contrast, ♀ clones allocated more dry matter to numerous lateral meristems when under stress and displayed lesser degrees of osmotic adjustment. Analysis indicated that the greatest degree of osmotic adjustment in leaves occurred in hybrid 242 ♂, the ♂ parent, and in 239 ♂ to a lesser extent, whereas three of the four hybrids (240 ♀, 239 ♂, 242 ♂) displayed substantial adjustments to water stress in roots. Given that the TRIC ♀ parent did not display osmotic adjustment in either tissue, the hybrids' water stress tolerance capabilities were likely conferred by the DELT ♂ parent.

Major Project Reports:

Tschaplinski, T.J., G.A. Tuskan, and C.A. Gunderson, "Water Stress Tolerance of Black Cottonwood and Eastern Cottonwood Clones and Four of Their Hybrid Progeny. I. Growth, Water Relations and Gas Exchange," (submitted to the *Canadian Journal of Forest Research*).

Tschaplinski, T.J., and G.A. Tuskan, "Water Stress Tolerance of Black Cottonwood and Eastern Cottonwood Clones and Four of Their Hybrid Progeny. II. Metabolites and Inorganic Ions That Constitute Osmotic Adjustment," (submitted to the *Canadian Journal of Forest Research*).

Summary Date: December 1992

Research on Improving the Productivity of Switchgrass (*Panicum virgatum*) as a Biofuels Crop: Genotypic Diversity in Carbon Acquisition, Allocation and in the Utilization of Limiting Water and Nutrient Resources

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Contract Number: In-house

Contract Period: 06/92 – 05/93

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: -0- (DOE/ORNL)
FY 1992: $50,000 (DOE/ORNL)

Objective:

To examine the role that genotypic diversity plays in determining photosynthesis, aboveground and belowground respiration, patterns of carbon allocation, water- and nutrient-use efficiencies, and the growth of native and commercial switchgrass genotypes.

Approach/Background:

This project uses growth-analysis techniques combined with physiological measures to investigate the linkages between physiology and genetics within switchgrass genotypes. Once these processes are sufficiently documented, they can be exploited in plant breeding programs to achieve dependable biomass productivity.

Status/Accomplishments:

Switchgrass seeds were collected; this collection represents a diverse group of commercially developed upland and lowland ecotypes, native accessions of switchgrass from within the United States and elsewhere, and improved varieties in their last few years of testing prior to commercial release. We will continue to expand this collection as opportunities arise.

An initial six genotypes of switchgrass (Alamo, Kanlow, Cave-in-Rock, Trailblazer, Pathfinder, and a native accession from Florida) were planted in the greenhouse during July 1992. Thirty-nine days after planting, measurements of photosynthesis and dark respiration were made. Following these measurements, the shoot was clipped at a height of 10 cm, and plants were returned to the greenhouse to initiate a second growth cycle. Aboveground biomass was dried, weighed, and ground, and samples were set aside for nitrogen analysis. Forty-one days later, the entire measurement sequence was repeated to characterize the productivity and physiological status of these plants during the regrowth period. Plants were then destructively harvested and divided into shoots, stubble, and roots. Dry weights were determined and used to examine patterns of carbon allocation aboveground and belowground, and in the calculation of root-to-shoot ratios for each genotype.

Preliminary analyses indicate that the gas-exchange characteristics of the six genotypes did not differ to any significant extent. All genotypes had similar photosynthetic and respiratory rates. Despite these similarities, genotypic differences in aboveground productivity were observed.

Based on these results, seeds of the genotypes Alamo, Kanlow, Cave-in-Rock, and Pathfinder have been planted in a second experiment, along with the genotypes Shelter and Wabasso.

Major Project Reports: None

Summary Date: December 1992

Resource Analysis for Energy Production, Land Use, and Environment

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Contract Number: In-house

Contract Period: 10/91 – 09/92

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: $130,000 (DOE/ORNL)
FY 1992: $150,000 (DOE/ORNL)
FY 1992: $ 70,000 (EPA)
FY 1992: $ 35,000 (TVA)

Objective:

To improve understanding of the interactions between environmental and economic constraints on energy crop production and how those interactions vary with geographic regions, government policy, and spatial scale; to produce models of production supply and cost.

Approach/Background:

In the first analyses, only land capability (quality of land and current land use) was used to predict (on a national basis) how much land might grow energy crops and how much biomass might be produced on those lands. In the second phase, economic decisions that would be made by the owners of land were modeled to develop a cost-supply curve for woody biomass feedstocks for a particular energy conversion facility in East Tennessee. Economic decisions were modeled using a general linear model which maximized agricultural return in the facility region. The model assumed that farmers would grow energy crops on specific lands when the price they could get for energy crops assured them a greater net return than the most profitable conventional crop. Overall production within the region was limited by the amount and quality or type of land.

In the latest phase there are three tasks—(1) to repeat the facility-scale modeling exercise at three locations in the Midwest, (2) to model the cost-supply schedule for woody and herbaceous crops across the entire TVA region (a 276-county area encompassing 9.3 million acres of cropland and pasture), and (3) to build a Geographic Information System and associated landscape model of land use and environmental features to simulate landscape level shifts in environmental parameters with the adoption of energy crops. The first two tasks are well under way, and the third task is just beginning.

Status/Accomplishments:

There are 392 million acres within the conterminous United States that are capable of growing energy crops with yields ≥ 3 dry tons per year. Much of this land is in the Midwest. Two billion tons of woody or herbaceous feedstock material could be produced on this land annually.

The price of biofuel feedstock to a facility is the summation of the costs to transport the material from the farm to the facility and the price paid to the farmer for the feedstock. Longer distances increase hauling costs, while high yields for conventional crops increase the price paid to the farmer. Agricultural crop subsidies to corn and wheat increase the energy crops' price required to induce farmers to shift their land to energy crop production. In the East Tennessee region, crop subsidies increased the cost to supply the facility with energy crop feedstock by more than 10%.

Major Project Reports:

Graham, R.L., "An Analysis of the Land Base for Energy Crops in the Conterminous United States."

Graham, R.L., "Biomass Fuel Costs Predicted for East Tennessee Power Plant."

Summary Date: December 1992

Cottonwood Germ Plasm Maintenance

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Principal Investigator: C.G. Tauer

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Contract Number: 19X-5595OV

Contract Period: 02/86 – 04/93

Contract Funding (Source):
FY 1990: $2,700 (DOE/ORNL)
FY 1991: $3,000 (DOE/ORNL)
FY 1992: $3,000 (DOE/ORNL)

Objective:

To establish, field test, maintain, and share a wide range of southern representatives of the *Populus* genus of the United States (mostly *Populus deltoides*); to broaden the genetic base for traditional and biotechnological genetic methodology.

Approach/Background:

The research and development work performed under this agreement is part of the Indo–United States Poplar Studies. The DOE funds have allowed establishment, maintenance, and measurement of the India *Populus* clones on a single site in the United States. This will ensure the long-term availability of this *Populus* collection for additional testing and breeding in the United States.

Status/Accomplishments:

Two plantings of five replicates each were established in February 1988. The main planting contains 126 of the original 166-clone collection. Each clone is represented by one 4-tree row-plot per replicate. Twenty-two additional clones are maintained in small numbers in a clone bank. Eighty-nine clones are also represented in a second clone-spacing planting. Both plantings are maintained and measured annually. These data have been sent to the India group. In addition, cuttings have been sent to several interested parties in the United States and elsewhere.

Major Project Reports: None

Summary Date: December 1992

Breeding and Selection of New Switchgrass, *Panicum virgatum*, Varieties for Increased Biomass Production

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Department of Agronomy
Oklahoma Agricultural Experiment Station
Stillwater, OK 74078-5269

Principal Investigator: C.M. Taliaferro

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Contract Number: 19X-SL127C

Contract Period: 05/92 – 04/97

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: -0- (DOE/ORNL)
FY 1992: $78,000 (DOE/ORNL)

Objective:

To increase yield potential in switchgrass populations synthesized from germ plasms of (1) lowland tetraploid southern types (LTST), (2) lowland tetraploid northern types (LTNT), (3) upland hexaploid southern types (UHST), and (4) upland hexaploid northern types (UHNT).

Approach/Background:

Two major switchgrass types, lowland and upland, are differentiated on the basis of their edaphic requirements. Within each of these groups are numerous ecological and chromosomal ploidy variants. The lowland and upland switchgrasses are predominantly tetraploids (2n = 4x = 36 chromosomes) and hexaploids (2n = 6x = 54 chromosomes), respectively. Recurrent Restricted Phenotypic Selection (RRPS) will be practiced within each of the four synthesized breeding populations mentioned above. The four breeding populations are derived from commercial cultivars, breeding lines, and other germ plasm of known value and breeding behavior at the inception of the project. Additional germ plasm will be acquired from existing collections or from indigenous wild stands and evaluated for standard descriptors. Germ plasm identified as having superior genetic value will be incorporated into the breeding populations.

Status/Accomplishments:

Germ plasm of LTST, LTNT, UHST, and UHNT plant types was acquired in March, and seedlings were started in the greenhouse. Plants were transplanted to field nurseries in late June or early July. Each nursery contains approximately 900 plants spaced on 1-m centers. Conditions were excellent and plants in each of the four nurseries began producing seed heads in mid-September. During the last week of September, plants deemed superior in growth on the basis of visual rating were identified in each of the four nurseries. Five flowering culms were taken from each selected plant within a nursery and placed in a container of water in isolation in greenhouses for the purpose of intercrossing the plants. Seed obtained from intercrossing the selected plants will be used to generate the next selection population. Biomass production will be measured for each plant in each of the four nurseries at the end of the growing season. These data will be used to assess effectiveness of visual selection and to screen approximately 10% of selected plants from each of the four populations.

A germ plasm nursery was started during the summer of 1992 and presently contains 25 accessions. Arrangements have been made to obtain switchgrass accessions from the Soil Conservation Service, the USDA, the USDA National Germ Plasm Collection, and university plant breeders.

Major Project Reports: None

Summary Date: December 1992

Evaluation of Potential Herbaceous Biomass Crops on Marginal Lands

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Contractor:
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West Lafayette, IN 47907-1150

Principal Investigator: K.D. Johnson

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Contract Number: 19X-27412

Contract Period: 04/90 – 11/91

Contract Funding (Source):
FY 1990: $30,000 (DOE/ORNL)
FY 1991: $15,000 (DOE/ORNL)
FY 1992: -0- (DOE/ORNL)

Objective:

To evaluate the agronomic and economic feasibility of a variety of production and storage systems for herbaceous biomass crops.

