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Biofuels: Project Summaries

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by the
National Renewable Energy Laboratory
a U.S. DOE national laboratory
Golden, CO 80401-3393

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Preface

Domestic transportation fuels are almost exclusively derived from petroleum and account for about two-thirds of total U.S. petroleum consumption. In 1993, 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, which 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 Energy Policy Act of 1992 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 to 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 1993 (October 1, 1992 through September 30, 1993). Each summary sheet contains an account of project funding, objectives, accomplishments and current status, and significant publications.

Ethanol

Pretreatment

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

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

Principal Investigator: D. Hsu

Telephone: (303) 384-6117

Contract Number: DE-AC02-83CH10093

Contract Period: 10/92-09/94

Contract Funding (Source):

FY 1993: \$340,532 (DOE)
FY 1994: \$252,000 (DOE)

Objective:

Develop efficient, cost-effective pretreatment techniques for preparation of enzymatically digestible lignocellulosic biomass.

Approach/Background:

Cellulose in lignocellulosic biomass is not readily digestible by cellulase enzymes; a pretreatment step is necessary to render it digestible. Dilute sulfuric acid pretreatment was previously shown to be effective for hardwoods, herbaceous crops, and agricultural residues. Preliminary results of a two-stage dilute-acid pretreatment scheme using a percolation process show that, in addition to generating digestible pretreated solids residues, yields of xylose equivalents are significantly increased. The preliminary results are in need of confirmation and a larger-scale system is required to generate data for process performance verification, mass-balance closing, and process economics analysis.

Status/Accomplishments:

The two-stage dilute-acid percolation pretreatment of hybrid poplar particles was further investigated using the small bench-scale percolation system. Yields of xylose equivalents as high as 92% of theoretical were consistently obtained. The pretreated solids residues were highly digestible and fermentable. SSF studies show favorable results—ethanol yields of 80 to 90+% of theoretical in 48 h were observed.

Larger percolation systems with reactor sizes 20-30 times greater than the small bench-scale reactor investigated to date were designed and are being assembled for further investigation of the percolation pretreatment process.

A patent application, covering a number of innovative concepts of using percolation process for pretreatment of lignocellulosic biomass, was filed.

Major Project Reports:

Hsu, T.; Torget, R.; Farmer, J. (1993) *Dilute-Acid Percolation Pretreatment of Hardwood*. Golden, CO: National Renewable Energy Laboratory.

Torget, R.; Hsu, T. (1993). *Two-Temperature Dilute-Acid Prehydrolysis of Hardwood Xylan Using a Percolation Process*. Golden, CO: National Renewable Energy Laboratory.

Summary Date: March 1994

Kinetic and Modeling Investigation Of Dilute-Acid Pretreatment

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Auburn University
Auburn, AL 36849

Principal Investigator: Y.Y. Lee

Telephone: (205) 844-2019

Contract Number: XAW-3-13441-01

Contract Period: 07/91-09/94

Contract Funding (Source):

FY 1992: \$113,361 (DOE)
FY 1993: \$109,646 (DOE)
FY 1994: \$126,330 (DOE)

Objective:

Develop an understanding of the effects of operating variables on yields of xylose from hemicellulose in NREL's two-stage dilute sulfuric acid pretreatment process.

Approach/Background:

During pretreatment of lignocellulose biomass, such as hardwoods, herbaceous crops, and agricultural residues, hemicellulosic sugars (primarily xylose) are released. Through experimental and modeling studies, the interrelationship between particle size, temperature, residence time, acid concentration, and the yield of xylose can be determined. This study uses hybrid poplar, switchgrass, and corn stover to establish kinetic models that predict yields of xylose through dilute sulfuric acid pretreatment. Up-flow percolation reactor configurations, taking into account intra-particle and inter-particle mass and heat transfer phenomena, are being investigated.

Status/Accomplishments:

Kinetic models describing the release of xylose during dilute-acid pretreatment have been developed. These models, based on experimental data and theoretical kinetic analysis, have demonstrated that the use of a two-stage pretreatment scheme allows for variable temperature profiles that enhance the process performance. Work currently is ongoing to improve the models by incorporating the generation of oligomeric xylose as an intermediate product, mass and heat diffusion factors, and non-ideal flow characteristics to more accurately predict dilute-acid pretreatment process performance.

Major Project Reports:

Kim, B.J.; Lee, Y.Y. (1993). *Modeling of Percolation Process in Dilute-Acid Hydrolysis/Pretreatment of Biphasic Hemicellulose*. Golden, CO: National Renewable Energy Laboratory. Work performed by Auburn University, Auburn, AL.

Kim, B.J.; Lee, Y.Y.; Torget, R. (in press) "Modified Percolation Process in Dilute-Acid Hydrolysis of Biphasic Hemicellulose." *Applied Biochem. & Biotechnol.*

Kim, B.J.; Lee, Y.Y.; Torget, R. (1993). "Optimal Temperature Policy of Percolation Process as Applied to Dilute-Acid Hydrolysis of Biphasic Hemicellulose." *Applied Biochem. & Biotechnol.* Vol. 39/40, pp. 119-129.

Kim, B.J.; Lee, Y.Y.; Torget, R. (1993). *Simulation of a Modified Percolation Process in Dilute-Acid Hydrolysis of Biphasic Hemicellulose*. Golden, CO: National Renewable Energy Laboratory. Work performed by Auburn University, Auburn, AL.

Lee, Y.Y.; Kim, B.J.; Torget, R. (1993). *An Optimal Temperature Policy of Percolation Process as Applied to Dilute-Acid Hydrolysis of Biphasic Hemicellulose*. Golden, CO: National Renewable Energy Laboratory. Work performed by Auburn University, Auburn, AL.

Summary Date: March 1994

Alternate Pretreatment Study— Ammonia Recycled Percolation

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Auburn University
Auburn, AL 36849

Principal Investigator: Y.Y. Lee

Telephone: (205) 844-2019

Contract Number: XAW-3-11181-02

Contract Period: 01/93–02/95

Contract Funding (Source):

FY 1993: \$114,422 (DOE)
FY 1994: \$168,334 (DOE)

Objective:

Identify and develop pretreatment approaches that could improve ethanol production performance over the observed NREL base-case dilute sulfuric acid pretreatment process.

Approach/Background:

Pretreatment of lignocellulosic biomass is one of the key steps to a successful biomass-to-ethanol conversion process. Until 1992, resources focused on a dilute sulfuric acid pretreatment process, which has become part of the NREL base-case ethanol process. Although proven technically effective and economically promising, the dilute sulfuric acid process has several areas for improvement including reduced yields of furfural and HMF from, respectively, xylose and glucose; substances that may be toxic to cellulase enzymes and ethanol-fermenting yeasts; reduced levels of xylan remaining in pretreated solids residue; enhanced enzymatic digestibility of pretreated solids residue; and reduction or elimination of the generation of gypsum. This subcontract is one of the several that were estab-

lished to conduct research and development on other promising pretreatment processes. Ammonia recycled percolation process is one that has not previously been reported in the literature.

Status/Accomplishments:

Through the first-year study of milled whole-tree hybrid poplar, near-optimal pretreatment conditions have been established. Lignin removal of up to 50% was observed. Concomitantly, close to 50% removal of xylan was observed. Maximum enzymatic digestibility of the pretreated solids residues approached that of filter paper (95%).

In an effort to produce streams of relatively pure xylose and solubilized lignin, a pretreatment scheme of a hot-water percolation step, followed by ammonia recycled percolation, is being developed.

Major Project Reports:

Yoon, H.H.; Wu, Z.W.; Lee, Y.Y. (submitted)
"Ammonia Recycled Percolation Process for Pretreatment of Biomass Feedstock." *Applied Biochem. & Biotechnol.*

Summary Date: March 1994

Alternate Pretreatment Study—Dilute Acid and Organosolv

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Colorado State University
Fort Collins, CO 80523

Principal Investigators: H. Schroeder and
J. Linden

Telephone: (303) 491-7768

Contract Number: XAW-3-11181-04

Contract Period: 01/93–04/94

Contract Funding (Source):

FY 1993: \$99,830 (DOE)

Objective:

Identify and develop pretreatment approaches that could improve ethanol production performance over the observed NREL base-case dilute sulfuric acid pretreatment process.

Approach/Background:

Pretreatment of lignocellulosic biomass is one of the key steps to a successful biomass-to-ethanol conversion process. Until 1992, resources were focused on a dilute sulfuric acid pretreatment process, which has become part of the NREL base-case ethanol process. Although proven technically effective and economically promising, the dilute sulfuric acid pretreatment process has several areas for improvement: yields of furfural and HMF from, respectively, xylose and glucose; substances that may be toxic to cellulase enzymes and ethanol-fermenting yeasts; reduced levels of xylan remaining in pretreated solids residue; enhanced enzymatic digestibility of pretreated solids residue; and reduction or elimination of the generation of gypsum. This subcontract is one of the several that were established to conduct research and development

on other promising pretreatment processes. Phosphoric acid pretreatment and oxalic acid pretreatment with or without the presence of methanol were contracted to be studied.

Status/Accomplishments:

Work on NREL quality control standards has been performed and is continuing. Concomitantly, numerous pretreatment runs using either phosphoric or oxalic acid, most without the presence of methanol, were conducted. A limited number of organosolv runs were also conducted. Samples are currently being analyzed and simultaneous saccharification and fermentation (SSF) tests are being conducted.

Major Project Reports:

Schroeder, H.A.; Linden, J.C. (1993). *Evaluation of Alternative Pretreatment and Biomass Fractionation Processes*. Golden, CO: National Renewable Energy Laboratory. Work performed by Colorado State University, Fort Collins, CO.

Summary Date: March 1994

Alternate Pretreatment Study—Dilute Nitric Acid

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Forest Products Laboratory
University of California at Berkeley
1301 South 46th Street
Richmond, CA 94804

Principal Investigator: D. Brink

Telephone: (510) 215-4214

Contract Number: XAW-3-11181-01

Contract Period: 03/93–03/94

Contract Funding (Source):

FY 1993: \$159,376 (DOE)

Objective:

Identify and develop pretreatment approaches that could improve ethanol production performance over the observed NREL base-case dilute sulfuric acid pretreatment process.

Approach/Background:

Pretreatment of lignocellulosic biomass is one of the key steps to a successful biomass-to-ethanol conversion process. Until 1992, resources focused on a dilute sulfuric acid pretreatment process, which has become part of the NREL base-case ethanol process. Although proven technically effective and economically promising, the dilute sulfuric acid process has several areas for improvement: reduced yields of furfural and HMF from, respectively, xylose and glucose; substances that may be toxic to cellulase enzymes and ethanol-fermenting yeasts; reduced levels of xylan remaining in pretreated solids residue; enhanced enzymatic digestibility of pretreated solids residue; and reduction or elimination of the generation of gypsum. This subcontract is one of the several that were established to con-

duct research and development on potentially more promising pretreatment processes. Dilute nitric acid pretreatment of whole-tree hybrid poplar chips using a 0.5-ton/day pilot facility was contracted to be studied.

Status/Accomplishments:

The pilot facility, last used in the processing of rice straw slurried in dilute acid, required extensive reconfiguration, recalibration of sensors, and maintenance to run the wood feedstock. Following shakedown runs of the demonstration unit, runs were conducted to establish steady-state conditions to provide the pretreatment residues required for SSF evaluations. Temperatures, acid concentrations, and feed rates were varied in these runs. From the runs, seven steady-state periods were selected for chemical analysis and production of ethanol by SSF. It was found that the cellulose content of the pretreatment residues generally ranged from 57% to 60%. Seven-day SSF evaluations on these residues have achieved ethanol yields above 90% of theoretical.

Major Project Reports:

Brink, D.L.; Merriman, M.M.; Herrmann, C.C. (1993). *Dilute Nitric Acid Pretreatment and Enzymic Hydrolysis of Lignocellulosic Materials*. Golden, CO: National Renewable Energy Laboratory. Work performed by Forest Products Laboratory, Richmond, CA.

Summary Date: March 1994

Preparation of Standardized Pretreated Biomass

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Major Project Reports: None

Summary Date: March 1994

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Hazen Research, Inc.
4601 Indiana Street
Golden, CO 80403

Principal Investigator: C. Kenney

Telephone: (303) 279-4501

Contract Number: YAC-4-14107-01

Contract Period: 02/94–02/95

Contract Funding (Source):

FY 1994: \$63,963 (DOE)

Objective:

Prepare, according to standard dilute-acid pretreatment protocols, pretreated biomass solids and prehydrolyzates for use by NREL in-house research groups and subcontractors.

Approach/Background:

Several NREL research groups and subcontractors require pretreated biomass solids or liquors for research activities. It is desirable that consistent pretreated solids and liquors be generated for use. To ensure large quantities of consistent pretreated products be prepared, a 25-gallon pretreatment reactor (paddle reactor) system was designed and fabricated. Hazen Research, Inc., was selected to complete the assembly and shakedown of the system and to prepare standardized pretreated products to meet demand.

Status/Accomplishments:

The assembly of the paddle-reactor pretreatment system is currently underway.

Optimization of Dilute-Acid Pretreatment Of Selected Biomass Feedstocks

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Oregon State University
Corvallis, OR 97331-6602

Principal Investigators: M. Penner and
A. Hashimoto

Telephone: (503) 737-6513

Contract Number: XR-2-11186-1

Contract Period: 10/92-03/94

Contract Funding (Source):
FY 1993: \$120,081 (DOE)

Objective:

Establish optimal dilute sulfuric acid pretreatment conditions for selected biomass feedstocks that will result in maximum yields of ethanol equivalents (ethanol equivalents being proportional to the sum of the fermentable sugars produced in the course of pretreatment and glucose produced through enzymatic saccharification of the pretreated residue).

Approach/Background:

Previous research has shown that removal of hemicellulose by dilute sulfuric acid prehydrolysis of lignocellulosic biomass renders cellulose accessible to cellulase enzymes. The extent of prehydrolysis depends upon temperature, time, and acid concentration, and the reaction can be modeled as two parallel pseudo first-order reactions through which xylose is produced as well as (partially) degraded to non-fermentable compounds. The enzymatic digestibility of the remaining cellulose has been shown, for a number of hardwoods, agricultural residues, and herbaceous crops, to be directly related to the extent of hemicellulose

removal from the pretreated material. Potential ethanol yield is dependent upon both the yield of xylose from hemicellulose and the enzymatic digestibility of the cellulose. Thus, a high yield of xylose and a high cellulose enzymatic digestibility are essential for economic viability. Milled hybrid poplar and switchgrass were selected for this study.

Status/Accomplishments:

Experimental pretreatment runs varying temperature (140° to 180°C), acid concentration (0.6 to 1.2 wt %), and time (2-60 min) were conducted for the selected biomass feedstocks. A matrix of 40-50 data points was generated for each feedstock. Kinetic models were developed and apparent optimum conditions for maximum yields of xylose were identified. These optimum points are being experimentally verified in complete mass-balance studies.

Major Project Reports:

Penner, M.H.; Hashimoto, A.G. (1993).
Optimization of Dilute-Acid Pretreatment of Selected Biomass. Golden, CO: National Renewable Energy Laboratory. Work performed by Oregon State University, Corvallis, OR.

Summary Date: March 1994

Alternate Pretreatment Study—Ammonia Fiber Explosion

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

RA Energy, Ltd.
11615 Angus Road, Suite 211
Austin, TX 78759

Principal Investigator: V. Latimer

Telephone: (512) 502-9080

Contract Number: XAW-3-11181-06

Contract Period: 07/93–07/94

Contract Funding (Source):

FY 1993: \$112,481 (DOE)

Objective:

Identify and develop pretreatment approaches that could improve ethanol production performance over the observed NREL base-case dilute sulfuric acid pretreatment process.

Approach/Background:

Pretreatment of lignocellulosic biomass is one of the key steps to a successful biomass-to-ethanol conversion process. Until 1992, resources focused on a dilute sulfuric acid pretreatment process, which has become part of the NREL base-case ethanol process. Although proven technically effective and economically promising, the dilute sulfuric acid process has several areas for improvement: reduced yields of furfural and HMF from, respectively, xylose and glucose; substances that may be toxic to cellulase enzymes and ethanol-fermenting yeasts; reduced levels of xylan remaining in pretreated solids residue; enhanced enzymatic digestibility of pretreated solids residue; and reduction or elimination of the generation of gypsum. This subcontract is one of the several that were established to conduct research and development on potentially more

promising pretreatment processes; a patented ammonia fiber explosion (AFEX) pretreatment process, using whole-tree hybrid poplar, was contracted to be investigated.

Status/Accomplishments:

Work on NREL quality control standards has been performed and is continuing. Concomitantly, appropriate AFEX pretreatment protocols have been under development.

Major Project Reports: None

Summary Date: March 1994

Alternate Pretreatment Study—Lime Pretreatment

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Texas A&M University
College Station, TX 77843-3124

Principal Investigator: M. Holt/apple

Telephone: (409) 845-9708

Contract Number: XAW-3-11181-03

Contract Period: 08/93–08/94

Contract Funding (Source):

FY 1993: \$118,667 (DOE)

Objective:

Identify and develop pretreatment approaches that could improve ethanol production performance over the observed NREL base-case dilute sulfuric acid pretreatment process.

Approach/Background:

Pretreatment of lignocellulosic biomass is one of the key steps to a successful biomass-to-ethanol conversion process. Until 1992, resources focused on a dilute sulfuric acid pretreatment process, which has become part of the NREL base-case ethanol process. Although proven technically effective and economically promising, the dilute sulfuric acid process has several areas for improvement: yields of furfural and HMF from, respectively, xylose and glucose; substances that may be toxic to cellulase enzymes and ethanol-fermenting yeasts; reduced levels of xylan remaining in pretreated solids residue; enhanced enzymatic digestibility of pretreated solids residue; and reduction or elimination of the generation of gypsum. This subcontract is one of several that were established to conduct research and development on potentially more promising pretreatment processes.

Status/Accomplishments:

Switchgrass was selected as the first feedstock substrate to be investigated (it will be followed by hybrid poplar and corn stover). Work on NREL quality control standards has been performed and is continuing. Concomitantly, appropriate lime pretreatment protocols have been under development. Lime pretreatment conditions have been identified that produce nearly theoretical yields of sugars from switchgrass in 3 days using 5 IU/g cellulase loadings.

Major Project Reports: None

Summary Date: March 1994

Alternate Pretreatment Study—Alkaline Peroxide Extrusion

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Xylan, Inc.
111 Southwest Street
Mankato, KS 66956

Principal Investigator: G. Tyson

Telephone: (913) 794-2283

Contract Number: XAW-3-11181-05

Contract Period: 01/93–01/95

Contract Funding (Source):

FY 1993: \$98,673 (DOE)
FY 1994: \$98,000 (DOE)

Objective:

Identify and develop pretreatment approaches that could improve ethanol production performance over the observed NREL base-case dilute sulfuric acid pretreatment process.

Approach/Background:

Pretreatment of lignocellulosic biomass is one of the key steps to a successful biomass-to-ethanol conversion process. Until 1992, resources focused on a dilute sulfuric acid pretreatment process, which has become part of the NREL base-case ethanol process. Although proven technically effective and economically promising, the dilute sulfuric acid process has several areas for improvement: reduced yields of furfural and HMF from, respectively, xylose and glucose; substances that may be toxic to cellulase enzymes and ethanol-fermenting yeasts; reduced levels of xylan remaining in pretreated solids residue; enhanced enzymatic digestibility of pretreated solids residue; and reduction or elimination of the generation of gypsum. This subcontract

is one of the several that were established to conduct research and development on other more-promising pretreatment processes. Alkaline peroxide extrusion pretreatment of whole-tree hybrid poplar chips, coarsely milled switchgrass, and coarsely milled corn stover were contracted to be investigated.

Status/Accomplishments:

Optimal pretreatment conditions are being determined. Based on the cellulose content in dry starting hybrid poplar, the alkaline peroxide extrusion pretreated biomass produced yield of ethanol of 32% of theoretical conversion.

Work on switchgrass and corn stover is underway.

Major Project Reports:

Tyson, G.J. (1993). *Evaluation of Alternate Pretreatment and Biomass Fractionation Processes*. Golden, CO: National Renewable Energy Laboratory. Work performed by Xylan, Inc., Mankato, KS.

Summary Date: March 1994

Pentose-Fermentation Biocatalyst Development

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

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

Principal Investigator: S. Picataggio

Telephone: (303) 384-6107

Contract Number: DE-AC02-83CH10093

Contract Period: 10/92-09/94

Contract Funding (Source):

FY 1993: \$420,000 (DOE)
FY 1994: \$653,000 (DOE)

Objective:

Develop novel ethanologenic microorganisms capable of rapid and efficient fermentation of the pentose sugars in lignocellulosic hydrolysates to ethanol.

Approach/Background:

While many microorganisms can efficiently ferment the glucose component in cellulosic biomass to ethanol, the conversion of pentose sugars in the hemicellulose fraction to ethanol is difficult and remains one of the economic bottlenecks in the current conversion scheme. Despite the development of recombinant strains with improved xylose fermentation performance, high ethanol yields and productivities from cellulosic hydrolysates and increased ethanol concentrations are key targets that have yet to be achieved. These performance targets drive the development of novel recombinant microorganisms capable of rapid, high-yield fermentation of xylose and other pentose sugars to ethanol. These novel biocatalysts will be developed through the metabolic engineering of key biochemical pathways to redirect intracellular carbon-flow to ethanol as the sole fermentation product.

Status/Accomplishments:

A comprehensive literature survey was completed to identify the most-promising microorganisms for metabolic engineering. Candidate microorganisms were evaluated by comparison of their known metabolic characteristics to a prioritized list of fermentation performance criteria anticipated to be essential in a commercial process. Each trait was weighted according to whether it was an essential or a desirable, but dispensable, trait. As the result of this survey, the bacteria *Zymomonas mobilis* and *Lactobacillus* were identified as the most promising candidates for metabolic engineering. Research to define and genetically manipulate the key biochemical pathways in these microorganisms is in progress.

Major Project Reports:

Picataggio, S.; Zhang, M.; Finkelstein, M. (1993). *Identification of Ethanologens for Metabolic Engineering*. Golden, CO: National Renewable Energy Laboratory.

Summary Date: March 1994

Pentose Bioprocessing

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

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

Principal Investigators: S. Picataggio and
J. McMillan

Telephone: (303) 384-6107

Contract Number: DE-AC02-83CH10093

Contract Period: 10/92-09/94

Contract Funding (Source):

FY 1993: \$368,000 (DOE)
FY 1994: \$252,000 (DOE)

Objective:

Develop technologies that efficiently convert pentose sugars from pretreated hemicellulose hydrolyzates to ethanol.

Approach/Background:

Microorganisms that can directly ferment D-xylose to ethanol have been identified and others have been genetically engineered. Some ferment xylose to ethanol at yields above 90% of theoretical when grown on laboratory media containing pure xylose. However, the potential of using such organisms to convert hemicellulose hydrolyzates to ethanol is unknown and must be evaluated.

Status/Accomplishments:

Comparative evaluation studies were performed on neutralized poplar wood hemicellulose hydrolyzate to rank candidate xylose-fermenting microorganisms according to efficacy. Two promising microorganisms are recombinant *E. coli* ATCC 11303 (pLOI297) and wildtype *P. stipitis* NRRL Y-7124.

The organisms are now being studied at the bench-scale to more thoroughly characterize and optimize performance.

Complementing the comparative evaluation effort, a versatile state-of-the-art, bench-scale fermentation data acquisition and control system (DAS) designed specifically for low-gas, flow-rate processes was constructed and tested. The system was demonstrated and performs well under both aerobic and anaerobic fermentor operation, with carbon mass balances over aerobic and anaerobic testing intervals closing to within 5%. It was also shown that this system is capable of quantifying aeration requirements for microaerophilic bioprocesses.

Additional shake-flask experiments are planned to assess conversion performance on feedstocks other than hardwood poplar, as well as to evaluate other promising microorganisms when they become available. When appropriate, the DAS will be used to verify performance observed at the shake-flask scale. In preparation for these activities, a comprehensive literature review on hydrolyzate conversion has been completed.

Major Project Reports:

Boynton, B.L.; Newman, M.M.; McMillan, J.D. (1993). *Comparative Evaluation of Candidate Xylose-Fermenting Microorganisms on Hybrid Poplar DN34 Hemicellulose Hydrolyzate*. Golden, CO: National Renewable Energy Laboratory.

McMillan, J.D. (1993). *Construction and Validation of a Bench-Scale Fermentation Data Acquisition and Control System*. Golden, CO: National Renewable Energy Laboratory.

McMillan, J.D. (1993). *Conversion of Hemicellulose Hydrolyzates to Ethanol*. Golden, CO: National Renewable Energy Laboratory.