Approach/Background:

As technology for economically utilizing biomass from herbaceous crops for energy is developed, it is also necessary to investigate cost-effective production practices and storage methods.

Status/Accomplishments:

Investigation of ways to reduce dry matter losses and compositional changes while storing herbaceous feedstocks is needed. Our objectives were to evaluate storage losses and compositional changes associated with switchgrass and tall fescue packaged as large round hay bales.

Storage surface and wrap type had the greatest impact on dry matter recovery and not compositional changes of the herbaceous feedstock. Storing large round bales on crushed rock was superior on sod for recoverability of unweathered dry matter and total dry matter. Switchgrass had less loss than tall fescue. Binding bales with twine compared with netting resulted in 6.6% less total recoverable switchgrass dry matter. Small increases in fiber and nitrogen composition were apparent in samples taken as probings from the outer bale surface exposed to air. Unweathered switchgrass had lower fiber content compared with weathered samples, with the greatest difference in the acid detergent fiber fraction (5.4%). Inside storage of large round bales does not appear to be economical as lower storage cost alternatives are available for the storage of herbaceous biomass.

Major Project Reports:

Johnson, K.D., D.K. Greene, and J.J. Volenec, "Potential Herbaceous Biomass Crops in the Midwest."

Johnson, K.D., J.H. Cherney, D.K. Greene, and J.J. Volenec, "Evaluation of Switchgrass and Sorghum Biomass Potential."

Summary Date: December 1992

Genetic Biomass and Growth Analysis of Clonal Silver Maple (*Acer saccharinum* L.) in Several Locations

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Contractor:
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Principal Investigators: J.E. Preece, W.C. Ashby, and P.L. Roth

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Contract Number: 86X-95908C

Contract Period: 04/90 – 06/94

Contract Funding (Source):
FY 1990: $50,000 (DOE/ORNL)
FY 1991: $80,000 (DOE/ORNL)
FY 1992: $81,000 (DOE/ORNL)

Objective:

To select and test four outstanding clones each of silver maple (*Acer saccharinum* L.) from 15 of 26 different provenances collected throughout the eastern United States and southeast Canada during the spring and early summer of 1987. These 60 clones were outplanted in replicated studies in 1991 in Minnesota, and on both an upland and a bottomland site in Carbondale, Illinois. Plantings in spring 1992 were in Kansas and Minnesota.

Approach/Background:

This project utilizes genetic and physiological techniques to maximize biomass production of silver maple as a short-rotation woody crop.

Status/Accomplishments:

Micropropagated silver maple plantlets were planted in a bottomland and an upland plantation near Carbondale, Illinois, during June 1991 and in Kansas and Minnesota during the spring of 1992. Ten blocks of 60 clones (four clones each from 15 provenances, with three plantlets per clone), were planted in a randomized complete block design through a 1.1-m wide, 0.03-mm (1.25-mil) thick, white-on-black plastic mulch. Although there was a severe drought during the summer of 1991, there was a 95% and 93% survival rate in upland and bottomland plantations, respectively. Survival rates in Kansas and Minnesota at the end of the 1992 growing season were greater than 85%. During the first growing season, trees grew taller on the upland site; however, bottomland trees nearly caught up in height in the second growing season. The mulch resulted in negligible soil erosion, conserved soil moisture, and facilitated weed control.

During the large-scale production phase, the use of *in vitro* root initiation of microshoots, followed by transplanting to preformed peat rooting plugs (Techniculture®) in high humidity trays and close monitoring of the new plantlets, expedited the micropropagation operation. Plant vigor, survival, and establishment were improved by selecting only high-quality microshoots to be used for rooting. Following rooting and acclimatization, plantlets in plugs were transplanted (with no shock or delay in growth) into RootMaster® containers in the greenhouse. Plantlets were then hardened in a lath house, or by overwintering in the field, prior to transplanting into the plantations. Container-grown plantlets at least 30-cm tall established better in the field than shorter plantlets.

Major Project Reports:

Ashby, W.C., D.F. Bresnan, P.L. Roth, J.E. Preece, and C.A. Huetteman, "Nursery Establishment, Phenology and Growth of Silver Maple Related to Provenance."

Preece, J.E., C.A. Huetteman, W.C. Ashby, and P.L. Roth, "Micro- and Cutting Propagation of Silver Maple. I. Results with Adult and Juvenile Propagules."

Preece, J.E., C.A. Huetteman, W.C. Ashby, and P.L. Roth, "Micro- and Cutting Propagation of Silver Maple. II. Genotype and Provenance Affect Performance."

Summary Date: December 1992

Bioenergy from Willow

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Research Foundation
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Principal Investigators: E.H. White and
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Contract Number: 19X-SK934V

Contract Period: 05/92 – 03/93

Contract Funding (Source):
FY 1990: -0-
FY 1991: -0-
FY 1992: $12,000 (DOE/ORNL)

Objective:

Establish stool beds for nursery scale-up of genetically improved hybrid willow clones for future demonstration farm plantings. Develop a strategy for the establishment of hybrid willow farms in the northeastern United States.

Approach/Background:

The first objective will be met by planting unrooted dormant cuttings of superior hybrid willow clones (developed by the University of Toronto) at one or more locations. The second objective will be met by hosting a workshop with experts on renewable energy from local, regional, and national organizations to form a consensus on how dedicated willow bioenergy plantations fit into the overall energy supply of the northeastern United States.

Status/Accomplishments:

The University of Toronto has agreed to provide unrooted cuttings of genetically improved willows for clone-site trials and will begin scaling up selected clones for a demonstration farm. Clone-site trials will supply critical information and could potentially be used to provide cuttings for the demonstration farm.

Clone-site information is critical to developing a plan for establishing a large bioenergy farm. Site preparation was completed for planting clone-site trials in Massena and Tully, New York, in the spring of 1993. The Massena site was mowed, raked, and treated with a mixture of glyphosate and the octyl ester of 2,4-dichlorophenoxyacetic acid, both at the 2 lb. ai/ac rate, to kill all vegetation on the site. Oxyfluorfen was applied at the 2 lb ai/ac rate as a pre-emergent herbicide cap. The Tully site was treated similarly, except that it was plowed, disked, and raked after confirming the effectiveness of the contact herbicides, but prior to application of oxyfluorfen. Both sites are representative of large acreages of abandoned agricultural land in New York State.

Major Project Reports: None

Summary Date: December 1992

An Evaluation of the Herbaceous Biomass Potential in the Eastern United States

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Contractor:
University of Tennessee
Institute of Agriculture
Knoxville, TN 37901-1071

Principal Investigator: B.C. English

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Contract Number: 11B-99732C-S60

Contract Period: 05/91 – 06/93

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: $65,000 (DOE/ORNL)
FY 1992: $62,000 (DOE/ORNL)

Objective:

To evaluate herbaceous biomass potential in the eastern United States with regard to the agricultural sector. This appraisal will include estimates on environmental impacts and estimates on economic feasibility on a regional basis. A second objective was added to the project this last year. A National Energy Modeling Component for ethanol is to be developed first derived from corn and then, pending funding, derived from biomass resources.

Approach/Background:

The project employs the Agricultural Resource Interregional Modeling System (ARIMS). The primary component of this model is a linear programming model designed to simulate the supply of this nation's agricultural commodities. The model as it currently exists is a cost minimization model. It minimizes the transportation and production costs of 16 crops and three major livestock types, plus ethanol production. An ethanol production sector has been incorporated into the model and is currently in operation evaluating the impacts of a corn-to-ethanol program. Once biomass activities are completed, then demand for biomass will be incorporated into the model.

The second component of the analytical framework is a simulation model of the agricultural sector. This model, POLYSIM, is a national policy simulation model of aggregate U.S. agriculture, with submodels for seven major crops and seven livestock categories. Incorporated into POLYSIM is an ethanol model capable of determining the impacts that large-scale ethanol production will have on other crop prices and supplies in the model.

These two models, operating interactively, will assist in determining national and regional market effects and the agricultural sector impacts of introducing large-scale ethanol or other biomass industries.

Status/Accomplishments:

The project has completed the initial phase, with ARIMS model development nearing completion. Biomass rotations need updating, incorporating recent findings. The POLYSIM model is up and running, with the ethanol sector in place.

Major Project Reports: None

Summary Date: December 1992

Analyses of Black Locust Progeny Tests

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Contract Number: 11X-SK935V

Contract Period: 05/92 – 05/93

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: -0- (DOE/ORNL)
FY 1992: $8,000 (DOE/ORNL)

Objective:

To evaluate 15-year-old black locust progeny tests for growth, insect damage, and form. To analyze and summarize the above-mentioned data, drawing general conclusions regarding the performance of black locust in east Tennessee and make specific recommendations pertaining to the selection of certain seed sources for future work.

Approach/Background:

This project evaluates existing black locust progeny tests for genetically superior trees in terms of growth, borer resistance, and form.

Status/Accomplishments:

Plantations currently exist on the University of Tennessee's Forestry Experiment Station near Tullahoma, Tennessee, and on a Campbell County strip mine in Tennessee near the Kentucky border. The Tullahoma planting has been periodically measured and can be easily evaluated. The Campbell County planting was inspected for survival and found to have a high percentage of trees living. A search for identification tags was made, and the beginning of each replication was marked with a treated post or flagging or both. Discrepancies exist between the map and the few tags that were found. The majority of the discrepancies can be resolved, although there is at least one instance in which trees will not be measured because of confusion as to the seed source. Diameter, form, and borer damage were measured on three replications.

Major Project Reports: None

Summary Date: December 1992

An Economic and Technical Analysis of Selected Issues Related to Energy Crop Production

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Contract Number: 11B-99732C S19

Contract Period: 11/90 – 09/93

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: -0- (DOE/ORNL)
FY 1992: $33,000 (DOE/ORNL)

Objective:

To (1) estimate the energy invested in fertilizers and pesticides used in agricultural production, (2) estimate the costs of biofuel production from selected biomass crops grown in different production zones of the United States, (3) estimate the cost of transporting biomass feedstocks, and (4) model the soil environmental impact of growing herbaceous energy crops on Iowa farms.

Approach/Background:

For each objective, a review of the literature is conducted to identify a suitable analytical method and data source including DOE annual reports, The Fertilizer Institute's manufacturers survey data, the U.S. Department of Agriculture's Marketing Service data on trucking rates, and other published reports.