Summary Date: March 1994

Determine Nutrient Requirements

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractors:

The Trustees of Dartmouth College
6210 Raven House
Hanover, NH 03755-3580

The University of Toronto
27 King's College Circle
Toronto, Ontario M5S 1A1
Canada

Purdue Research Foundation
Hovde Hall
West Lafayette, IN 47907

Principal Investigators: L.R. Lynd (Dartmouth);
H.G. Lawford (Toronto); G.T. Tsao (Purdue)

Telephone: L.R. Lynd, (603) 646-2231;
H.G. Lawford, (416) 978-7096;
G.T. Tsao, (317) 494-7024

Contract Numbers: Dartmouth: XAP-3-11195-01
Toronto: AAP-4-11195-03
Purdue: XAP-3-11195-02

Contract Period: 12 months
Dartmouth: 09/93-08/94
Toronto: 01/94-12/94
Purdue: 09/93-08/94

Contract Funding (Source):

FY 1993:	Dartmouth:	\$102,000	(NREL)
	Toronto:	\$ 79,000	(NREL)
	Purdue:	\$107,000	(NREL)

Objective:

Develop cost-effective media formulations for efficient high-yield conversion of hemicellulose hydrolyzates to ethanol.

Approach/Background:

A critical need exists to more accurately determine minimum nutrient requirements and to develop cost-effective media formulations for high-yield conversion of hemicellulose hydrolyzates to ethanol. This is because nutrient costs must be minimized to realize favorable process economics. Therefore, a multiple-award multi-year subcontract has been awarded to establish nutrient requirements for a variety of xylose-fermenting organisms. Sub-contractors will first determine nutrient requirements for productive, high-yield ethanolic fermentation of hydrolyzates and then focus on developing cost-effective media formulations.

Status/Accomplishments:

Three subcontracts are now underway. Researchers are investigating nutrient requirements for both ethanologenic bacteria (thermophilic *Clostridium thermo-saccharolyticum* at Dartmouth College; mesophilic recombinant *E. coli* at the University of Toronto) and yeasts (wildtype *Pichia stipitis*, *Candida shehatae*, as well as proprietary recombinant yeast, at Purdue University). Studies to define minimum nutrient requirements for fermentation of pure xylose are in progress. Once completed, conversion of hydrolyzates supplemented with minimal medium components will be evaluated. After establishing nutrient requirements for rapid and effective fermentation of hydrolyzates, efforts to formulate cost-effective process media will commence.

Major Project Reports: None

Summary Date: March 1994

Genetic Engineering of Xylose-Fermenting Yeasts

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

University of Wisconsin
Madison, WI 53706

Principal Investigator: T. Jeffries

Telephone: (608) 231-9453

Contract Number: XAU-4-11193-02

Contract Period: 11/93-10/94

Contract Funding (Source):

FY 1994: \$120,000 (DOE)

Objective:

Develop, through the application of genetic engineering techniques, an improved ethanologenic yeast capable of efficient xylose fermentation.

Approach/Background:

Efficient conversion of the xylose in the hemicellulose fraction to ethanol remains one of the economic bottlenecks in the current conversion scheme. *Candida shehatae* and *Pichia stipitis* are the yeasts most capable of xylose fermentation, although at rates and yields that are presently less than required for commercial production. These yeasts use a two-step pathway in which xylose is first reduced by an NAD(P)H-dependent xylose reductase to xylitol, which is then oxidized to xylulose by an NAD-dependent xylitol dehydrogenase. The different cofactor specificities of these two enzymes limit the efficiency by which these yeasts convert xylose to xylulose and subsequently to ethanol. Generally, the xylose-assimilating yeasts are capable of achieving moderate ethanol yields and final ethanol concentrations, but demonstrate relatively low ethanol productivities, especially in the absence of oxygen. This research is directed at improving the fermenta-

tion performance of these yeasts by using genetic engineering to increase their ethanol productivity and the ethanol tolerance.

Status/Accomplishments:

A genetic transformation system based on complementation of a uracil-requiring mutant with the gene complementing this defect has been developed for *P. stipitis*. Research is in progress to apply this system to develop an improved xylose-fermenting *P. stipitis* by placing its alcohol dehydrogenase and pyruvate decarboxylase genes under the control of a xylose-inducible promoter.

Major Project Reports: None

Summary Date: March 1994

Development of Cellulose Conversion

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

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

Principal Investigators: K. Kadam and G. Philippidis

Telephone: (303) 384-6866

Contract Number: DE-AC02-83CH10093

Contract Period: 10/92-09/93

Contract Funding (Source):

FY 1991: \$230,000 (DOE)
FY 1992: \$295,000 (DOE)
FY 1993: \$313,000 (DOE)
FY 1994: \$338,000 (DOE)

Objective:

Design and optimize the cellulose-to-ethanol conversion process.

Approach/Background:

Cellulose can be efficiently converted to ethanol using cellulase enzymes and fermentative organisms in a single step—the simultaneous saccharification and fermentation process (SSF). A mathematical model of the SSF process has been developed. 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:

Key model parameters were determined through critical experimentation regarding (1) the growth and ethanol production characteristics of the fermentative organism, and (2) the kinetic behavior of the hydrolytic enzymes, cellulase, and β -glucosidase. The model was improved by considering the dependence of the rate of cellulose hydrolysis on

the surface area and structure of the lignocellulosic substrate. The model has been used in various applications, such as the design of a continuous SSF process (bench and pilot scale) and the evaluation of ethanol-removal schemes during the SSF.

The successful design and operation of a bench-scale continuous SSF system was a major breakthrough. Continuous saccharification work was initially performed to identify and correct operating problems. A subsequent process was developed for the first time and the material balance was satisfactorily closed. Manipulation of the dilution rate and other process parameters has led to promising ethanol production levels.

Major Project Reports:

Philippidis, G.P. (1993). "Conversion of Cellulosic Biomass to Fuel Ethanol." *Energy from Biomass*; Vol. 16, pp. 545-557.

Philippidis, G.P.; Smith, T.K.; Wyman, C.E. (1993). "Study of the Enzymatic Hydrolysis of Cellulose During the SSF Process for Production of Fuel Ethanol." *Biotechnol. Bioeng.*; Vol. 41, pp. 846-853.

Philippidis, G.P.; Smith, T.K. (1993). *Milestone Report: Results of Bench-Scale Continuous Saccharification*. Golden, CO: National Renewable Energy Laboratory.

Philippidis, G.P.; Spindler, D.D.; Wyman, C.E. (1992). "Mathematical Modeling of Cellulose Conversion to Ethanol by the Simultaneous Saccharification and Fermentation Process." *Appl. Biochem. Biotechnol.*; Vol. 34/35, pp. 543-556.

Philippidis, G.P.; Wyman, C.E. (1992). "Production of Alternative Fuels: Modeling of Cellulosic Biomass Conversion to Ethanol." Chapter in *Recent Advances in Biotechnology*. Edited by F. Vardar-Sukan and S. Sukan. The Netherlands: Kluwer Academic Publishers.

Kadam, K.L.; Schmidt, S.; Smith, T.K. (1993). *Milestone Report: Preliminary Results on Promising Hexose-Fermenting Organisms*. Golden, CO: National Renewable Energy Laboratory.

Kadam, K.L.; Smith, T.K.; Philippidis, G.P. (1993). *Milestone Report: Continuous SSF System Design and Operation for Material Balance Studies*. Golden, CO: National Renewable Energy Lab.

Summary Date: March 1994

Continuous Conversion of Cellulosic Biomass to Ethanol

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Thayer School of Engineering
Dartmouth College
8000 Cummings Hall
Hanover, NH 03755-8000

Principal Investigator: L. Lynd

Telephone: (604) 646-2231

Contract Number: XAE-3-13005-01

Contract Period: 01/93–01/94

Contract Funding (Source):

FY 1992: \$196,801 (DOE)
FY 1993: \$232,560 (DOE)

Objective:

Develop direct microbial conversion (DMC) process and evaluate continuous solids retention bioreactors (CSRBs) for biomass conversion to ethanol.

Approach/Background:

DMC combines enzyme production and sugar-to-ethanol conversion in one operation. *Clostridium thermocellum* is the organism of choice. Because of its comprehensive nature, DMC is a long-term technology.

Preferential solids retention during the biomass-to-ethanol conversion may improve ethanol productivity. Under mixing conditions, large, cellulose-rich particles tend to reside toward the bottom of the fermentor. In contrast, the small, lignin-rich particles stay at the top of the solids bed and are washed out in the continuous overflow stream. A continuous solids retention bioreactor (CSRB) scheme increases residence time of

undigested particles—thereby increasing productivity.

Status/Accomplishments:

Investigated DMC process with *Clostridium thermocellum* using pretreated poplar as substrate. DMC issues are formation of an exopolymer, specificity of the organism, and overall productivity of the fermentation. The formation of the exopolymer, which seems to be common among *Clostridia*, was addressed by co-cultivation of *Clostridium thermocellum* and *Clostridium saccharolyticum*. Specificity and overall productivity were addressed by screening for efficient *C. thermocellum* strains. Several *Clostridium* strains were evaluated, and all produced different amounts of exopolymer including those not requiring vitamins. A current focus is making a transition to continuous DMC.

CSRB issues are selection of proper configuration, establishing differential solids retention, and demonstrating efficacy of chosen CSRB configuration. Several configurations were evaluated. Preliminary data indicate CSRB is a viable concept. A mathematical model was developed that explains the CSRB data well; the model will be further refined.

Major Project Reports:

South, C.R.; Lynd, L.R. (1993). *Analysis of Conversion of Particulate Biomass to Ethanol in Continuous Solids Retention and Cascade Bioreactors*. Golden, CO: National Renewable Energy Laboratory. Work performed by Thayer School of Biochemical Engineering, Hanover, N.H.

South, C.R.; Hogsett, D.A.; Lynd, L.R. (1993). *Modeling Simultaneous Saccharification and Fermentation of Lignocellulose to Ethanol in Batch and Continuous Reactors*. Golden, CO: National Renewable Energy Laboratory. Work performed by Thayer School of Biochemical Engineering, Hanover, N.H.

South, C.R.; Hogsett, D.A.; Lynd, L.R. (1993). *Continuous Fermentation of Cellulosic Biomass to Ethanol*. Golden, CO: National Renewable Energy Laboratory. Work performed by Thayer School of Biochemical Engineering, Hanover, N.H.

Summary Date: December 1993

Cellulase Development

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

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

Principal Investigator: S. Thomas

Telephone: (303) 384-6187

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/94

Contract Funding (Source):

FY 1993: \$1,317,000 (DOE)
FY 1994: \$1,555,000 (DOE)

Objective:

Purify, characterize, and compare cellulases from a variety of organisms to determine which have the highest specific activity, the best match to anticipated hydrolysis and fermentation conditions, and the ability to act synergistically to enhance the rate of hydrolysis of the solid substrate, cellulose.

Using recombinant DNA technology, develop cost-effective, highly productive, industrially acceptable microbial strains for the production of well-balanced, multi-enzyme cellulase systems for efficient hydrolysis of cellulose.

Approach/Background:

Identification of extremely active sources of cellulase enzymes for the depolymerization of cellulose is an important first step in the development of an economical process. Cloning chosen cellulase genes from microorganisms that produce high-specific-activity cellulases into a very rapidly growing host (i.e., a bacterium) will permit production of the most-active cellulase systems known. The ability to select and overproduce these cellulases will significantly lower the cost of production

of cellulases and/or significantly increase the rate of hydrolysis of lignocellulosic biomass, consequently reducing the cost of production of ethanol for fuels.

Status/Accomplishments:

In FY 1993, the gene for the *Acidothermus cellulolyticus* E1 endoglucanase was completely sequenced, permitting the construction of several expression vectors for the highly active endoglucanase in both *E. coli* and *Streptomyces lividans*. Purified enzymes supplied by subcontractors associated with this activity, and our own work, have permitted extensive comparative biochemical studies to be carried out, resulting in the ability to rationalize which genetic constructions should be constructed first. Several endoglucanase, exoglucanase and β -glucosidase genes cloned at NREL and elsewhere have been shown to function in heterologous host bacteria, such as *E. coli* and *S. lividans*. Initial multi-gene expression systems are being designed and assembled for testing.

Major Project Reports:

Himmel, M.E.; Tucker, M.P.; Adney, W.S.; Grohmann, K. (1993). *Thermostable E1 Endoglucanase from Acidothermus cellulolyticus*. U.S. Patent No. 5,275,944.

Tucker, M.P.; Grohmann, K.; Himmel, M.E., Mohagheghi, A. (1992). *Thermostable Purified Endoglucanase from Thermophilic Bacterium Acidothermus cellulolyticus*. U.S. Patent No. 5,110,735.