Status/Accomplishments:

Objective 1.—Manufacturers' energy survey data is being used to estimate energy requirements of fertilizers. The weighted average energy uses in the production of nitrogen and phosphorous nutrients under 1987 technology were estimated in GJ/mt 51.93 and 10.29, respectively. Energy requirements for nitrogen and potash nutrients have substantially declined during the 1979 to 1987 period, causing a significant reduction in agriculture's dependence on nonrenewable fossil resources. Unit energy consumption in producing the active ingredients of major U.S. herbicides, insecticides, and fungicides was in GJ/mt 214.93, 245.06, and 356.39, respectively.

Objective 2.—Harvesting is the major cost in biomass production. Three main factors need to receive attention to reduce the total harvesting cost: (1) that the unit cost of harvesting declines as biomass yield increases, (2) that the yield depends on the number of cuttings every year (in the case of switchgrass) and the time of cutting, and (3) that the harvesting cost is also a function of annual machinery use. A methodology is being developed to evaluate these trade-offs, and harvesting cost and machinery requirement data for evaluating these trade-offs are being collected.

Objective 3.—Preliminary results show that transportation costs, at present, will not be a large component of the biofuel costs, but could be in the future. A model is being further refined.

Objective 4.—Findings indicate that although sorghum for biomass appears to be more economical, it is also more erosive than switchgrass. Analysis of the major Iowa soils (35 soils in total) is planned to achieve estimates of the farm-level environmental benefits of herbaceous biomass crops.

Major Project Reports:

Bhat, M.G. and B.C. English, "Biofuels from Energy Crops in the United States: A Regional Economic Analysis."

Bhat, M.G., B.C. English, A. Turhollow, and H. Nyangito, "Energy in Synthetic Agricultural Inputs: Revisited."

Bhat, M.G., B.C. English, and M. Ojo, "Regional Cost of Transporting Biomass Feedstocks."

Bhat, M.G. and B.C. English, "Biofuels from Energy Crops in the United States: A Regional Economic Analysis."

Summary Date: December 1992

Regional Supply of Biomass: An Evaluation of Three Midwestern Sites

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Contract Number: 11B-99732C-S60

Contract Period: 04/92 – 04/93

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: -0- (DOE/ORNL)
FY 1992: $67,000 (DOE/ORNL)

Objective:

To evaluate herbaceous biomass potential in three midwestern sites—Cass County, North Dakota; Olmstead County, Minnesota; and Orange County, Indiana.

Approach/Background:

Develop and test baseline linear programming models for each of the three sites. Using the baseline LP, incorporate biomass production and develop a regional supply curve for biomass. Provide impacts on the agricultural sector in each of the three regions and impacts on the environment. Evaluate biomass policy scenarios and describe the impacts these scenarios have on the supply curve.

Status/Accomplishments:

The regional land base in all three regions has been identified. Soils for agricultural production have been developed. Work on the Linear Programming model has been initiated. Traditional crops grown in each region are identified along with a written regional description.

Major Project Reports: None

Summary Date: December 1992

Development of *In Vitro* Culture Systems for Switchgrass (*Panicum virgatum*)

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Contractor:
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Principal Investigator: B.V. Conger

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Contract Number: 19X-SL129C

Contract Period: 06/92 – 06/93

Contract Funding (Source):
FY 1990: -0-
FY 1991: -0-
FY 1992: $52,000 (DOE/ORNL)

Objective:

To develop efficient and repeatable regeneration systems from cell and tissue cultures of switchgrass (*Panicum virgatum*).

Approach/Background:

The most efficient, repeatable, and preferred mode of regeneration in cereals and grasses is somatic embryogenesis. This project deals with identifying genotypes and developing protocols that will result in somatic embryogenesis from leaf and suspension cultures in switchgrass.

Status/Accomplishments:

More than 100 plants each of Alamo and Cave-in-Rock were established in a greenhouse to serve as explant material. They were grown under standard conditions and repotted once during the 90-day period.

Plants were cut back to either the crown or the first node. Young shoots (secondary tillers) of approximately 3- to 6-cm length (originating either from the crown or stem node) were collected and used as initial explants. They were sterilized and cut transversely into segments of 0.3- to 0.5-cm length. These were arranged serially from the basal to the distal portions. Within each tiller segment, the whorled leaf pieces were separated from each other and plated from the outer to the innermost. Calli were produced from all plants. The most important factor was leaf piece position. Callus initiation was observed 2 to 4 weeks after inoculation on all pieces of the basal two tiller segments and, on rare occasions, on the innermost leaf piece of the third segment. After the transfer of the calli to the media without growth regulators (or supplemented only with gibberellic acid), green spots and roots were observed after 2 weeks. Green shoots developed on three Alamo genotypes; however, they had abnormal morphology and grew slowly.

Whole tillers were cut just above the crown. Beginning at the base, individual nodes were excised from each tiller and the identity of their position maintained. Nodes were either explanted whole or split longitudinally into two pieces before plating. New shoots were produced from the nodes within 6 to 14 days of culture. However, there were differences in response between different tillers and between different nodes within a tiller. After 1 month, two or three new shoots were produced from a single node. These could be successfully used as explants. In a few cases, leaves or inflorescences were produced instead of shoots. The direct production of inflorescences is unique and of special interest. These could be used for in vitro fertilization experiments, another culture, or to induce somatic embryogenesis.

Major Project Reports: None

Summary Date: December 1992

Evaluation of Switchgrass Cultivars and Cultural Methods for Biomass Production in the South Central United States

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Project Manager: S.B. McLaughlin

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Contractor:
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Telephone: (817) 968-4144

Contract Number: 19X-SL128C

Contract Period: 05/92 – 04/97

Contract Funding (Source):
FY 1990: -0-
FY 1991: -0-
FY 1992: $93,000 (DOE/ORNL)

Objective:

To obtain data on adaptation of switchgrass (*Panicum virgatum* L.) cultivars in the south central United States, to develop guidelines for optimum germination and storage of switchgrass seed, to develop germ plasm with reduced seed dormancy, to develop germ plasm with improved establishment, and to develop improved management practices for switchgrass biomass production.

Approach/Background:

A multi-environment approach is used to determine cultivar performance. Genetic and physiological techniques are used to modify seed dormancy and improve the establishment of switchgrass as a biomass crop.

Status/Accomplishments:

Nine switchgrass cultivars were planted at six diverse sites in Texas (Stephenville, Knox City, Dallas, Beeville, Temple, and College Station) in April 1992. Fair to good stands were established at each site except Stephenville. Replanting may be necessary at Stephenville in 1993. Estimates of seeding-year biomass production will be obtained at Knox City, Dallas, and College Station in November 1992. An experiment on switchgrass response to nitrogen, phosphorus, and row spacing was established at Stephenville in April 1992 and at Beeville in September 1992. A biomass harvest was made at Stephenville in September 1992, and it indicated that switchgrass was still responding to nitrogen fertilizer at levels of 200 kg ha^{-1}. An experiment on harvest schedule effects on switchgrass persistence was established at Dallas and Stephenville in April 1992. Good stands have been obtained, and data collection will begin in 1992. The seed of switchgrass selected for low- and high-crown node elevation will be harvested in November and planted in the spring of 1992 to determine if selection has improved establishment characteristics of switchgrass.

Major Project Reports: None

Summary Date: December 1992

Biomass Research and Training Program

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Tuskegee, AL 36088

Principal Investigator: A. Weaver

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Contract Number: 19X-55997C

Contract Period: 05/91 – 12/91

Contract Funding (Source):
FY 1990: $33,000 (DOE/ORNL)
FY 1991: $26,000 (DOE/ORNL)
FY 1992: $11,000 (DOE/ORNL)

Objective:

To study various urea nitrogen fertilizer regimes required for optimum tree growth and nutrition in an American sycamore (*Platanus occidentalis* L.) plantation (consisting of approximately 4000 trees) for at least 4 years.

Approach/Background:

Research in a sycamore tree plantation was initiated in 1987 at Tuskegee University. Comprehensive analytical studies in the short-rotation intensive culture sycamore tree planation are conducted to evaluate specific tree requirements for optimum urea fertilizer inputs to achieve maximum tree growth and sustained productivity. Studies have included various urea nitrogen fertilizer applications, tree growth and physiology, leaf phenology and physiology, coppicing physiology, soil N transformations, ecosystem N retention and requirements, nondestructive harvesting of selected trees and physiology, and insect and disease development.

Status/Accomplishments:

In 1992, the sycamore tree plantation was in its fourth year of post-coppicing growth. Urea N was applied during April 1988, 1989, and 1990. The urea N treatments consisted of (1) a one-shot application (450 kg N/ha) in 1988 only, (2) annual-even (150 kg N/ha) each year, (3) annual-balloon with increasing doses applications (50, 150, or 250 kg N/ha) each year for 3 years, (4) multiple applications (50 kg N/ha) done three times per year, and (5) controls (no N applications). However, no urea N fertilizer applications were done in 1991. Data on tree growth and physiology, tree nutrition, ecosystem N retention and requirements, nondestructive harvesting of selected trees, and insect and disease development have been collected. The evaluation of data is ongoing.

Major Project Reports: None

Summary Date: December 1992

Genetic Variation Among Switchgrasses for Agronomic Traits, Forage Quality, and Biomass Fuel Production

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Contractor:
USDA, Agricultural Research Service
Lincoln, NE 68583-0910

Principal Investigators: K.P. Vogel and K.J. Moore

Telephone: (402) 464-3872

Contract Number: DE-AI05-900R21954

Contract Period: 09/90 – 09/93

Contract Funding (Source):
FY 1990: $17,600 (DOE/ORNL)
FY 1991: $44,000 (DOE/ORNL)
FY 1992: $48,000 (DOE/ORNL)

Objective:

To determine the magnitude of genetic variability for agronomic and biomass fuel production traits and the stability of those traits across the midwestern states for two sets of switchgrass germ plasm. One set will consist of the raw germ plasm collected from remanent prairie sites in the Midwest, plus check cultivars, and the other set will consist of elite germ plasm from the largest switchgrass breeding program in the United States.