Summary Date: March 1994

New Methods for Characterizing Cellulases

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

University of California—Davis
258 Cruess Hall
Davis, CA 95626

Principal Investigator: S. Shoemaker

Telephone: (916) 752-2922

Contract Number: XA-1-11225-01

Contract Period: 10/91–01/95

Contract Funding (Source):

FY 1991: \$116,790 (DOE)
FY 1991: \$148,777 (DOE)
FY 1994: \$169,849 (DOE)

Objective:

Develop useful standard methodologies for the kinetic characterization of endo-1,4-glucanases.

Approach/Background:

Considerable controversy exists concerning the actual activities of many cellulases reported in the scientific literature. Much disagreement focuses on the question of enzyme purity; however, both assay type and protocol have been indicated as causes of confusion. A standard assay procedure must be developed so that different labs can produce reliable data which can be compared directly with data from other sources.

Status/Accomplishments:

A viscometric assay procedure has been developed and improved to the point where a standard viscometric method has been submitted for project-wide use. These assays, which were developed using model substrates, will now be extended for use on relevant lignocellulosic substrates. Further

improvements will focus on development of a new assay which will be based on direct molecular-weight determinations of substrate molecules during the course of reaction.

Major Project Reports:

University of California. (October 1993). *Annual Report for Subcontract XA-1-11225-1; Period of Performance, 11/92 - 9/93*. Golden, CO: National Renewable Energy Laboratory. Work performed by University of California, Davis, CA.

Summary Date: March 1994

Identification of Leading Cellulase Producers

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

cosidase activity relative to cellulase activity will
receive special attention.

Major Project Reports: None

Summary Date: March 1994

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Department of Microbiology
Colorado State University
Fort Collins, CO 80523

Principal Investigator: R. Tengerdy

Telephone: (303) 491-6163

Contract Number: XAC-3-11185-01

Contract Period: 07/93-07/94

Contract Funding (Source):

FY 1991: \$121,582 (DOE)

Objective:

Identify the best possible cellulase-producing microbial systems that synthesize and secrete high-specific-activity cellulases at high production rates, using pretreated biomass as the carbon source for microbial growth.

Approach/Background:

A large collection of filamentous, lignocellulolytic fungi and actinomycetes resides at the Technical University of Budapest (TUB). This collection will be screened in batch culture for cellulase and β -glucosidase activities. Strains that produce the most cellulase activity in batch culture will be carried on to fed-batch experiments to assess potential for high productivity.

Status/Accomplishments:

Over 400 strains have been screened in batch culture and ranked according to cellulase enzyme titer. Various ratios of cellulase to β -glucosidase activities are represented in this ranking. As commercial *T. reesei* preparations are notoriously deficient in β -glucosidase, those strains showing enhanced β -glu-

Cellulases from *Thermomonospora fusca*

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Cornell University
Department of Biochemistry
Ithica, NY 14853

Principal Investigator: D. Wilson

Telephone: (607) 255-5706

Contract Number: XAC-3-13260-01

Contract Period: 11/91-03/95

Contract Funding (Source):

FY 1991: \$ 94,878 (DOE)
FY 1991: \$124,852 (DOE)
FY 1994: \$100,000 (DOE)

Objective:

Purify and characterize large quantities of active endoglucanases and exoglucanases from the cellulolytic bacterium, *Thermomonospora fusca*. Isolate and characterize cellulase genes from *T. fusca*.

Approach/Background:

T. fusca is a thermotolerant bacterium that produces endo- β -1,4-glucanases with very high specific activities. In addition, it is one of the few bacterial systems that has been shown to express an active, highly synergistic exo- β -1,4-glucanase. Proteins are purified by classical chromatography techniques from both native and recombinant cultures of microorganisms.

Status/Accomplishments:

Milligram quantities of endoglucanases and an exoglucanase have been purified from both native and recombinant sources by standard chromatographic techniques. An excellent x-ray crystal structure has been solved for the *T. fusca* endoglu-

canase, E₂ and shows remarkable structural homology with the previously solved x-ray structure for *T. reesei* CBH II core protein. *T. fusca* genes for five different endo- and exocellulases have been cloned and sequenced.

Major Project Reports:

Irwin, D.C.; Spezio, M.; Walker, L.P.; Wilson, D.B. (1993). "Activity Studies of Eight Purified Cellulases: Specificity, Synergism and Binding Domain Effects." *Biotech. Bioeng.*; Vol. 42, pp. 1002-1013.

Jung, E.D.; Lao, G.; Irwin, D.; Barr, B.K.; Benjamin, A.; Wilson, D.B. (1993). "DNA Sequences and expression in *Streptomyces lividans* of an Exoglucanase Gene and an Endoglucanase Gene from *Thermomonospora fusca*." *Appl. Env. Microbiol.*; Vol. 59, pp. 3032-3043.

Spezio, M.; Wilson, D.B.; Karplus, P.A. (1993). "Crystal Structure of the Catalytic Domain of a Thermophilic Endocellulase." *Biochemistry*; Vol. 32, pp. 9906-9916.

Walker, L.P.; Belair, C.D.; Wilson, D.B.; Irwin, D.C. (1993). "Engineering Cellulase Mixtures by Varying the Mole Fraction of *Thermomonospora fusca* E₅ and E₃, *Trichoderma reesei* CBH I, and *Caldocellum saccharolyticum* β -glucosidase." *Biotech. Bioeng.*; Vol. 42, pp. 1019-1028.

Summary Date: March 1994

Maintain Standard Cellulase Stock

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Major Project Reports: None**Summary Date:** March 1994**Project Manager:** J. Mielenz**Telephone:** (303) 275-4489**Contractor:**

Environmental BioTechnologies
4040 Campbell Ave.
Menlo Park, CA 94025

Principal Investigator: D. Munnecke**Telephone:** (415) 462-6700**Contract Number:** YAW-3-13046-01**Contract Period:** 12/92-11/94**Contract Funding (Source):**

FY 1993: \$33,779 (DOE)

FY 1994: \$23,986 (DOE)

Objective:

Store, distribute, and monitor a standard cellulase preparation, purchased from a third party.

Approach/Background:

This subcontract provides a project-wide service function for users of cellulase within the Ethanol Project. The purpose is to distribute (free of charge) quantities of a uniform cellulase preparation to both in-house users and other subcontractors whose work requires the use of a cellulase preparation. Cellulase and β -glucosidase activities are monitored monthly using standardized assay procedures and reported to NREL.

Status/Accomplishments:

Four 200-gallon drums of a single lot of a commercial cellulase preparation were purchased, properly stored, and monitored monthly for stability of the enzyme preparation. Quantities of this enzyme preparation were distributed to many cellulase users on request to NREL.

Mass Transfer in Viscous Suspensions During Cellulase Production

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

North Carolina State University
Chemical Engineering
College of Engineering
Box 7905
Raleigh, NC 27695-7905

Principal Investigator: D. Ollis

Telephone: (919) 515-2324

Contract Number: XAU-3-11184-01

Contract Period: 04/93-12/94

Contract Funding (Source):

FY 1991: \$126,690 (DOE)

Objective:

Fungal cellulases are currently the most promising cellulose-hydrolyzing enzymes. Consequently, understanding of oxygen and nutrient mass-transfer limitations during aerobic mycelial (fungal) growth is imperative to optimizing enzyme production and improving the overall biomass conversion process. The objective of this work is to develop a knowledge base addressing mass transfer in cellulase fermentation.

Approach/Background:

Cellulase enzyme production by aerobic fungi accompanies the propagation of microorganisms, which is known to proceed via formation and growth of mycelia. Mycelial growth results in transformation of the cell broth from a newtonian mixture into a highly viscous suspension, where oxygen and nutrient transfer rates may be severely reduced. To improve cell growth and support enzyme production, aeration and agitation strategies

need to be formulated that will ensure adequate transfer of oxygen and nutrients to the mycelia without adverse effects on cellulase activity. The success of such strategies relies on understanding and quantification of mass transfer of oxygen and nutrients in a cellulase production system.

Status/Accomplishments:

A 14-liter bench-scale fungal cultivation system was designed and installed that allows accurate measurement of oxygen concentrations, as well as agitation and aeration power, during cellulase production. Initial shakedown experiments were performed to test the measurement techniques. A procedure for measuring broth viscosity was developed using the whole fermenter as a viscometer; this method will complement measuring viscosity of samples in an off-line mode. A cup-and-vane device was adapted to an off-line chamber to allow measurement of suspension rheology. Calibration was done with simulated broths such as 0.4% xanthan gum solutions, and rheological parameters were estimated using a cone-and-plate rheometer. Some preliminary rheological studies were conducted with fermentation broth of *T. reesei* Rut C-30 to gain qualitative understanding of its behavior. Quantitative data on the effect of provided power (aeration and mixing) on dissolved oxygen concentration, cell growth, and cellulase productivity will be collected and used in developing correlations for $k_L a$ and other parameters.

Major Project Reports: None

Summary Date: December 1993

Investigate Biochemistry of Cellulase/Cellulose Interaction

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Oak Ridge National Laboratory (ORNL)
P.O. Box 2008
Oak Ridge, TN 37831

Principal Investigator: J. Woodward

Telephone: (615) 574-6826

Contract Number: DG-1-11138-01

Contract Period: 10/91-05/95

Contract Funding (Source):

FY 1992: \$ 99,800 (DOE)
FY 1993: \$ 99,800 (DOE)
FY 1994: \$100,000 (DOE)

Objective:

Determine the function and precise role of cellulase functional domains (catalytic and cellulose-binding) in the hydrolysis of cellulose.

Approach/Background:

The nature of the chemistry at the site of interaction between the binding domains of cellulases and the substrate is currently unknown. Elucidation of the roles played by cellulase catalytic and binding domains would aid in the development of improved cellulase enzymes through protein-engineering technology.

Status/Accomplishments:

Separation procedures have been developed for obtaining pure enzyme components from crude *Trichoderma reesei* enzyme preparations, including CBH I, CBH I core protein (catalytic domain), CBH II, CBH II core protein (cp), and *Aspergillus niger* EG. Studies are being carried out to determine adsorption coefficients for these components

in lignocellulose substrates. The CBH II cp displays an inherent endoglucanase-type ability to hydrolyze soluble, amorphous substrates. CBH I is specifically inhibited (instantaneous and reversible) by low concentrations of palladium complexes such as Na_2PdCl_6 .

Major Project Reports:

Donner, T.R.; Evans, B.R.; Affholter, K.A.; Woodward, J. (in press). "The Role of the Cellulose-Binding Domain of Cellobiohydrolase I in Cellulose Hydrolysis," Chapter in *Bioconversion of Fuels*. Edited by M.E. Himmel and J.O. Baber. Washington, D.C.: ACS Symposium Series.

Woodward, J. (1992). *Annual Progress Report: Biochemistry of Cellulase/Substrate Interactions*. Golden, CO: National Renewable Energy Laboratory. Work performed by Oak Ridge National Laboratory, Oak Ridge, TN.

Woodward, J.; Brown, J.P.; Evans, B.R.; Affholtes, K.A. (in press). "Papain Digestion of Crude *Trichoderma reesei* Cellulase: Purification and Properties of Cellobiohydrolase I and II Core Proteins." *Biotechnol. Applied Biochem.*

Woodward, J.; Tate, J.; Herrmann, P.C.; Evans, B.R. (1993). "Comparison of Ellman's Reagent with *N*-(1-pyrenyl) malaimide for the Determination of Free Sialphidryl Groups in Reduced Cellobiohydrolase I from *Trichoderma reesei*." *J. Biochemical Biophys. Methods*; Vol. 26, pp. 121-127.

Summary Date: March 1994

Optimization of Synergy Among Cellulases

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Oak Ridge National Laboratory (ORNL)
P.O. Box 2008
Oak Ridge, TN 37831

Principal Investigator: J. Woodward

Telephone: (615) 574-6826

Contract Number: DW-2-12289-1

Contract Period: 01/93-01/95

Contract Funding (Source):

FY 1993: \$ 99,800 (DOE)
FY 1994: \$120,000 (DOE)

Objective:

Obtain fundamental knowledge about cellulase-catalyzed hydrolysis of lignocellulosic materials with respect to the phenomenon of synergism among cellulase components.

Approach/Background:

A synergistic effect is commonly observed when endo- β -1,4-glucanases act on solid cellulosic substrates in the presence of exo- β -1,4-glucanases. This phenomenon is seen not only when enzymes from the same system are incubated together, but also when exoglucanases from one biological system are mixed with endoglucanases from a completely different species. Synergy is observed between bacterial and fungal-derived enzymes. Despite numerous experiments that demonstrate the widespread nature of synergy between cellulases, no clear explanation for this phenomenon has yet emerged. Clearly, since synergy among cellulases influences the rate at which cellulosic substrates can be saccharified, it is important to understand the basic parameters which control it.