Approach/Background:

Two separate experiments are being conducted at each of three sites: Mead, Nebraska; Ames, Iowa; and West Lafayette, Indiana. Experiment 1 is evaluating the raw germ plasm accessions collected from remnant midwestern prairies in 1989. Greenhouse grown seedlings of each accession were transplanted into replicated plots at each location in the spring of 1990. An accession is the germ plasm from a single remnant prairie. Check cultivars are included as controls. Experiment 2 consists of replicated sward trials of 13 experimental strains and seven check cultivars, and it was seeded in the spring of 1990. In 1991 and 1992, the experiments were harvested for biomass yield. Forage samples will be collected for forage quality analyses. These analyses will include tests on converting the forage to a biomass fuel. I. Carlson (Iowa State) and K. Johnson (Purdue University) are cooperators via Specific Cooperative Agreements with The United States Department of Agriculture–Agricultural Research Service.

Status/Accomplishments:

All research is on schedule, and the objectives are being achieved. All experiments were successfully established in 1990. Establishment year forage yields were harvested at two sites—Ames and West Lafayette. In 1991, two harvests were made on all experiments at all locations. Forage was sampled from all plots, at all harvests, at all locations. In 1992, the first harvest has been completed at all locations. An additional harvest will be made at all locations on the elite germ plasm experiment after a killing frost, which usually occurs after mid-October.

All forage quality work for 1991 has been completed, and all 1991 results have been summarized and submitted to the Oak Ridge National Laboratory. Forage quality work is in progress for the 1992 samples that have been collected to date.

The results to date indicate that there is substantial genetic variation for biomass fuel traits, forage yield, and quality among switchgrasses adapted to the midwestern states. Forage yields of the highest yielding strains averaged more than 6 tons/acre in 1991. In 1992, some of the strains will probably produce more than 7 tons of biomass per acre.

Major Project Reports:

Hopkins, A.A., K.P. Vogel, K.J. Moore, K.J. Johnson, and I.T. Carlson, "Variation among Switchgrasses from Remanent Prairies for Agronomic and Biofuel Traits."

Vogel, K.P., K.J. Moore, and A.J. Hopkins, "Genetic Variation among Switchgrasses for Agronomic Traits, Forage Quality, and Biomass Fuel Production."

Summary Date: December 1992

Increasing the Productivity of Biomass Plantations of Alder and Cottonwood in the Pacific Northwest

Directing Organization:
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Project Manager: J. Tuskan

Telephone: (615) 576-8141

Contractor:
USDA Forest Service
Pacific Northwest Research Station
3625 93rd Avenue SW
Olympia, WA 98512

Principal Investigator: D.S. DeBell

Telephone: (206) 956-2345

Contract Number: DE-AI05-81OR20914

Contract Period: 02/90 – 08/92

Contract Funding (Source):
FY 1990: $133,000 (DOE/ORNL)
FY 1991: $ 32,000 (DOE/ORNL)
FY 1992: $ 89,000 (DOE/ORNL)

Objective:

To develop guidelines for crop management and identify morphological and physiological traits that might be used for evaluating and improving cultural practices and for selecting superior genotypes.

Approach/Background:

Red Alder (*Alnus rubra*), black cottonwood (*Populus trichocarpa*), and various *Populus* hybrids are the trees most suitable for short-rotation bioenergy plantations in Oregon and Washington. Biological information is needed to provide guidelines for growing productive, cost-effective crops. Research plantations of alder and cottonwood are providing information on the effects of several short-rotation intensive culture practices, applied singly and in combination. The practices include spacing, fertilizers, irrigation, and weed-control treatments.

Status/Accomplishments:

At the end of the sixth (1991) growing season, red alder trees in Plantation I averaged 8.9 m in height and 7.7 cm in diameter in the 2-m, regularly irrigated plots. Growth and survival were less at the closer (0.5-m and 1-m) spacings; plots receiving low and irregularly-scheduled irrigation had reduced growth (at all three spacings) and increased survival (at the two closer spacings).

At the end of the sixth (1991) growing season in the *Populus* plots of Plantation I, one clone (hybrid 11-11) continues to outperform the other clone (Dula 01) at all spacings. At the 2-m spacing, mean 6-year height and diameter were 17.2 m and 12.8 cm for 11-11, and 11.8 m and 9.9 cm for Dula. Individual tree growth was markedly reduced at the closer spacings for both clones; only 16% of 11-11 trees have died in the closest spacing.

Plantation III, which has four *Populus* clones planted in pure and mixed clonal blocks at three spacings (0.5, 1.0, and 1.5 m), has continued to have excellent growth. Heights in pure clonal blocks measured midsummer during the third growing season (1992) ranged from 6.2 m (the closest spacing) to 10.3 m (the widest spacing). Results showed no differences in total yield between polyclonal blocks and the mean of monoclonal blocks. Pure clonal blocks have less variation in tree size than the polyclonal blocks where the more competitive clones have outgrown, and are now shading, their less competitive neighbors.

Major Project Reports:

DeBell, D.S., G.W. Clendenen, and J.W. Zasada, "Growing *Populus* Biomass: Comparison of Woodgrass versus Wider-Spaced Short-Rotation Systems."

DeBell, D.S. and C.A. Harrington, "Deploying Genotypes in Short-Rotation Plantations: Mixtures and Pine Cultures of Clones and Species."

Radwan, M.A., Y. Tanaka, A. Dobkowski, and W. Fangen, "Production and Assessment of Red Alder Planting Stock."

Summary Date: December 1992

Increasing Yields of Poplar Energy Plantations

Directing Organization:
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Oak Ridge, TN 37831-6352

Project Manager: J. Tuskan

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Contractor:
USDA Forest Service
North Central Forest Experiment Station
Rhinelander, WI 54501

Principal Investigator: J.G. Isebrands

Telephone: (715) 362-7474

Contract Number: DE-A105-80OR20763

Contract Period: 05/91 – 04/92

Contract Funding (Source):
FY 1990: $120,000 (DOE/ORNL)
FY 1991: $117,400 (DOE/ORNL)
FY 1992: $148,000 (DOE/ORNL)
plus $50,000/capital equipment

Objective:

To improve poplar (*Populus* spp.) yield through selection based on physiology research and to improve critical plant traits through biotechnology.

Approach/Background:

Scientific information is being developed for increasing plantation yields with selected studies on tree physiology, biotechnology, process modeling, and clonal screening.

Status/Accomplishments:

The development phase of an ecophysiological growth process model of poplar (ECOPHYS) was completed. The model was used to develop photosynthesis sampling strategies in field-grown poplars as part of air pollution studies.

Data were analyzed from first-year field tests of somaclonally selected herbicide-tolerant poplars. Roundup-tolerant trees were treated for a second year in FY 1992, as in commercial plantation production. All treatment plots (Roundup- and Oust-tolerant trees) were measured for the second year, and data are currently being analyzed. Results from first year tests show commercially important levels of tolerance for all Oust- and Roundup-tolerant clones. The best performing clones lacked herbicide-induced foliar injury and had darker green foliage. All clones had greater numbers of branches compared with parent clones. Second-year field data include evaluations of disease and frost susceptibility.

Physiological characterization experiments were conducted to verify the productivity of somaclonally selected and genetically engineered herbicide-tolerant hybrid poplars. A manuscript was prepared that describes productivity traits of genetically engineered poplars including whole tree biomass, maximum assimilation rates, and herbicide-induced chlorophyll degradation. Studies were also completed to determine light compensation points, dark respiration rates, and photosynthesis curves. Similar studies were performed on somaclonally selected trees. There were few differences in productivity characteristics between genetically engineered trees and the parent clones. In comparison, somaclonally selected trees showed significant differences in numerous traits such as biomass accumulation and photosynthetic efficiency.

Major Project Reports:

Host, G.E., "An Ecophysiological Whole Tree Approach to Modeling Tree Growth."

Riemenschneider, D.E., B.G. McMahon, and M.E. Ostry, "Use of Selection Indices to Increase Tree Height and to Control Damaging Agents in 2-year-old Balsam Poplar."

Wolf, A.T., T.E. Burk, and J.G. Isebrands, "Sampling Schemes for Estimating Total-tree Photosynthesis in *Populus* Clones—A Modeling Approach."

Summary Date: December 1992

Short-rotation Woody Crop Trials for Energy Production in the North Central United States

Directing Organization:
Oak Ridge National Laboratory (ORNL)
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Project Manager: J. Tuskan

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Contractor:
USDA Forest Service
North Central Forest Experiment Station
Grand Rapids, MN 55744

Principal Investigator: E.A. Hansen

Telephone: (218) 326-7109

Contract Number: DE-AI05-89OR21871

Contract Period: 03/91 – 03/92

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: $120,000 (DOE/ORNL)
FY 1992: $149,000 (DOE/ORNL)

Objective:

To establish large (8-ha) plantations to obtain estimates of biomass yields and production costs of wood energy in the North Central region of the United States.

Approach/Background:

Considerable research information exists on growing high yields of some woody species, particularly *Populus*. In a project jointly funded by DOE/ORNL, the U.S. Department of Agriculture Forest Service, and the Electric Power Research Institute, this information is being tested in large commercial-sized field plantings.

Status/Accomplishments:

Plantations at eight sites, from the eastern Dakotas into western Wisconsin, were tended during this past year. This involved disking and herbicide application for weed control at all sites. Tree survival and growth were good. Data collection and analyses from the 1992 season were completed, showing biomass production up to 6.7 Mg/ha in the 5-year-old plantations. The best 5-year-old clone in the small test plots of the clonal trials was exceeding 13.0 $Mg \cdot ha^{-1} \cdot year^{-1}$. The greatest yields were obtained on the high water table lands in east central Minnesota and on the deep loess soils of western Wisconsin. Plantations in the more arid areas of western Minnesota had slower growth.

Data from 4- and 5-year-old hybrid trials were analyzed to identify the best performing clones. Performance of individual clones tended to be uniform across the region; a clone that was one of the fastest growers at one site tended to be among the best clones at all sites. Soil data were collected at all sites to relate biomass production to site characteristics. Older hybrid poplar plantations were located and soil samples collected to determine soil carbon accretion under hybrid poplar. Tree leaf samples were collected at all plantations to determine nutritional status. Test plots were fertilized with nitrogen at all plantations to determine tree growth response to fertilizer. Tissue culture research is progressing toward developing a septoria-resistant variety of clone NE-308, a particularly drought-resistant clone.

Major Project Reports:

Hansen, E.A., "Poplar Woody Biomass Yields: A Look to the Future."

Hansen, E.A., "Biological Opportunities to Increase Tree Biomass Accumulation and Yield from Timberland."

Strong, T. and E.A. Hansen, "Response of Three *Populus* Species to Drought."

Summary Date: December 1992

Perennial Species for Optimum Production of Herbaceous Biomass in the Piedmont

Directing Organization:
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Project Manager: A. Turhollow

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Contractor:
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

Principal Investigators: D.J. Parrish, D.D. Wolf, W.L. Daniels, J.S. Cundiff, and D.H. Vaughan

Telephone: (703) 231-9778

Contract Number: 19X-27413

Contract Period: 02/85 -- 02/90

Contract Funding (Source):
FY 1990: $21,000 (DOE/ORNL)
FY 1991: -0- (DOE/ORNL)
FY 1992: -0- (DOE/ORNL)

Objective:

To investigate eight herbaceous biomass candidates on marginal Piedmont sites and seek ways to optimize economic production of the most promising candidate(s).

Approach/Background:

Energy cropping can reduce dependence on finite petrofuels while cycling carbon—thereby reducing atmospheric degradation. With appropriate energy-crop species and management practices, land in the Piedmont could produce feedstocks for liquid fuels. (The Piedmont is currently underutilized agriculturally because of inherent soil and site limitations.)

Status/Accomplishments:

In FY 1992, Virginia Tech completed a 7-year screening study of eight potential herbaceous biofuels species. Switchgrass was consistently the most productive of the candidate species. Average yields for switchgrass ranged from 8 to 16 Mg/ha during the years following establishment. Switchgrass yields did not appear to be as dependent on growing season rainfall as were yields of the other crops. Economic and erosional analyses showed distinct differences among the candidate species; culture of annual species resulted in significantly higher soil losses than with perennial species, and switchgrass was shown to be superior both for economic and erosional considerations.

A 4-year N-management and cutting-frequency experiment that was completed in FY 1992 sought to optimize biomass production of switchgrass. On one site, there was no benefit of added N during the 4 years of the study. At the other two locations, the optimum level of N fertilization appeared to be no more than 50 kg/ha. Crimson clover (*Trifolium incarnatum*) and hairy vetch (*Vicia villosa*) intercropped into the stubble of harvested switchgrass appeared to supply all of the N needs of switchgrass, but management of the legumes in the spring was crucial to prevent excessive competition with the switchgrass. Cutting-frequency studies showed a loss of standing switchgrass biomass between early September and early November. The reduction in harvestable biomass appeared to be caused by the translocation of dry matter to belowground parts. Harvesting in early September yielded more biomass within a year, but it reduced biomass yields in succeeding years when compared with early November harvests. This poses a dilemma for potential producers of switchgrass biomass.

Major Project Reports:

Parrish, D.J., D.D. Wolf, W.L. Daniels, D.H. Vaughan, and J.S. Cundiff, "A 5-Year Study of Herbaceous Biomass Species in the Piedmont."

Parrish, D.J. and D.D. Wolf, "Managing Switchgrass for Biomass."

Parrish, D.J., D.D. Wolf, and W.L. Daniels, "Perennial Species for Optimum Production of Herbaceous Biomass in the Piedmont (Management Study, 1987–1991): Final Report to Contractor."

Summary Date: December 1992

Genetic Improvement and Evaluation of Black Cottonwood for Short-Rotation Biomass Production

Directing Organization:
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Oak Ridge, TN 37831-6352

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Contractors:
University of Washington
Seattle, WA 98195

Western Washington Research and
Extension Center
Puyallup, WA 98371

Principal Investigators: R.F. Stettler, P.E. Heilman, T.M. Hinckley, and H.D. Bradshaw, Jr.

Telephone: (206) 543-2723

Contract Number: 19X-43382C

Contract Period: 09/89 – 08/92

Contract Funding (Source):
FY 1990: $182,000 (DOE/ORNL)
FY 1991: $218,000 (DOE/ORNL)
FY 1992: $222,000 (DOE/ORNL)

Objective:

To (1) genetically improve black cottonwood (*Populus trichocarpa*) for biomass production, (2) elucidate critical components of productivity and the ways in which they can be manipulated genetically and environmentally, and (3) encourage poplar culture in the Northwest.

Approach/Background:

This project provides benchmark data on the potential productivity of black cottonwood, the fastest growing hardwood of the Pacific Northwest, when grown under short-rotation intensive culture.

Status/Accomplishments:

To date, 240 markers have been mapped, covering an estimated 90% of the *Populus* genome at a resolution of 20 centiMorgans. This map has adequate marker density to permit the identification of quantitative trait loci relevant to biomass productivity. A replicated plantation of F_2 and B_1 material at Puyallup, now in its second year, serves to provide a vast array of phenotypic data for this purpose. Stoolbeds have been established to produce an adequate supply of three-generation pedigree materials for large plantations in diverse environments.

During the past year, a comprehensive analysis of clonal evaluation trials was conducted, screening more than 1300 clones at 14 field sites, both east and west of the Cascades. Results showed the majority of well-performing clones, all F_1 hybrids, to be quite site specific in their productivity.

Major Project Reports:

Bradshaw, H.D., Jr., and G.S. Foster, "Marker-Aided Selection and Propagation Systems in Trees: Advantages of Cloning for Studying Quantitative Inheritance."

Dunlap, J.D., P.E. Heilman, and R.F. Stettler, "Genetic Variation and Productivity of *Populus trichocarpa* T. and G. and its Hybrids. Part 5—The Influence of Ramet Position on Three-Year Growth Variables."

Friend, A.L., G. Scarascia-Mugnozza, J.G. Isebrands, and P.E. Heilman, "Quantification of Two-Year-Old Hybrid Poplar Root Systems: Morphology, Biomass, and ^{14}C Distribution."

Hinckley, T.M., H. Richter, and P.J. Schulte, "Water Relations."

Scarascia-Mugnozza, G. "Physiological and Morphological Determinants of Yield in Intensively Cultured Poplars (*Populus* spp.)."

Summary Date: December 1992

Economic Analysis Task

Directing Organization
Oak Ridge National Laboratory (ORNL)
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Project Manager: A. Turhollow

Telephone: (615) 576-8144

Contractor: Not applicable (NA)

Principal Investigator: NA

Telephone: NA

Contract Number: NA

Contract Period: NA

Contract Funding (Source):
FY 1990: -0- (DOE/ORNL)
FY 1991: $182,000 (DOE/ORNL)
FY 1992: $243,000 (DOE/ORNL)

Objective:

To conduct economic analyses of issues relevant to herbaceous energy crops and short-rotation woody crops and to provide direction and oversight to subcontracted research that deals with economic issues.

Approach/Background:

This project utilizes an agricultural economist and expert subcontractors to conduct economic analyses.

Status/Accomplishments:

In FY 1992 there were two projects subcontracted with the University of Tennessee, one dealing with developing supply curves for energy crops at a national scale and the other dealing with the development of supply curves for issues such as the cost of transporting energy crops and the energy inputs associated with agricultural inputs (i.e., fertilizers and pesticides). Development of the supply curves at a national scale is being done in conjunction with the Energy Information Administration of DOE.

Presentations were made on the economics of short-rotation woody crops production, the cost of producing feedstocks for ethanol production, and environmental and institutional issues associated with the use of ethanol as a motor fuel. Chapters in two reports were written on the economics of energy crops production and the economics of short-rotation woody crop production; a journal article on the environmental and institutional aspects of the use of ethanol as a motor fuel was written.

An ORNL staff member spent 4 months at the Office of Technology Assessment working on a chapter on the economic and environmental impacts of large-scale biomass production and use. This effort will continue into FY 1993.

Major Project Reports:

Turhollow, A.F., "Economics of Dedicated Energy Crop Production."

Turhollow, A.F., "Economic Considerations for the Production of Wood for Energy."

Turhollow, A.F. and S. Kanhouwa, "Factors Affecting the Market Penetration of Biomass-Derived Liquid Transportation Fuels."

Summary Date: December 1992

Biofuels Feedstock Storage

Terrestrial Feedstocks/Process Interaction
Terrestrial Feedstocks/Fuel Production Interface

Directing Organization:
National Renewable Energy Laboratory (NREL)
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Project Manager: A. Wiselogel

Telephone: (303) 231-7816

Contractor:
National Renewable Energy Laboratory
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Principal Investigators: A. Wiselogel and D.K. Johnson

Telephone: (303) 231-7816; 231-7633

Contract Number: In-house

Contract Period: 01/92 – 01/93

Contract Funding (Source):
FY 1992: $462,750 (DOE)

Objective:

The primary objective of this task is to assess agricultural residues, herbaceous energy crops, and short-rotation woody crops as lignocellulosic feedstocks for ethanol conversion.

Approach/Background:

To meet the primary objective, several secondary objectives have been established: improve methodology by developing rapid feedstock analysis techniques and standard materials and methods; define the feedstock quality characteristics for ethanol conversion and determine management of feedstock storage, handling, and pretreatment techniques; determine the important sources of variation for feedstock characteristics; and produce decision-making tools to assist in the development of a commercial ethanol from biomass industry.

Status/Accomplishments:

Four biomass samples, from *Populus deltoides*, *Pinus radiata*, wheat straw, and bagasse, have become National Institute of Standards and Technology (NIST) reference materials through collaboration between NREL and NIST.

Significant progress has been made in the development of a new rapid analysis method for biomass characterization utilizing pyrolysis mass spectrometry and factor analysis.

The variables used to determine feedstock quality for conversion to ethanol are: total convertible carbohydrates (e.g., total monosaccharide potential for ethanol or for catalytic upgrading), percent lignin (as a source of heat for ethanol production or syn-gas potential), and ethanol production potential. Feedstock quality determination is part of the storage experiments that were initiated for eight feedstocks relevant to the ethanol program. All of the storage experiments are on schedule.

Experiments to determine sources of variation in feedstock quality will use variety trials and progeny tests already established at the U.S. Department of Agriculture and state university research facilities. The initial assessment will generate data for the development of a model that describes the impact of storage on feedstock quality, composition, and ethanol potential. Currently, data management for the ambient and storage environment data sets and some of the pretreatment and SSF/fermentation data is under way. A review of Geographic Information Systems formats, hardware requirements, capabilities, and available supplementary data sets (political boundaries, climate, transportation, and vegetation) is progressing with the help of NREL staff and outside experts.

Major Project Reports:

Agblevor, F.A., B. Rejai, and R.J. Evans, "Pyrolysis Analysis and Catalytic Upgrading of Lignocellulosic Materials by Molecular Beam mass Spectrometry.