Status/Accomplishments:

Individual cellulase components from *Trichoderma reesei*, *Aspergillus niger*, and *Cellulomonas fimi* cellulase preparations were purified. Core proteins (catalytic domains) from both of the exo- β -1,4-glucanases (CBH I and CBH II) were prepared by cleavage with papain followed by chromatographic separations. Pairwise combinations of endoglucanases and exoglucanases were examined for their ability to exhibit synergy. The effect of β -glucosidase purified from *A. niger* was also examined. Synergy between *C. fimi* Cen D and *T. reesei* CBH I has been shown to occur at pH 5.0 where Cen D is catalytically inactive.

Major Project Reports:

Hamilton, L.A.; Woodward, J. (January 1994). Annual Technical Progress Report: Optimize *Trichoderma reesei* Cellulase Components for Synergy. Golden, CO: National Renewable Energy Laboratory. Work performed by Oak Ridge National Laboratory, Oak Ridge, TN.

Summary Date: March 1994

Cellulases from *Clostridium thermocellum*

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

University of Rochester
Department of Chemical Engineering
Gavett Hall, Room 206
Rochester, NY 14627

Principal Investigator: D. Wu

Telephone: (716) 275-8499

Contract Number: XAC-3-13419-01

Contract Period: 08/93-08/94

Contract Funding (Source):

FY 1993: \$ 106,080 (DOE)

Objective:

Purify and characterize large quantities of active endoglucanases from the cellulolytic, anaerobic bacterium, *Clostridium thermocellum*. Isolate and characterize cellulase genes from *C. thermocellum*.

Approach/Background:

C. thermocellum produces a cellulase system consisting of more than a dozen different polypeptides, which act together as a particulate system, referred to as a cellulosome, at the cell surface. This strategy for cellulose degradation is common to many anaerobic cellulolytic organisms and provides an important alternative to the freely soluble cellulases being investigated by other sub-contractors.

Status/Accomplishments:

A genomic library of *C. thermocellum* DNA has been constructed in *E. coli*. Genes for the anchor-age/scaffolding protein, CelL, and a key catalytic subunit, CelS, have been cloned and sequenced. An

important endoglucanase gene, *celD*, has been cloned and expressed in a recombinant system to purify large quantities of the CelD protein for biochemical characterization at NREL.

Major Project Reports:

Wang, W.K.; Kruus, K.; Wu, J.H.D. (1993)
"Cloning and DNA Sequence of the Gene Coding for *Clostridium thermocellum* Cellulase S₁ (CelS), a Major Cellulosome Component." *J. Bacteriol.* Vol. 175, pp. 1293-1302.

Summary Date: March 1994

Cellulases from *Microbispora bispora*

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Rutgers University
Department of Microbiology
Lipman Hall, Room 333A
New Brunswick, NJ 08903

Principal Investigator: D. Eveleigh

Telephone: (908) 932-9829

Contract Number: XO-2-11201-01

Contract Period: 01/92-04/95

Contract Funding (Source):

FY 1991: \$ 89,979 (DOE)
FY 1991: \$ 62,880 (DOE)
FY 1994: \$100,000 (DOE)

Objective:

Purification and characterization of large quantities of active endo- β -1,4-glucanases, exo- β -1,4-glucanases, and 1,4- β -D-glucosidases from the cellulolytic, thermotolerant bacterium, *Microbispora bispora*. Isolation and thorough characterization of cellulase and β -glucosidase genes from *M. bispora*.

Approach/Background:

M. bispora is a thermotolerant, composting bacterium that produces high-specific-activity endo- β -1,4-glucanases and a cellobiose activity, which is extremely resistant to feedback inhibition by glucose. It has also been shown to express at least one exo- β -1,4-glucanase. Proteins are purified by classical chromatography techniques from both native and recombinant cultures of microorganisms.

Status/Accomplishments:

Milligram quantities of endoglucanase A have been purified from a recombinant source by standard

chromatographic techniques and supplied to NREL for testing. Milligram quantities of a putative cellobiohydrolase have been purified from *M. bispora* cultures and supplied to NREL for testing and verification. *M. bispora* genes for an endoglucanase and two β -glucosidases have been cloned and sequenced.

Major Project Reports:

Wright, R.M.; Yablonsky, M.D.; Shalita, Z.; Goyal A.K.; Eveleigh, D.E. (1992). "Cloning, Characterization, and Nucleotide Sequence of *Microbispora bispora* Bgl B: a Thermostable Beta-glucosidase Expressed in *Escherichia coli*." *Appl. Environ. Microbiol.*; Vol. 58, pp. 3455-3465.

Summary Date: March 1994

Cellulases from *Thermotoga neapolitana*

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Major Project Reports: None**Summary Date:** March 1994**Project Manager:** J. Mielenz**Telephone:** (303) 275-4489**Contractor:**

Rutgers University
Department of Microbiology
Lipman Hall, Room 333A
New Brunswick, NJ 08903

Principal Investigator: D. Eveleigh**Telephone:** (908) 932-9829**Contract Number:** XC-2-11179-01**Contract Period:** 01/92-05/95**Contract Funding (Source):**

FY 1991: \$30,000 (DOE)
FY 1991: \$67,498 (DOE)
FY 1994: \$40,000 (DOE)

Objective:

Purify and characterize large quantities of active endoglucanases and 1,4- β -D-glucosidases from the bacterium, *Thermotoga neapolitana*. Isolate and characterize endo- β -1,4-glucanase and 1,4- β -D-glucosidase genes from *T. neapolitana*.

Approach/Background:

T. neapolitana is a hyperthermophilic eubacterium isolated from a deep-sea ocean vent which produces highly thermotolerant, very high specific-activity endo- β -1,4-glucanases (CMCases). Proteins are purified by classical chromatography techniques.

Status/Accomplishments:

Milligram quantities of *T. neapolitana* endoglucanase B have been purified by standard chromatographic techniques and supplied to NREL for testing.

Analyze Complex Samples and Develop Methods

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

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

Principal Investigator: C. Ehrman

Telephone: (303) 384-6185

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/94

Contract Funding (Source):

FY 1993: \$300,000 (DOE)
FY 1994: \$464,000 (DOE)

Objective:

Provide precise and accurate analysis of complex samples for the activities of the Ethanol Project and develop and validate methods in answer to new analytical needs. This analytical information is used to meet the specific technical objectives of the various research groups within the project.

Approach/Background:

This task provides a certified analytical service to the project by verifying the analytical results using well-researched and documented procedures, using task-maintained and validated instruments, and following sound QA/QC principles. This task produces high-quality analytical data through the use of analytical measurements that are accurate, reliable, objective, and meet established quality control principles. Submitted samples are analyzed using standard analytical procedures, as well as new methods developed specifically in response to a new analytical need.

Status/Accomplishments:

In FY 1993, over 170 chemical analysis work orders were processed. These work orders represented more than 2000 different samples and a wide range of analyses—all of which originated from project researchers and subcontractors. New instruments were brought on-line and validated, including a laser diffraction particle-size analyzer and a high-performance anion-exchange chromatograph with pulsed amperometric detection. Major method development projects were completed, including methods for the analysis of alkaline-pretreated solids and liquors, fermentation residues, and enhanced liquid chromatography protocols for the analysis of soluble sugars, total sugars, organic acids, and biomass degradation products.

Major Project Reports:

Adney, W.S.; Ehrman, C.I.; Baker, J.O.; Thomas, S.R.; Himmel, M.E. (1993). *Cellulase Assays: Methods from Empirical Mathematical Models*. Golden, CO: National Renewable Energy Laboratory.

Ehrman, C.I.; Himmel, M.E. (1993). *Simultaneous Saccharification and Fermentation of Pretreated Biomass: Improving Mass Balance Closure*. Golden, CO: National Renewable Energy Laboratory.

Vinzant, T.B.; Ponfick, L.; Nagle, N.J.; Ehrman, C.I.; Reynolds, J.B.; Himmel, M.E. (1993). *SSF Comparison of Selected Woods from Southern Sawmills*. Golden, CO: National Renewable Energy Laboratory.

Summary Date: March 1994

Develop and Maintain Project QA/QC Program

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

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

Principal Investigator: C. Ehrman

Telephone: (303) 384-6185

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/94

Contract Funding (Source):

FY 1994: \$170,000 (DOE)

Objective:

Develop a programmatic QA/QC service that will result in the highest possible levels of work quality, reproducibility, and utility. This service will develop a detailed Quality Assurance Plan (QAP), a manual containing standardized analytical procedures, validated conformance evaluation standards, and protocols for tracking and validating instrument performance.

Approach/Background:

The incorporation of sound quality control and quality assurance practices into the day-to-day activities of an analytical laboratory is a necessary step in the production of high quality analytical data and in the improvement of overall project performance. The quality control aspect describes the activities designed specifically to provide a quality product, whereas the quality assurance aspect describes planned activities designed to ensure that the quality control activities are being properly implemented.

Status/Accomplishments:

A detailed ethanol project QAP has been issued and steps to assimilate the plan into daily activities of

the project have been implemented. The laboratory analytical procedures manual has been updated with new laboratory analytical procedures as the task has developed and validated new analytical methods. New conformance evaluation standards have been identified and validated as tools for accessing the quality of generated analytical results.

Major Project Reports:

Brigham, J.S.; Ehrman, C.I.; Templeton, D.W.; Ruiz, R.O. (1993). *Ethanol Project Quality Assurance Plan*. Golden, CO: National Renewable Energy Laboratory.

Ehrman, C.I.; Templeton, D.W.; Ruiz, R.O.; Brigham, J.S. (1993). *Chemical Analysis and Testing Task Laboratory Analytical Procedures Manual*. Golden, CO: National Renewable Energy Laboratory.

Summary Date: March 1994

Compositional Analysis of Lignocellulosic Samples

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

Hauser Chemical Research, Inc.
5555 Airport Boulevard
Boulder, CO 80301

Principal Investigator: D. Timmons

Telephone: (303) 443-4662

Contract Number: RAC-4-14108

Contract Period: 03/94-03/95

Contract Funding (Source):

FY 1994: \$148,000 (DOE)

Objective:

Use an outside analytical testing laboratory to provide precise and accurate compositional analysis of routine lignocellulosic samples of interest to the ethanol project. This analytical information will supplement the more complex and/or nonroutine analyses conducted by the Chemical Analysis and Testing (CAT) task. The data will be used by various research groups with the project to meet specific technical objectives defined in the annual operating plan.

Approach/Background:

Routine feedstock, pretreated biomass, and the solid fraction of fermentation residues are to be analyzed for total solids, Klason lignin, acid-soluble lignin, cellulose (as glucose), hemicellulosic sugars, and ash by following a core group of CAT-task laboratory analytical procedures. During the course of these analyses, the quality control protocols described in the approved QA/QC plan must be followed. The results of each group of analyses are reported to the CAT task for evaluation and data reduction.

Status/Accomplishments:

This subcontract has just been awarded. The subcontractor has successfully completed the analysis of the conformance evaluation standards and has delivered their QA/QC test results. After receipt and approval of the Experimental Plan and the QA/QC Plan, submittal of routine samples for compositional analysis will begin.

Major Project Reports: None

Summary Date: March 1994

Process Integration

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

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

Principal Investigators: G. Philippidis and
Q. Nguyen

Telephone: (303) 384-6888

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/94

Contract Funding (Source):

FY 1994: \$2,041,000 (DOE)

Objective:

Ensure that a fully integrated process functions reliably and economically when operated on realistic lignocellulosic 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 operational realities that a fully integrated commercial process will face.

Approach/Background:

Much of the research conducted over the last several years has been done at the bench scale using idealized substrates. Furthermore, the individual elements of the overall process were investigated in an isolated fashion, rather than being integrated into a complete feedstock-to-product process. This approach was taken to understand the scientific basis of each process step. The approach of the Process Integration task is based on the need 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 and process engineering needs from CRADA projects, 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 physical and chemical interactions that will be found in a commercial process; and (3) guide the design, operation, and optimization of a pilot plant (PDU), which will demonstrate a chemically and mechanically integrated biomass conversion process and develop design data for the engineering demonstration unit (EDU).

Status/Accomplishments:

In addition to bench-scale testing carried out under CRADAs (1992-present), the Process Integration group has been working on evaluating the effects of real feedstocks on the fermentation steps in the ethanol conversion process. Experiments have successfully demonstrated early steps of the xylose and simultaneous saccharification and fermentation (SSF) processes based on realistic hybrid poplar feedstock, pretreated with dilute acid. Xylose fermentation turned out to be far more difficult to achieve. Successful growth on hydrolysate liquor was finally accomplished through an extensive program of gradual adaptation. The SSF step was demonstrated using hydrolyzed wood chips from the Tennessee Valley Authority in several batch fermentations at the 150-L scale. A continuous multistage bioreactor cascade system was successfully operated using municipal solid waste (MSW). So far, that system has demonstrated realistic means for mixing high-solids slurries and controlling microbial contamination. In parallel, studies are underway to investigate the nutrient requirements of the SSF process and the feasibility of nutrient/biocatalyst recycling.

Major Project Reports: None

Summary Date: March 1994

Process Development Unit

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: J. Mielenz

Telephone: (303) 275-4489

Contractor:

John Brown Engineers and Constructors
300 South Riverside Plaza
Suite 1100
Chicago, IL 60606

Principal Investigators: B. Duff, Q. Nguyen, and
G. Philippidis

Telephone: (303) 384-6862

Contract Number: YS-2-1161-1

Contract Period: 05/92-06/95

Contract Funding (Source):

FY 1992\$ 2,394,000 (DOE)
FY 1993\$10,419,000 (DOE)
FY 1994\$ 3,300,000 (DOE)

Objective:

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 40-100-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 individual unit operations. 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 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 realistic operating conditions and observe the various equipment-size dependent phenomena. The PDU must be designed with a high degree of flexibility and instrumentation to accommodate various process schemes and feedstock substrates.

Because the eventual goal is commercialization, the PDU will be a facility where new ethanol production technology will be tested for NREL's industrial partners.

Status/Accomplishments:

Work performed during Phase I of this subcontract included vendor testing, a conceptual design, and a preliminary engineering design. Preliminary engineering ended in May 1993 with complete process flow diagrams, process and instrumentation diagrams, and a cost estimate. Procurement of long-lead-time equipment began with the purchase of fermenters in February 1993.

Phase II of this work—detailed design and installation of equipment—began in June 1993. Detailed design generated complete installation and layout drawings of the PDU and ended in January 1994. Procurement activities began in September 1993 and continued through March 1994. Construction activities began in October 1993, with concrete work and installation of structural steel. Major equipment installation, piping, and electrical work began in February 1994. Major construction activities are expected to be complete by June 1994, at which time startup activities and initial experimental investigations will begin. A detailed experimental plan has been designed to investigate key issues of the technology.

The PDU has been designed to continuously process a variety of lignocellulosic feedstock to ethanol. The plant currently includes equipment for feed handling, pretreatment, fermentation, distillation, and solids separation.

Major Project Reports: None

Summary Date: March 1994

Methanol and Higher Alcohols

Carbon Dioxide Recycling

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: D. Tyndall

Telephone: (303) 275-4483

Contractor:

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

Principal Investigator: D. DuBois

Telephone: (303) 384-6171

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/93

Contract Funding (Source):

FY 1994: \$280,000 (DOE)

Objective:

Develop modified electrodes capable of reducing CO₂ to methanol in aqueous environments. During conversion to alcohol fuels, 40%-60% of the carbon content of biomass is rejected to the atmosphere. Reduction of CO₂ to methanol using electricity generated by photovoltaic arrays could double the output of alcohol fuels for a given land mass and increase the overall efficiency with which solar energy is converted to fuels. It would also provide a method for storing photovoltaically derived electricity as a liquid fuel of high energy density.

Approach/Background:

In previous research, a new class of catalysts for the electrochemical reduction of CO₂ to CO was developed. (The reduction of CO₂ to CO represents the first step in the reduction of CO₂ to methanol.) These catalysts operate efficiently (i.e., at low overpotentials and with high turnover rates) in organic solvents. Practical applications of these catalysts will require (1) that catalysts operate in aqueous environments to reduce resistance losses common to organic solvents, and (2) modification of electrode surfaces with catalysts to promote catalyst recovery and minimize the amount of

catalyst required. To obtain these objectives, current research is demonstrating that these catalysts can be suitably modified to operate under aqueous conditions and that these catalysts can be attached to electrode surfaces. This research requires the preparation, characterization, and testing of catalysts with substituents appropriate for producing water solubility and/or appropriate for electrode modification.

Status/Accomplishments:

A number of modified catalysts were prepared that were soluble in aqueous solutions. Generally, these modifications resulted in loss of catalytic activity. However, it was found that if the catalysts were modified in the appropriate position, catalytic activity in aqueous solutions could be maintained. Although the selectivities for CO production at this stage of the research is only modest, 30%-40%, there appears to be no intrinsic barrier to modifying these catalysts so that high selectivities may be achieved. Recent research efforts have focused on the development of electrodes with small amounts of catalyst attached directly to the electrode surface. Glassy carbon electrodes have been modified with catalysts with trimethoxysilyl functional groups. These electrodes catalyze reduction of CO₂ to CO with approximately 10% selectivity at the present time. Research is in progress to improve these selectivities.

Major Project Reports: None

Summary Date: April 1994

Indirectly Heated Biomass Gasifier—Phase I

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: D. Tyndall

Telephone: (303) 275-4483

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: 12/91–09/94

Contract Funding (Source):

FY 1991: \$1,072,451 (DOE)
FY 1993: \$1,518,868 (DOE)

FY 1993-1994 Cost Sharing:

\$250,000 (Solar Turbine); \$18,000 (Weyerhaeuser);
\$12,000 (Sauder Woodworking)

Objective:

Aid in the development of a commercially viable fuels production process based on indirect biomass gasification. Phase I provides additional operating data for an existing gasifier; incorporates a slip-stream hot-gas conditioning train into gasifier operation; and performs a detailed design study of gasifier modification for pressurized operation.

Approach/Background:

Biomass gasification involves the production of a medium- or high-Btu gas, rich in hydrogen or carbon monoxide, which is then catalytically converted into fuels. Indirect gasifiers produce a solid carbon-rich char that is typically reacted with air in a separate combustor to produce heat. Indirect gasifiers typically produce a gas rich in carbon monoxide with low carbon dioxide levels. Downstream synthesis-gas modification is required

to produce a synthesis gas with a suitable hydrogen: carbon monoxide ratio, and a low level of inert light hydrocarbons such as methane. 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 with hybrid poplar and switchgrass to produce a medium-Btu synthesis gas and to determine process yields as a function of temperature and steam rate.

A method to control ash agglomeration when firing high alkali containing biomass fuels was identified. This method should be applicable to both gasification and combustion processes.

A slip stream catalytic hot-gas conditioning chamber was designed, installed, and operated. A novel proprietary hot-gas conditioning catalyst (DN34) was identified that shows promising results for both tar cracking and water gas shift activity.

Catalysts similar to DN34 have been screened in a preliminary fashion. Additional screening tests will optimize catalyst performance for both hot-gas conditioning and water-gas shift reactions.

Major Project Reports:

Gebhard, S.C.; Wang, D.; Overend, R.P.; Paisley, M.A. (in press). "Catalytic Conditioning of Synthesis Gas Produced by Biomass Gasification." *Biomass and Bioenergy*.

Paisley, M.A.; Litt, R.D. (1993). "Hot-Gas Conditioning of Biomass-Derived Synthesis Gas." Presented at First Biomass Conference of the Americas, Burlington, VT, August 30-September 2.

Paisley, M.A.; Litt, R.D. (1993). "Evaluation of Energy Plantation Crops in a High-Throughput Indirectly Heated Biomass Gasifier," Presented at First Biomass Conference of the Americas, Burlington, VT, August 30-September 2.

Summary Date: April 1994

Indirectly Heated Biomass Gasifier—Phase 2

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: D. Tyndall

Telephone: (303) 275-4483

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: 12/91–09/94

Contract Funding (Source):

FY 1991: \$1,072,451 (DOE)
FY 1993: \$1,518,868 (DOE)

FY 1993-1994 Cost Sharing:

\$250,000 (Solar Turbine); \$18,000 (Weyerhaeuser);
\$12,000 (Sauder Woodworking)

Objective:

Aid in the development of a commercially viable fuels production process based on indirect biomass gasification. Phase 2 extends hot-gas conditioning system data base; formulates technical specifications for scale-up of hot-gas conditioning system; and provides an acceptable gas stream to fuel an unmodified gas turbine system.

Approach/Background:

Biomass gasification involves the production of a medium- or high-Btu gas, rich in hydrogen or carbon monoxide, which is then catalytically converted into fuels. Indirect gasifiers produce a solid carbon-rich char that is typically reacted with air in a separate combustor to produce heat. Indirect gasifiers typically produce a gas rich in carbon monoxide with low carbon dioxide levels. Downstream modification is required to produce a synthesis gas with a suitable hydrogen:carbon monoxide ratio, and a low level of inert light

hydrocarbons such as methane. Biomass gasification systems typically use scrubbers to remove tar and residual char.

Status/Accomplishments:

Designed and fabricated full-scale hot-gas conditioning chamber capable of processing the PRU's entire synthesis gas output. Tests evaluating the performance of DN34 or other hot-gas conditioning catalysts are planned.

Completed detailed design study evaluating feasibility of system operation at elevated pressure—including design of necessary system modifications, procurement and construction time estimates, and detailed cost estimates.

Additional process systems installed in PRU including synthesis-gas scrubbing systems, synthesis-gas compressor, and 200-kW gas turbine. System shakedown operation completed. System operation scheduled for late FY 1994.

Initial experiments performed to characterize gasifier product gas using NREL's transportable molecular-beam mass spectrometer (TMBMS). Successfully interfaced the TMBMS with gasifier and collected preliminary data on unprocessed syngas and syngas catalytically conditioned with DN34. Research goal is to develop capability of the TMBMS for real-time quantitative analysis.

Major Project Reports:

Paisley, M.A.; Breault, R.W.; Bain, R. (1992). "Design and Economics of Electricity Production from an Indirectly Heated Biomass Gasifier." Presented at 5th Annual National Biofuels Conference and Exposition, Newton, MA, October 19-22.

Breault, R.W. (1992). *Design and Economics of Electricity Production from an Indirectly Heated Biomass Gasifier*. Golden, CO: National Renewable Energy Laboratory. Work performed by Battelle Memorial Institute, Columbus, OH.

Paisley, M.A.; Litt, R.D.; Taylor, D.R.; Tewksbury, T.L.; Hupp, D.E.; Wood, R.D. (November 1993). *Phase Completion Report: Operation and Evaluation of an Indirectly Heated Biomass Gasifier*. Golden, CO: National Renewable Energy Laboratory. Work performed by Battelle Memorial Institute, Columbus, OH.

Summary Date: April 1994

Syngas Conditioning—Gasifier Stream Characterization

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: D. Tyndall

Telephone: (303) 275-4483

Contractor:

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

Principal Investigator: S. Gebhard

Telephone: (303) 384-6249

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92–09/93

Contract Funding (Source):

FY 1994: \$155,000 (DOE)

Objective:

Support syngas conditioning catalyst research by providing important information about the composition of unprocessed biosyngas in real time by directly analyzing biosyngas from PDU-scale biomass gasifiers.

Approach/Background:

In syngas conditioning catalyst development work, tests are routinely performed on the micro scale and on the engineering scale. The integration of micro-scale catalyst research with engineering-scale catalyst and reactor evaluation produces an effective parallel program of catalyst development and process scale-up. A crucial link between these scales is knowing the real unprocessed syngas composition. This link is provided by syngas analysis with the transportable molecular beam mass spectrometer (TMBMS).

The TMBMS permits direct, real-time sampling and analysis of unprocessed and catalytically conditioned syngas streams. All gas and vapor phase compounds are detected simultaneously.

Status/Accomplishments:

A transfer line system interfacing the TMBMS with the Battelle gasifier was designed and successfully installed at the PDU site.

The TMBMS was successfully tested during a subsequent shakedown run with the gasifier using hog fuel feed. The data showed that the TMBMS can be used to monitor biomass gasification.

Mass spectra were obtained for unprocessed syngas and syngas processed over DN34 and a similar catalyst DN37. Benzene, toluene, phenol, styrene, cresol, indene, naphthalene, 1- and 2-methyl-naphthalene, acenaphthylene phenanthrene, anthracene, and pyrene were among the more-abundant tar compounds found in unprocessed syngas.

The concentration of tar compounds was reduced by catalytic conditioning. Small amounts (few hundred ppm) of benzene, naphthalene, and higher-molecular-weight aromatic compounds were detected in the outlet gas from both catalytic reactors. This is consistent with previous micro-scale catalyst testing with DN34 at NREL using a syngas and synthetic gasifier tar. Different operating conditions will be explored in micro-scale experiments.

Major Project Reports:

Gebhard, S.C.; Gratson, D.A.; French, R.J.; Ratcliff, M.A. (1993). *TMBMS Performance on the Battelle Columbus Laboratory Indirectly Heated Biomass Gasifier*. Golden, CO: National Renewable Energy Laboratory.