Agblevor, F.A., B. Rejai, D. Wang, A.E. Wiselogel, H. Chum, and F. Keany, 1992, "Thermochemical Conversion of Biomass to Fuels and Chemicals: The Effect of Storage Conditions on Pyrolysis Products."

Milne, T.A., H.L. Chum, F.A. Agblevor, and D.K. Johnson, "Standardized Analytical Methods Activity Report."

Wiselogel, A.E., D.K. Johnson, and H.L. Chum, "Interface Between Production and Conversion Technologies for the Development of Dedicated Biomass Feedstocks."

Summary Date: December 1992

Evaluation of Feedstock Pretreatability and Saccharification/Fermentation

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Colorado State University
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Fort Collins, CO 80523

Principal Investigators: J. Linden and H. Schroeder

Telephone: (303) 491-7768

Contract Number: XC-1-11142-1

Contract Period: 09/91 – 07/93

Contract Funding (Source):
FY 1991: $189,246 (DOE)
FY 1992: $189,216 (DOE)

Objective:

To determine the suitability of potential sources of biomass as ligno-cellulosic feedstocks for conversion to ethanol. The scope of this research involves analyzing herbaceous, woody, and agricultural residues, and determining sources of extraneous variation and stability during storage. Other work being done under this subcontract includes corn stover storage effects and the use of ensilage as an enzymatic pretreatment.

Approach/Background:

NREL is evaluating a range of grasses and woody feedstocks to determine their potential as feedstock for ethanol conversion. There are several elements to this question, including the effect of growth and storage conditions on the ethanol conversion process; the effect of feedstock type on the ethanol conversion process; the pretreatability (via dilute acid hydrolysis) of feedstocks; and, finally, the fermentability of feedstocks after pretreatment with dilute acid. Ensilage, which involves the use of enzymes during the storage of silage to enhance fermentability, has been studied using cellulase enzymes.

Status/Accomplishments:

The subcontractor has conducted pretreatment tests on 95 samples of feedstock collected by NREL in storage studies being conducted throughout the United States. In addition, pretreatment tests have been conducted on so-called reference materials submitted by NREL. Feedstocks tested include American sycamore, Black Locust, Hybrid Poplar, Sericea, switchgrass, Aspen, and wheat straw. Fermentability studies have been conducted on 61 pretreated samples of biomass. Significant variability in these results points out the need for improvement in the protocol being used by the subcontractor. Preliminary ensilage experiments with Genencor Laminex cellulase preparations have shown an ability to enhance ethanol conversion.

Major Project Reports:

Schroeder, H. and J. Linden, "Evaluation of Feedstock Pretreatability and Saccharification/Fermentation Suitability as a Function of Harvesting and Storage."

Summary Date: December 1992

Effects of Storage on *Robinia Pseudoacacia*

Directing Organization:
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Contractor:
Iowa State University
251 Beardshear Hall
Ames, IA 50011-1021

Principal Investigator: R. Hall

Telephone: (515) 294-1166

Contract Number: XA-1-11203-1

Contract Period: 10/91 – 10/92

Contract Funding (Source):
FY 1991: $31,250 (DOE)

Objective:

To monitor, sample, and harvest *Robinia Pseudoacacia* wood to study the effects of storage on biomass wood quality.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time, depending on the size of the conversion facility, its location, and the number of feedstocks that it will be able to utilize at a given location. On storage, these various feedstocks may undergo both mass and compositional changes that impact the yield of the fuels produced. These changes (as a function of season, harvest mode, and storage time) are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, and for testing of their pretreatability (using the dilute acid process) and the suitability of the resulting substrates for conversion to ethanol via simultaneous saccharification and fermentation (SSF).

Status/Accomplishments:

All samples have been collected and submitted to NREL for analysis and testing.

Major Project Reports:

Hall, R., "Effects of Storage on *Robinia Pseudoacacia.*"

Summary Date: December 1992

Effects of Storage on Sweet and Forage Sorghum

Directing Organization:
National Renewable Energy Laboratory (NREL)
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Project Manager: A. Wiselogel

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Contractor:
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1559 Agronomy
Ames, IA 50011-1021

Principal Investigator: I.C. Anderson

Telephone: (515) 294-9651

Contract Number: XA-1-11237-1

Contract Period: 10/91 – 10/92

Contract Funding (Source):
FY 1991: $23,420 (DOE)

Objective:

To monitor, sample, and harvest sweet and forage sorghum to study the effects of storage on biomass feedstock quality.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time, depending on the size of the conversion facility, its location, and the number of feedstocks that it will be able to utilize at a given location. On storage, these various feedstocks may undergo both mass and compositional changes that impact the yield of the fuels produced. These changes (as a function of season, harvest mode, and storage time) are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, and for testing of their pretreatability (using the dilute acid process) and the suitability of the resulting substrates for conversion to ethanol via simultaneous saccharification and fermentation.

Status/Accomplishments:

The study is complete. All samples have been submitted to NREL for analysis and testing.

Major Project Reports:

Anderson, I. and D. Buxton, "Effects of Silage Storage on Sweet and Forage Sorghum for Biomass to Ethanol and Thermochemical Fuels Projects."

Summary Date: December 1992

Effects of Storage on Bagasse

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: A. Wiselogel

Telephone: (303) 231-7816

Contractor:
Hawaiian Commercial and Sugar Company
P.O. Box 266
Puunene Avenue
Puunene, HI 96784

Principal Investigator: R. Kwok

Telephone: (808) 877-0081

Contract Number: HA-1-11211-1

Contract Period: 08/91 – 02/92

Contract Funding (Source):
FY 1991: $9,775 (DOE)

Objective:

To monitor, sample, and harvest bagasse to study the effects of storage on biomass feedstock quality.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time, depending on the size of the conversion facility, its location, and the number of feedstocks that it will be able to utilize at a given location. On storage, these various feedstocks may undergo both mass and compositional changes that impact the yield of the fuels produced. These changes (as a function of season, harvest mode, and storage time) are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, and for testing of their pretreatability (using the dilute acid process) and the suitability of the resulting substrates for conversion to ethanol via simultaneous saccharification and fermentation.

Status/Accomplishments:

The study is complete. Samples have been submitted to NREL for analysis and testing.

Major Project Reports:

Keany, F., A. Wiselogel, and F. Agblevor, "Effects of Storage on Bagasse."

Summary Date: December 1992

Effects of Storage on Eucalyptus

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: A. Wiselogel

Telephone: (303) 231-7816

Contractor:
Bioenergy Development Corporation
888 Kalanianaole Avenue
Hilo, HI 96720

Principal Investigator: T. Crabb

Telephone: (808) 961-0411

Contract Number: AA-2-11210

Contract Period: 01/92 – 12/92

Contract Funding (Source):
FY 1991: $15,784 (DOE)

Objective:

To determine the impact of proposed storage methodology on eucalyptus for the Biomass-to-Ethanol and Thermochemical Fuels Projects.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time, depending on the size of the conversion facility, its location, and the number of feedstocks that it will be able to utilize at a given location. On storage, these various feedstocks may undergo both mass and compositional changes that impact the yield of the fuels produced. These changes (as a function of season, harvest mode, and storage time) are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, and for testing of their pretreatability (using the dilute acid process) and the suitability of the resulting substrates for conversion to ethanol via simultaneous saccharification and fermentation.

Status/Accomplishments:

The sample collection is on schedule.

Major Project Reports: None

Summary Date: December 1992

Effects of Storage on Sericea lespedeza

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: A. Wiselogel

Telephone: (303) 231-7816

Contractor:
Auburn University
Department of Agronomy and Soils
202 Funchess Hall
Auburn University, AL 36849

Principal Investigator: D.I. Bransby

Telephone: (205) 844-3935

Contract Number: HW-1-11176-1

Contract Period: 09/91 – 03/92

Contract Funding (Source):
FY 1991: $11,619 (DOE)
FY 1992: $ 1,948 (DOE)

Objective:

To monitor, sample, and harvest Sericea lespedeza to study the effects of storage on biomass feedstock quality.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time, depending on the size of the conversion facility, its location, and the number of feedstocks that it will be able to utilize at a given location. On storage, these various feedstocks may undergo both mass and compositional changes that impact the yield of the fuels produced. These changes (as a function of season, harvest mode, and storage time) are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, and for testing of their pretreatability (using the dilute acid process) and the suitability of the resulting substrates for conversion to ethanol via simultaneous saccharification and fermentation.

Status/Accomplishments:

All 48 samples from both harvests planned for this study have been collected and submitted to NREL for analysis and testing.

Major Project Reports: None

Summary Date: December 1992

Effects of Storage on American Sycamore

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: A. Wiselogel

Telephone: (303) 231-7816

Contractor:
Oak Ridge National Laboratory
P.O. Box 2008
Oak Ridge, TN 37831-6034

Principal Investigator: C.A. Gunderson

Telephone: (615) 574-6318

Contract Number: DA-2-11209

Contract Period: 10/91 – 04/92

Contract Funding (Source):
FY 1991: $53,000 (DOE)

Objective:

To monitor, sample, and harvest American sycamore wood to study the effects of storage on biomass wood quality.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time, depending on the size of the conversion facility, its location, and the number of feedstocks that it will be able to utilize at a given location. On storage, these various feedstocks may undergo both mass and compositional changes that impact the yield of the fuels produced. These changes (as a function of season, harvest mode, and storage time) are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, and for testing of their pretreatability (using the dilute acid process) and the suitability of the resulting substrates for conversion to ethanol via simultaneous saccharification and fermentation.

Status/Accomplishments:

All samples have been collected and submitted to NREL for analysis and testing.

Major Project Reports: None

Summary Date: December 1992

Effects of Storage on Switchgrass for Biomass-to-Ethanol and Thermochemical Fuels Project

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: A. Wiselogel

Telephone: (303) 231-7816

Contractor:
Texas A&M University Research Foundation
Box 3578
College Station, TX 77843

Principal Investigator: M. Sanderson

Telephone: (817) 968-4144

Contract Number: HW-1-11164

Contract Period: 10/91 – 03/93

Contract Funding (Source):
FY 1991: $14,526 (DOE)
FY 1992: $ 5,000 (DOE)

Objective:

To determine the feedstock quality of switchgrass for conversion to ethanol and thermochemical products, and the impact of storage on that quality.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time, depending on the size of the conversion facility, its location, and the number of feedstocks that it will be able to utilize at a given location. On storage, these various feedstocks may undergo both mass and compositional changes that impact the yield of the fuels produced. These changes (as a function of season, harvest mode, and storage time) are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, and for testing of their pretreatability (using the dilute acid process) and the suitability of the resulting substrates for conversion to ethanol via simultaneous saccharification and fermentation.