Gebhard, S.C.; Gratson, D.A.; French, R.J. (1994). "Real-Time Mass Spectrometric Analysis of Synthesis Gas Produced by Biomass Gasification." Presented at 17th Annual Symposium of the Rocky Mountain Fuels Society, Golden, CO, March.

Summary Date: April 1994

Syngas Conditioning–Catalyst Development

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: D. Tyndall

Telephone: (303) 275-4483

Contractor:

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

Principal Investigator: S. Gebhard

Telephone: (303) 384-6249

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92–09/93

Contract Funding (Source):

FY 1993: \$390,000 (DOE)
FY 1994: \$422,000 (DOE)

Objective:

Develop method to condition biomass-derived syngas (biosyngas) for production of methanol and other renewable oxygenates. Replacement of water scrubbing followed by catalytic steam reforming to remove tars and methane holds promise of significant production cost reductions.

Approach/Background:

Unprocessed syngas from leading biomass gasifiers contains particulate matter, methane, other low-molecular-weight hydrocarbons, and tar. These impurities preclude the direct use of biosyngas for catalytic fuels synthesis. Additionally, the hydrogen:carbon monoxide ratio is not optimum for methanol synthesis.

Direct, one-step catalytic syngas conditioning replaces the expensive and energy-intensive steps of scrubbing to remove tar, followed by reheating for conventional methane steam reforming. In direct syngas conditioning, tars are eliminated, the concentration of light hydrocarbons reduced, and the hydrogen:carbon monoxide ratio adjusted in

one or two catalytic steps that operate at gasification temperatures.

Status/Accomplishments:

Constructed micro-scale fluidized bed gasifier. Interfaced gasifier with NREL molecular-beam mass spectrometer (MBMS). Switchgrass gasification examined using quartz chips and DN34 catalyst as the solids beds, with water and deuterium oxide for steam generation. No significant catalytic effect observed from DN34 in this configuration. Gas composition similar to that observed from the Battelle Columbus Laboratory's indirectly heated biomass gasifier.

Four rare-earth oxide (REO) modified forms of DN34 synthesized and tested in a plug-flow micro reactor interfaced with MBMS. Synthetic tar and syngas used. One catalyst exhibited improved water-gas shift activity compared with DN34.

Conducted two 50-hour lifetime tests of DN34. Synthetic syngas and tar (10 wt% naphthalene dissolved in toluene) used. Essentially complete naphthalene destruction observed during entire tests. Conversion of benzene (formed by steam dealkylation of toluene) went from 95% at the start of the test to 75% after 50 hours. Reproducibility between the two tests was excellent. Two NREL REO-modified DN34 catalysts were also tested for 8 hours each. One catalyst exhibited more superior tar destruction activity than observed for DN34 in its first 8 hours. Longer testing of this catalyst is planned.

Major Project Reports:

Gebhard, S.C.; Wang, D. (1993). *Evaluation of a Micro-Scale Fluidized-Bed Biomass Gasifier*. Golden, CO: National Renewable Energy Laboratory.

Gebhard, S.C. (1993) *Chemistry and Modification of DN34 Syngas Conditioning Catalysts*. Golden, CO: National Renewable Energy Laboratory.

Zhao, X.; Cowley, S.W. (1993). *Report for Subcontract RAE-3-133442: Catalyst Lifetime Testing for Methanol Syngas Conditioning*. Golden, CO: National Renewable Energy Laboratory.

Summary Date: April 1994

Ether Additives

Scale-Up NREL-Designed, 36-Ton-Per-Day Vortex Reactor

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: D. Tyndall

Telephone: (303) 275-4483

Contractor:

Interchem Environmental, Inc.
9135 Barton
Overland Park, KS 66214

Principal Investigators: J. Diebold (NREL)
and D. Johnson (Interchem)

Telephone: (303) 231-7250

Contract Number: AA-2-1165-1

Contract Period: 10/93-09/94

Contract Funding (Source):

Interchem cost-sharing not disclosed
FY 1991: \$125,000 (DOE)
FY 1993: \$125,000 (DOE)
FY 1994: \$250,000 (DOE)

Objective:

Demonstrate the scale-up of the NREL-designed 36-ton-per-day (TPD) vortex reactor and adapt it to operation with abrasive-containing feedstocks.

Approach/Background:

Through a cost-sharing agreement, NREL's reactor design has been transferred to Interchem, which is scaling up the vortex reactor. Using waste materials available at low or negative costs, Interchem's 36-TPD pyrolysis unit will be commercially viable. In this arrangement, Interchem is reimbursed by NREL for much of their engineering costs, but not equipment costs. Installation and early operation expenses are also partially defrayed. This approach to technology transfer ensures close interactions between NREL and Interchem.

A FY 1994 contract modification will cover the engineering and operational costs of designing

and operating an inert abrasive removal system based on work done at NREL and to be done at Hazen Research.

Status/Accomplishments:

Interchem produced shop drawings and then tried unsuccessfully to locate a fabricator. Due the similarities of the vortex reactor tube to a gun barrel, NREL's technology transfer office located a defense contractor that manufactured gun barrels. Interchem oversaw the fabrication of the scaled-up vortex reactor. An NREL engineer was also present during part of the manufacture and during the inspection of the completed reactor. The vortex reactor was successfully completed. Interchem is now having the pyrolysis furnace custom made to fit around the vortex reactor. The system is estimated to be completed in early FY 1995.

This technology transfer effort between NREL and Interchem was recently awarded the Federal Laboratory Consortium's 1994 Award for Excellence in Technology Transfer and was also recognized in a letter of commendation from the United States Secretary of Energy.

Major Project Reports:

Johnson, D.A.; Maclean, D.; Feller, J.; Diebold, J.; Chum, H. (in press) "Ablative Fast Pyrolysis: Prototype Plant." *Biomass and Bioenergy*.

Johnson, D.A.; Maclean, D.; Chum, H.L.; Overend, R.P. (1993). "Ablative Fast Pyrolysis: Converting Wood, Agricultural Wastes and Crops into Energy and Chemicals." Presented at the First Biomass Conference of the Americas, Burlington, VT, August 3-September 2.

Summary Date: April 1994

Thermochemical Conversion of Biomass To Fuel Products— Ethers Process Development

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: D. Tyndall

Telephone: (303) 275-4483

Contractor:

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

Principal Investigator: J. Diebold

Telephone: (303) 231-7250

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92–09/93

Contract Funding (Source):

FY 1994: \$1,003,000 (DOE)

Objective:

Design, fabricate, install, and operate an integrated system to thermochemically process up to 0.5-tons-per-day (TPD) of biomass or organic wastes to produce hydrocarbons for conversion to ethers.

Approach/Background:

Biomass is converted to oxygenated oil vapors using pyrolysis and then catalytically converted to olefins and a byproduct stream of aromatic chemicals. The olefins are subsequently passed to a second catalytic reactor to preferentially form butenes and pentenes. The olefin stream is isomerized to increase isobutene and reactive isopentenes before entering the final catalytic reactor to make the ether product.

NREL's pyrolysis process uses a unique 0.5-TPD vortex reactor. Based on previous experiments, a riser cracker reactor is best suited for the first catalytic step. If the process were integrated into an oil refinery, the hydrocarbon product stream from the first catalytic reactor could be used without further specialized processing. However, to enable stand-alone operation, other process steps are

scheduled to be added that will create in a fully integrated process demonstration unit.

Status/Accomplishments:

The removal of the char fines, which is critical to avoid poisoning the catalyst, is being demonstrated with a promising commercially made hot-gas bag-house.

A riser cracker sized to the vortex reactor has been designed, fabricated, and installed. The shakedown of this riser cracker is in progress using a commercial catalyst obtained from Mobil. Improved catalysts can be used as they become available. A catalyst regenerator is in the final design phase that will allow the continuous operation of the riser cracker with an equilibrium catalyst. Fabrication and installation of the regenerator is scheduled for FY 1994.

Major Project Reports:

Diebold, J.P.; Bridgwater, A.V.; Beckman, D.; Elliott, D.C.; Solantausta, Y. (1993). "IEA Technoeconomic Analysis of the Thermochemical Conversion of Biomass to Gasoline by the NREL Process." Chapter in *Advances in Thermochemical Biomass Conversion*. Edited by A.V. Bridgwater. Glasgow, UK: Blackie Academic; pp. 1325-1343.

Bain, R.; Diebold, J.P.; Overend, R.P.; Power, A.J.; Rejai, B. (1992). "The Production of Reformulated Gasoline Components from Biomass and RDF." Presented at IGT's Energy from Biomass and Wastes Symposium XVI, Orlando, FL, March 2-6.

Diebold, J.P.; Overend, R.P.; Rejai, B.; Power, A.J. (1992). "Reformulated Gasoline Components Production from Renewable Feeds," Chapter in *Liquid Fuels from Renewable Resources*. American Society of Agricultural Engineers; pp. 154-161.

Summary Date: April 1994

Thermochemical Conversion of Biomass to Fuel Products— Ethers Research

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: D. Tyndall

Telephone: (303) 275-4483

Contractor:

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

Principal Investigator: T. Milne

Telephone: (303) 231-1440

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92–09/93

Contract Funding (Source):

FY 1994: \$1,077,000 (DOE)

Objective:

Use fundamental principles of pyrolysis and catalysis to maximize the yield of iso-olefins from biomass for the production of oxygenates such as MTBE, ETBE, TAME, and TAEF.

Approach/Background:

Using NREL's molecular-beam mass spectrometer (MBMS), previous work has shown that the pyrolysis of biomass and refuse-derived fuel produces a reactive pyrolysis oil. This pyrolysis oil is then catalytically converted to deoxygenated products such as olefins and aromatics. The iso-olefins are converted to the ethers by reaction with either methanol or ethanol. Intermediate processing of olefin oligomerization and isomerization is expected to increase the yield of the tertiary ether.

Status/Accomplishments:

Catalytic processing of pyrolyzed plastics has continued using the MBMS as the organic product detector. The major plastics components present in municipal solid wastes (MSW) show the potential for high olefin yields. Polyolefinic plastics,

particularly from aliphatic olefins, produce the highest apparent yields of olefins compared to biomass. Some other plastics such as polyvinyl chloride and polyurethane produce high yields of aromatics. Lignocellulosic biomass produces high yields of single-ring aromatics. Other potential renewable feedstocks for this process were also evaluated.

Various experimental and commercial catalysts have been evaluated for the conversion of biomass pyrolysis vapors. Short tests with polyvinyl chloride and polyurethane over the zeolitic catalysts showed no detrimental destruction of the catalyst.

A dedicated Fuels MBMS has been designed, specified, and partially assembled, with all major capital equipment procured. This system will serve the thermo-chemical tasks, as well as the feedstock/conversion interface work.

A senior catalyst scientist from the petroleum industry came on board in May and is aggressively broadening the program by setting up catalyst synthesis capability, as well as designing and procuring a MAT system and laboratory-scale, continuous riser system for more realistic and quantifiable catalyst screening.

Major Project Reports:

Rejai, B.; French, R.; Wang, D.; Meglen, R.; Milne, T. (1993). *Refuse-Derived Fuel (RDF) Variability Study*. Golden, CO: National Renewable Energy Laboratory.

Klotz, M.; Milne, T.; Rejai, B. (1993). *Status Report on Olefin Oligomerization*. Golden, CO: National Renewable Energy Laboratory.

Rejai, B.; Wang, D. (1993). "Catalytic Conversion of Biomass and Wastes to Hydrocarbons: Model Compound Studies." Presented at the 1993 AIChE Annual Meeting, St. Louis, MO.

Summary Date: April 1994

Biodiesel

Culture Collection

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, DC 20585

Project Managers: L.M. Brown (FY 1993)
and J. Sheehan (FY 1994)

Telephone: (303) 275-4420

Contractor:

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

Principal Investigator: L.M. Brown

Telephone: (303) 384-7663

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92–09/93

Contract Funding (Source):

FY 1991: \$55,000 (DOE)
FY 1992: \$50,000 (DOE)
FY 1993: \$12,000 (DOE)

Objective:

Maintain a collection of microalgae that have significant potential with respect to biodiesel production.

Approach/Background:

To develop a technology base for the production of liquid fuels from microalgal biomass; it is desirable to have a pool of genetic resources (i.e., a collection of suitable strains) that can be used in organism development and culture studies. This is an important area that links efforts in genetic engineering to outdoor culture.

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. Cultures are transferred into fresh medium on a quarterly basis.

Methods continue to be refined in the areas of culture maintenance with emphasis on scaling down

the amount of culture medium used, using fewer medium formulations, developing formulations that are easier to prepare, and introducing automation to culture transfer.