Status/Accomplishments:

All switchgrass samples from two sets of harvests in the storage study have been collected and submitted to NREL for testing and analysis.

Major Project Reports: None

Summary Date: December 1992

Effects of Storage on *Populus Deltoids X Nigra*

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: A. Wiselogel

Telephone: (303) 231-7816

Subcontractor:
University of Minnesota
1100 Washington Avenue, South, Suite 201
Minneapolis, MN 55415

Principal Investigator: W. Johnson

Telephone: (218) 281-6510

Contract Number: XA-2-11198-1

Contract Period: 11/91 – 12/92

Contract Funding (Source):
FY 1991: $24,288 (DOE)

Objective:

To monitor, sample, and harvest *Populus Deltoids X Nigra* in order to study the effects of storage on biomass wood quality.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time, depending on the size of the conversion facility, its location, and the number of feedstocks that it will be able to utilize at a given location. On storage, these various feedstocks may undergo both mass and compositional changes that impact the yield of the fuels produced. These changes (as a function of season, harvest mode, and storage time) are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, and for testing of their pretreatability (using the dilute acid process) and the suitability of the resulting substrates for conversion to ethanol via simultaneous saccharification and fermentation.

Status/Accomplishments:

All eight samples from the first harvest and seven samples from the second harvest (up to week 13 samples), have been completed and submitted to NREL for analysis and testing.

Major Project Reports: None

Summary Date: December 1992

Analysis and Assessment

Impact of Ethanol Use on U.S. Refined Oil Markets

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C.J. Wallace

Telephone: (202) 484-1090

Contractor:
Meridian Corporation
4300 King Street
Alexandria, VA 23202-1508

Principal Investigator: D.I. Hertzmark

Telephone: (703) 998-3600

Contract Number: YS-2-12079

Contract Period: 10/91 – 12/92

Contract Funding (Source):
FY 1992: $50,000 (DOE)

Objective:

To examine impacts of incremental penetration of bioethanol into the transportation system. Price changes in fuels, changes in import levels, etc., were considered for overall impact on the U.S. economy.

Approach/Background:

A linear programming model REFORM was developed for current and future gasoline blending and supply in the United States. This model can readily answer questions about the responses of the U.S. refinery system if prices and output of key feedstock are altered or if overall demand is reduced.

Status/Accomplishments:

This document reports the projected impacts of a vast increase in ethanol production in the U.S. refinery industry. The premise of the work is a series of increases in ethanol production from current levels to more than 2.8 million barrels per day by 2010 to 2015.

Major Project Reports:

Hertzmark, D.I., "Impact of Ethanol Use on U.S. Refined Oil Markets."

Summary Date: December 1992

Conversion Technologies: Biomass to Ethanol

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C.J. Wallace

Telephone: (202) 484-1090

Contractor:
Meridian Corporation
4300 King Street, Suite 400
Alexandria, VA 22302-1508

Principal Investigator: R. Tshiteya

Telephone: (703) 998-3600

Contract Number: YS-2-12079

Contract Period: 10/91 – 10/92

Contract Funding (Source):
FY 1992: $50,000 (DOE)

Objective:

To serve as a desk companion for public policy decision-makers by presenting a range of scientific and engineering information on pathways for converting biomass feedstocks to ethanol.

Approach/Background:

The document contains a literature search, primary data collection and analysis, key issues and implications, and a glossary of useful terms and definitions.

Status/Accomplishments:

The process flows, expected outcomes, and technical difficulties for converting a full range of feedstocks (sugars, starch, and lignocellulosic material) to ethanol is presented. Fermentation of sugars and starches, acid hydrolysis of lignocellulosic feedstocks, and enzymatic hydrolysis of lignocellulosic feedstocks are covered in detail, ranging from process kinetics to alternative processing configurations and chemical dilutions. In addition, a separate chapter has been devoted to advanced and emerging concepts in biomass conversion to ethanol. Concepts covered include improvements in alternative microorganism for ethanol fermentation (to increase fermentation rates and ethanol yields), continuous processing systems versus the traditional batch technology, and conversion of the hemicellulose 5-carbon sugars fraction of the feedstock to ethanol. This chapter also focuses on process innovations and improvements that reduce energy usage (and thus process costs), such as alternative methods for fuel alcohol dehydrations. Finally, the possibility of using low-cost or no-cost waste feedstocks, such as municipal solid waste, is examined. This report was published in October 1992.

Major Project Reports:

Tshiteya, R.M., "Conversion Technologies: Biomass to Ethanol."

Summary Date: December 1992

Emission Characteristics of Alcohols and Alcohol-Fueled Vehicles

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C.J. Wallace

Telephone: (202) 484-1090

Contractor:
Meridian Corporation
4300 King Street, Suite 400
Alexandria, VA 22302-1508

Principal Investigator: R. Tshiteya

Telephone: (703) 998-3600

Contract Number: YS-2-12079-1

Contract Period: 10/91 – 12/92

Contract Funding (Source):
FY 1992: $50,000 (DOE)

Objective:

To serve as a desk companion for public policy decision-makers. The reference work focuses on airborne emissions created by transportation fuels, in general, and alcohol fuels/ethers, in particular.

Approach/Background:

This reference work is organized into four major topical areas. To facilitate usage, each section of the reference work has been designed to stand alone, with only infrequent cross-references to other sections. Key facts and definitions of terms are provided in a quick reference summary at the beginning of each major section. Extensive use of graphics has been made, whenever possible reproducing the full range of information presented in the original research reports.

Status/Accomplishments:

The major foci of this report are the performance characteristics of two primary fuel alcohols–ethanol (ethyl alcohol) and methanol (methylalcohol)—and the two major alcohol-based ethers that can potentially be used for gasoline blending—methyl tertiary butyl ether (MTBE) and ethyl tertiary butyl ether ((ETBE). The data are not evenly distributed among these four, however, with methanol and MTBE receiving the vast majority of the scientific testing, particularly for engine-related research. Large sets of data have been analyzed and synthesized here to address particular public policy questions, such as: What do we know about how alcohols and ethers can help reduce ambient levels of CO or hydrocarbons? Are there different sets of fuel/vehicle options that are more appropriate for emissions reductions in the near-term (2-5 years) and the long-term (10 years or more)? Because state and federal mobile source emissions regulations are largely written for particular criteria pollutants (CO, hydrocarbons, NO_x, and toxics), the effects of alcohol fuels and ethers in blends or as neat fuels on the production of each of these pollutants are summarized whenever those data are available. This document was published in June 1992.

Major Project Reports:

Tshiteya, R.M., "Emission Characteristics of Alcohols and Alcohol-Fueled Vehicles."

Summary Date: December 1992.

Biofuels Workshop

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C.J. Wallace

Telephone: (202) 484-1090

Contractor:
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: C.J. Wallace

Telephone: (202) 484-1090

Contract Number: In-house

Contract Period: 10/91 – 10/92

Contract Funding (Source):
FY 1992: $20,000 (DOE)

Summary Date: December 1992

Objective:

To provide a forum for the United States and Finland to exchange information on developments in the production and use of alternative liquid fuels from renewable sources and to explore issues and barriers relevant to collaborative ventures between the two countries.

Approach/Background:

The memorandum of understanding between DOE and the Ministry of Trade and Industry of Finland, dated October 23, 1990, calls for ". . . mutual interest in increasing the rational use of energy and in developing alternative energy sources."

Status/Accomplishments:

Workshop held in Washington, D.C., October 1991.

Major Project Reports: None

Analysis of the Financial and Investment Requirements for the Scale-up of Biomass Energy Crops

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C.J. Wallace

Telephone: (202) 484-1090

Contractor:
Meridian Corporation
4300 King Street, Suite 400
Alexandria, VA 22302-1508

Principal Investigator: J.W. Onstad

Telephone: (703) 998-3600

Contract Number: YS-2-12079

Contract Period: 10/91 – 10/92

Contract Funding (Source):
FY 1992: $50,000 (DOE)

Objective:

To expand the scope of previous analyses of the biomass feedstock by examining how U.S. agricultural markets actually operate.

Approach/Background:

Previous examinations of the feasibility of widespread biomass production have focused primarily on the total resource potential, according to the availability of cropland, and the costs of basic farming inputs and practices. In expanding on previous analysis, attempts were made to answer the question: Who will be the investors/participants in the new industry? Having identified these parties, the analysis is expanded to assess the risks and financial requirements of these participants. Finally, the cost factors associated with a comprehensive agricultural infrastructure, as applied to biomass production and marketing, are aggregated and compared to the previous cost projections for biomass production.

Status/Accomplishments:

The analysis presented in this report concludes that previous cost projections for the production of biomass are incomplete because (1) they have not incorporated the entire range of participants this market will likely entail, and (2) they do not account for the appropriate risks, rates of return, and fees that will be demanded by the many participants. No additional modeling (such as ARIMS or BLS) was performed for this study. However, by assuming certain levels of risk premiums and adding some of the current agricultural market fees to the results of other analyses, a probable range of prices for biomass was determined.

Major Project Reports:

Onstad, J.W., M.S. Lambrides, and B.S. McKenna, "Analysis of the Financial and Investment Requirements for the Scale-up of Biomass Energy Crops."

Summary Date: December 1992

Biofuels Systems Division Multiyear Program Plan: 1992–1996

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C.J. Wallace

Telephone: (202) 484-1090

Contractor:
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: C.J. Wallace

Telephone: (202) 484-1090

Contract Number: In-house

Contract Period: 10/91 – 10/92

Contract Funding (Source):
FY 1992: $65,000 (DOE)

Objective:

To provide an update of the multiyear plan to reflect new areas of emphasis, changes in subprogram priorities, technical advancements in the research programs, and changes reflected in the reorganization of the Conservation and Renewable Energy Office of DOE.

Approach/Background:

A goal-oriented approach was employed, starting with the specification of research milestones required to meet long-term program goals.

Status/Accomplishments:

The *Biofuels Systems Division Multiyear Program Plan: 1992–1996* was completed in October 1992. As in past years, the plan articulates programmatic directions, goals, expected accomplishments, and the research and development strategies employed in attaining these goals.

Major Project Reports:

NREL, *Biofuels Systems Division Multiyear Program Plan: 1992–1996.*

Summary Date: December 1992

Information Management Assessment

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C.J. Wallace

Telephone: (202) 484-1090

Contractor:
Meridian Corporation
4300 King Street, Suite 400
Alexandria, VA 22302-1508

Principal Investigator: A.F. Alvarez

Telephone: (703) 998-3600

Contract Number: XL-1-11177-1

Contract Period: 12/91 – 12/92

Contract Funding (Source):
FY 1992: $50,000 (DOE)

Objective:

To review and evaluate all phases of the Office of Transportation Technologies' (OTT's) response-recording-tracking systems, information processing procedures, correspondence controls, information distribution networks, and actuating process.

Approach/Background:

The study, involving a detailed assessment of current information management practices to assist OTT, is adequately and efficiently meeting the requests placed on the Office.

Status/Accomplishments: Project completed.

Major Project Reports:

Meridian Corporation, "Information Management Assessment."

Summary Date: December 1992

Biofuels Workshop II

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C.J. Wallace

Telephone: (202) 484-1090

Contractor:
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401-3393

Principal Investigator: C.J. Wallace

Telephone: (202) 484-1090

Summary Date: December 1992

Contract Number: In-house

Contract Period: 10/91 – 10/92

Contract Funding (Source):
FY 1992: $20,000 (DOE)

Objective:

To provide a forum for the United States and Finland to exchange information on developments in the production and use of alternative liquid fuels from renewable sources and to explore issues and barriers relevant to collaborative ventures between the two countries.

Approach/Background:

The memorandum of understanding between DOE and the Ministry of Trade and Industry of Finland, dated October 23, 1990, calls for ". . .mutual interest in increasing the rational use of energy and in developing alternative energy sources."

Status/Accomplishments:

Workshop held in Helsinki, Finland, August 1992.

Major Project Reports: None

International Energy Agency Bioenergy Agreement

Directing Organization:
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: C.J. Wallace

Telephone: (202) 484-1090

Contractor:
Meridian Corporation
4300 King Street, Suite 400
Alexandria, VA 22302-1508

Principal Investigator: C.J. Wallace

Telephone: (202) 484-1090

Contract Number: In-house

Contract Period: 10/91 – 10/92

Contract Funding (Source):
FY 1992: $145,000 (DOE)

Objective:

The United States belongs to the International Energy Agency's Bioenergy Agreement (IEA/BA). This agreement provides a mechanism for the participating countries to exchange information and to coordinate ongoing research programs in the area of bioenergy. The agreement includes cooperation in the areas of biomass production, harvesting, and conversion. Participation allows the United States to take advantage of the extensive research in other countries. Through this agreement, the United States gains access to a much larger base of information than it would have without the cooperation. This knowledge helps the United States to leverage its research dollars. At present, the United States pays only about 10% of the total costs of this work and receives the benefit of having other countries pay the remaining 90%.

Approach/Background:

The United States has participated in the IEA Bioenergy Agreement since its inception in 1978. At present, 15 countries participate in the cooperation. The cooperation consists of specific programs, called tasks, in four different areas. Task VII deals with biomass production, Task IV with biomass harvesting and supply, Task X with biomass utilization, and Task XI with municipal solid waste conversion. Within each task are individual projects called activities. These deal with topics such as bioconversion for ethanol production, gasification for methanol production, and others. The United States participates in all four tasks and several of the activities within each task. In addition, the United States leads the conversion work, Task X. As the Operating Agent, the United States leads the technical program and administers the funds provided by the participants. NREL will perform the Operating Agent function for Task X, effective January 1, 1993.

Status/Accomplishments:

These funds will be used to (1) pay 1992 U.S. contributions to the IEA Bioenergy Agreement, (2) sponsor joint workshops, and (3) host the 1992 IEA Bioenergy Agreement Executive Committee Meeting, Colorado Springs, Colorado, October 21–23, 1992.

Major Project Reports:

International Energy Agency, *Bioenergy Annual Report 1992*.

Summary Date: December 1992

Current Publications

Ethanol

Baker, J.O., K. Tatsumoto, K. Grohmann, J. Woodward, J.M. Wichert, S.P. Shoemaker, and M.E. Himmel, 1992, "Thermal Denaturation of *Trichoderma reesei* Cellulases Studied by Differential Scanning Calorimetry and Tryptophan Fluorescence," *Applied Biochemistry and Biotechnology*, Vol. 34/35, pp. 217–231.

Ehrman, T. and M.E. Himmel, "Chemical Analysis & Testing Standard Procedures: Determination of Total Solids/Moisture in Biomass; Two Stage Sulfuric Acid Hydrolysis for Determination of Carbohydrates; Determination of Klason Lignin in Biomass; Determination of Acid Soluble Lignin in Biomass; Determination of Ash in Biomass; Measurement of Cellulase Activities; Dilute Acid Pretreatment Methods; SSF Experimental Protocols," Alternative Fuels Division, NREL Technical Document, in development.

Eveleigh, D., 1992, "Purification of Cellulase, *M. bispora*," Final Report submitted to NREL.

Eveleigh, D., 1992, "Purification of Cellulase, *T. maritima*," Final Report submitted to NREL.

Hames, B., S.K. Black, F. Agblevor, R. Evans, D.K. Johnson, and H.L. Chum, 1991, "Measurement of the Functional Group Contents of Lignins Using FTIR and Partial Least Squares Regression," presented at the 6th International Symposium on Wood and Pulping Chemistry, April 30–May 5, 1991, Melbourne, Australia.

Himmel, M.E., W.S. Adney, and J.O. Baker, "Cellulase Assays: A Review," *Energy from Biomass and Wastes XVI* (D. Klass, ed.), IGT, Chicago, IL (in press).

Himmel, M.E., W.S. Adney, D.J. Mitchell, and J.O. Baker, "Isolation and Characterization of Two Forms of β-D-Glucosidase from *Aspergillus niger*," *Applied Biochemistry and Biotechnology*, Vol. 36/37 (in press).

Ingram, L. O. and K.T. Shammugam, 1991, "Development of Ethanologenic *E. coli* Expressing the Complete Ethanol Pathway from *Z. mobilis*," Draft Final Report submitted to NREL.

Johnson, D., Initial Results from Hydrotreating Low-Molecular-Weight Lignin Fractions with Platinum Impregnated Catalysts, Milestone Report.

Kim, B.J., Y.Y. Lee, and R. Torget, 1992, "On Optimal Temperature Policy of Percolation Process as Applied to Dilute-Acid Hydrolysis of Biphasic Hemicellulose," presented at the 14th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May 11–14, 1992. Submitted to *Applied Biochemistry and Biotechnology*.

Laymon, R.A., S.R. Thomas, M.P. Tucker, T. Vinzant, and M.E. Himmel, September 1992, "Codon Usage in *Acidothermus cellulolyticus*: Preliminary Comparisons," Poster III. 18, The Fifth Annual Colorado Biotechnology Symposium organized by CIRB, Colorado State University, Fort Collins, CO.

Lee, Y.Y., B.J. Kim, and R. Torget, May 1992, "On Optimal Temperature Policy of Percolation Process as Applied to Dilute-Acid Hydrolysis of Biphasic Hemicellulose," Scientific Note presented at 14th Annual Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN.

McMillan, J., September 1992, "Xylose Fermentation to Ethanol: A Review," Milestone Report.

Milne, T.A., H.L. Chum, F. Agblevor, and D.K. Johnson, 1992, "Standardized Analytical Methods," *Biomass and Bioenergy*, Vol. 2, pp. 341–366.

Philippidis, G.P., 1992, "Conversion of Cellulosic Biomass to Ethanol," presented at the Institute of Gas Technology Symposium on Energy from Biomass and Wastes, Orlando, FL, 1992.

Philippidis, G.P. and C.E. Wyman, 1992, "Production of Alternative Fuels: Modeling of Cellulosic Biomass Conversion to Ethanol," *Recent Advances in Industrial Applications of Biotechnology*, F. Vardar-Sukan and S. Sukan, eds., Kluwer Academic Publishers.

Philippidis, G.P. and C. E. Wyman, 1992, "Technology for Fuel Ethanol Production from Cellulosic Biomass," presented at the Biofor/Bioqual Annual Meeting on Implementation of Biological Processes for the Forest Industry and Environmental Quality, Vancouver, British Columbia, Canada.

Philippidis, G.P., T.K. Smith, and C.E. Wyman, 1992, "Study of the Enzymatic Hydrolysis of Cellulose during the Simultaneous Saccharification and Fermentation Process for Production of Fuel Ethanol," presented at the 14th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May 11–14, 1992.

Philippidis, G.P., D.D. Spindler, and C.E. Wyman, 1992, "Mathematical Modeling of Cellulose Conversion to Ethanol by the Simultaneous Saccharification and Fermentation Process." *Applied Biochemistry and Biotechnology,* Vol. 34/35, pp. 543–556.

Ponfick, L.R., T. Vinzant, N.J. Nagle, T.I. Ehrman, K.M. Magill, J.B. Reynolds, and M.E. Himmel, September 1992, "SSF Comparison of Selected Woods from Southern Saw Mills," poster session, The Fifth Annual Colorado Biotechnology Symposium organized by CIRB, Colorado State University, Fort Collins, CO.

Rice, P., September 1992, *Co-Products Market Assessment*, Final Report.

Schell, D.J., J.D. McMillan, G.P. Philippidis, N.D. Hinman, and C. Riley, 1992, "Ethanol From Lignocellulosic Biomass." Chapter 10 in *Advances in Solar Energy*. K.W. Böer, ed., Vol. 7. Boulder: American Solar Energy Society, Inc., pp. 373–448.

Shoemaker, S., September 1992, "Characterization of Endo-1,4-β-Glucanases by Viscosity," Draft Final Report submitted to NREL.

Torget, R., M. Himmel, and K. Grohmann, 1992, "Dilute Acid Pretreatment of Two Short Rotation Herbaceous Crops," *Applied Biochemistry and Biotechnology*, 1992.

Tucker, M.P., K. Grohmann, A. Mohagheghi, and M.E. Himmel, 1991, "Thermostable Purified Endoglucanase from Thermophilic Bacterium *Acidothermus cellulolyticus*," United States patent No. 5,110,735.

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Methanol and Higher Alcohols

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