Major Project Reports:

Brown, L.M.; Sprague, S.; Jarvis, E.E.; Dunahay, T.G.; Roessler, P.G.; Zeiler, K.G. (January 1994). *Biodiesel from Aquatic Species Project Report: FY 1993*. NREL/TP-422-5726. Golden, CO: National Renewable Energy Laboratory.

Brown, L.M.; Sprague, S.; Jarvis, E.E.; Dunahay, T.G.; Roessler, P.G.; Zeiler, K.G. (May 1993). *Biodiesel/Aquatic Species Project Report: FY 1992*. NREL/TP-422-5259. Golden, CO: National Renewable Energy Laboratory.

Summary Date: April 1994

Genetic Engineering

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue SW
Washington, D.C. 20585

Project Managers: L.M. Brown (FY 1993)
and J. Sheehan (FY 1994)

Telephone: (303) 275-4420

Contractor:

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

Principal Investigators: L.M. Brown (FY 1993)
and P.G. Roessler (FY 1994)

Telephone: (303) 384-6253

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/93

Contract Funding (Source):

FY 1991: \$120,000 (DOE)
FY 1992: \$110,000 (DOE)
FY 1993: \$408,000 (DOE)

Objective:

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

Approach/Background:

To produce biodiesel from microalgae in an economically feasible manner, it will be necessary to genetically manipulate microalgal strains in a way that leads to improved oil-production characteristics. This technology has the potential to provide a considerable portion of the nation's liquid fuel needs and to help reduce the accumulation of greenhouse gases in the atmosphere.

Status/Accomplishments:

Efforts in FY 1993 were aimed at creating systems to allow stable expression of genes introduced into algal cells. Selectable marker genes are being cloned that can either complement a genetic defect in the recipient cell or confer resistance to a selective agent such as an herbicide or antibiotic. Two genes were chosen for this work: nitrate

reductase (NR) and acetolactate synthase (ALS). Partial clones of NR and ALS were isolated from the green alga *Monoraphidium minutum* during FY 1993, and these partial clones were used as probes in efforts to isolate the full-length genes. Attempts to isolate the full-length NR gene have encountered a number of difficulties, and therefore, current efforts regarding *M. minutum* are focused on the ALS gene. In addition, a fusion between a bacterial antibiotic resistance gene and algal genetic control sequences has been constructed for use as a selectable marker for diatoms. Development of these selectable marker systems will allow introduction and stable inheritance of genes of interest for the metabolic engineering of microalgae for enhanced lipid production.

Efforts were also made regarding the introduction of DNA into algae. One promising method was developed that involves agitation of cells in the presence of DNA and silicon carbide whiskers.

Major Project Reports:

Brown, L.M.; Sprague, S.; Jarvis, E.E.; Dunahay, T.G.; Roessler, P.G.; Zeiler, K.G. (May 1993). *Biodiesel/Aquatic Species Project Report: FY 1992*. NREL/TP-422-5259. Golden, CO: National Renewable Energy Laboratory.

Brown, L.M.; Sprague, S.; Jarvis, E.E.; Dunahay, T.G.; Roessler, P.G.; Zeiler, K.G. (January 1994). *Biodiesel from Aquatic Species Project Report: FY 1993*. NREL/TP-422-5726. Golden, CO: National Renewable Energy Laboratory.

Dunahay, T.G. (1993). "Transformation of *Chlamydomonas reinhardtii* with Silicon Carbide Whiskers." *Biotechniques* (15:3); pp.452-454.

Roessler, P.G.; Brown, L.M.; Dunahay, T.G.; Heacox, D.A.; Jarvis, E.E.; Schneider, J.C.; Talbot, S.G.; Zeiler, K.G. (in press). "Genetic Engineering Approaches for Enhanced Production of Biodiesel Fuel from Microalgae." In *Bio-conversion for Fuels*. Edited by M. Himmel, J. Baker, and R. Overend. Washington D.C.: American Chemical Society.

Summary Date: April 1994

Lipid Biochemistry/Metabolic Engineering

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Managers: L.M. Brown (FY 1993)
and J. Sheehan (FY 1994)

Telephone: (303) 231-7830

Contractor:

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

Principal Investigator: P.G. Roessler

Telephone: (303) 384-6253

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/93

Contract Funding (Source):

FY 1992: \$190,000 (DOE)
FY 1993: \$ 80,000 (DOE)

Objective:

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

Approach/Background:

The production of genetically engineered microalgae having enhanced biodiesel production capabilities requires a knowledge of the pathways and enzymes involved in lipid accumulation. Once they are understood, it should be possible to manipulate these pathways to produce lipids of improved quantity and quality, thus improving the overall economics of generating liquid fuels through microalgal mass culture. This technology has the potential to provide a significant source of oil that will help to meet the demand for renewable liquid fuels such as clean-burning diesel substitutes.

Status/Accomplishments:

Work continued on attempts to improve the lipid yield from microalgal mass culture through manipulation of the enzyme acetyl-CoA carboxylase (ACCase), which has been shown to catalyze a key step in the lipid biosynthetic pathway. The gene

that encodes ACCase was cloned previously from the diatom *Cyclotella cryptica* by project personnel, representing the first time that this important gene was isolated from a photosynthetic organism. During FY 1993, an expressible form of the ACCase gene was produced for use in the genetic engineering of microalgae for enhanced lipid production. This involved the removal of two intervening sequences (introns) and the construction of vectors that will allow overexpression of the ACCase gene in various hosts, including bacteria, yeast, higher plants, and eventually algae. In the long term, future metabolic engineering studies using this gene may lead to significant commercial opportunities in both microalgae and oilseed crops.

Major Project Reports:

Brown, L.M.; Sprague, S.; Jarvis, E.E.; Dunahay, T.G.; Roessler, P.G.; Zeiler, K.G. (January 1994). *Biodiesel from Aquatic Species Project Report: FY 1993*. NREL/TP-422-5726. Golden, CO: National Renewable Energy Laboratory.

Brown, L.M.; Sprague, S.; Jarvis, E.E.; Dunahay, T.G.; Roessler, P.G.; Zeiler, K.G. (May 1993). *Biodiesel/Aquatic Species Project Report: FY 1992*. NREL/TP-422-5259. Golden, CO: National Renewable Energy Laboratory.

Roessler, P.G.; Bleibaum, J.L.; Thompson, G.A.; Ohlrogge, J.B. (in press). "Characteristics of the Gene that Encodes Acetyl-CoA Carboxylase in the Diatom *Cyclotella cryptica*." *Ann. New York Acad. Sci.*

Roessler, P.G.; Brown, L.M.; Dunahay, T.G.; Heacock, D.A.; Jarvis, E.E.; Schneider, J.C.; Talbot, S.G.; Zeiler, K.G. (in press). "Genetic Engineering Approaches for Enhanced Production of Biodiesel Fuel from Microalgae." In *Bio-conversion for Fuels*. Edited by M. Himmel, J. Baker, and R. Overend. Washington D.C.: American Chemical Society.

Roessler, P.G.; Ohlrogge, J.B. (1993). "Cloning and Characterization of the Acetyl-CoA Carboxylase Gene from the Diatom *Cyclotella cryptica*." *J. Biol. Chem.* Vol. 268; pp. 19254-19259.

Schneider, J.C.; Roessler, P. (in press). "Radio-labeling Studies of Lipids and Fatty Acids in *Nannochloropsis* (Eustigmatophyceae), an Oleaginous Marine Alga." *Journal of Phycology*.

Summary Date: April 1994

Biofuels Feedstock Interface

Effects of Ambient Environment On the Storage of Switchgrass in Iowa For Biomass-to-Ethanol and Thermochemical Projects

Directing Organization:

U.S. Department of Energy (DOE)
through the

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

Project Manager: A. Wiselogel

Telephone: (303) 275-4466

Contractor:

Iowa State University
Agronomy Department
Ames, IA 50011

Principal Investigator: I. Anderson

Telephone: (515) 294-9651

Contract Number: XAC-3-13277-03

Contract Period: 06/93-12/93

Contract Funding (Source):

FY 1993: \$49,449 (DOE)

Objective:

Determine the impact of proposed storage methodology on switchgrass for the biomass-to-ethanol and thermochemical fuels research.

Approach/Background:

This subcontract is the last phase of a 3-phase study to determine storage and handling impacts on feedstock quality. The first phase involved using several different harvesting methods, the second phase focused on sources of variation in feedstock quality, and this phase will provide climatic information plus supplemental handling data.

Status/Accomplishments:

Switchgrass has been harvested. The storage phase of the research is in its fifth month and switchgrass samples from the first quarter-year of storage have been sent to NREL for analysis. Weather and bale

environmental data are being collected and analyzed.

Major Project Reports:

Anderson, I. (1993). *Effects of Ambient Environment on the Storage of Switchgrass for Biomass-to-Ethanol and Thermochemical Projects QA/QC Plan*. Golden, CO: National Renewable Energy Laboratory. Work performed by Iowa State University, Ames, IA.

Summary Date: April 1994

Effects of Ambient Environment On the Storage of Switchgrass in Kentucky For Biomass-to-Ethanol and Thermochemical Projects

Directing Organization:

U.S. Department of Energy (DOE)
through the

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

Project Manager: A. Wiseloglel

Telephone: (303) 275-4466

Contractor:

University of Kentucky
College of Agriculture
Lexington, KY 40546-0091

Principal Investigator: M. Collins

Telephone: (606) 257-7310

Contract Number: XAC-3-13277-02

Contract Period: 06/93-12/93

Contract Funding (Source):

FY 1993: \$45,000 (DOE)

Objective:

Determine the impact of proposed storage methodology on switchgrass for the biomass-to-ethanol and thermochemical fuels research. Determine impacts of plant structure on drying rates.

Approach/Background:

This subcontract is the last phase of a 3-phase study to determine storage and handling impacts on feedstock quality. The first phase involved using several different harvesting methods, the second phase focused on sources of variation in feedstock quality, and this phase will provide climatic information plus supplemental handling data.

Status/Accomplishments:

Switchgrass has been harvested and analysis of environmental and cultural impacts on drying rates have been completed. The storage phase of the research is in its fifth month and switchgrass

samples from the first quarter-year of storage have been sent to NREL for analysis. Weather and bale environmental data are being collected and analyzed.

Major Project Reports:

Collins, M. (1993). *Effects of Ambient Environment on the Storage of Switchgrass for Biomass-to-Ethanol and Thermochemical Projects QA/QC Plan*. Golden, CO: National Renewable Energy Laboratory. Work performed by University of Kentucky College of Agriculture, Lexington, KY.

Summary Date: April 1994

Effects of Ambient Environment On the Storage of Switchgrass in Texas For Biomass-to-Ethanol and Thermochemical Projects

Directing Organization:

U.S. Department of Energy (DOE)
through the

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

Project Manager: A. Wiseloglel

Telephone: (303) 275-4466

Contractor:

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

Principal Investigator: M. Sanderson

Telephone: (817) 968-4144

Contract Number: XAC-3-13277-01

Contract Period: 06/93-12/93

Contract Funding (Source):

FY 1993: \$67,809 (DOE)

Objective:

Determine the impact of proposed storage methodology on switchgrass for the biomass-to-ethanol and thermochemical fuels research. Determine biomass loss due to harvesting and baling. Determine amount and constituents of runoff water from bales. Develop an energy balance. Study the use of near infrared spectrophotometry (NIRS) as a possible rapid-analysis technique to measure feedstock quality.

Approach/Background:

This subcontract is the last phase of a 3-phase study to determine storage and handling impacts on feedstock quality. The first phase involved using several different harvesting methods, the second phase focused on sources of variation in feedstock quality, and this phase will provide climatic information plus supplemental handling data.

Status/Accomplishments:

Switchgrass has been harvested and analysis of harvesting and transportation losses have been completed. The storage phase of the research is in its fifth month and switchgrass samples from the first quarter-year of storage have been sent to NREL for analysis. Weather and bale environmental data are being collected, as well as water runoff samples. The runoff samples are being analyzed for oxygen content and potential pollutants.

Major Project Reports:

Sanderson, M. A. (1993). *Effects of Ambient Environment on the Storage of Switchgrass for Biomass-to-Ethanol and Thermochemical Projects QA/QC Plan*. Golden, CO: National Renewable Energy Laboratory. Work performed by Texas A&M University Research Foundation, College Station, TX.

Summary Date: April 1994

Effects of Ambient Environment On the Storage of Switchgrass in Virginia For Biomass-to-Ethanol and Thermochemical Projects

Directing Organization:

U.S. Department of Energy (DOE)
through the

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

Project Manager: A. Wiselogle

Telephone: (303) 275-4466

Contractor:

Virginia Polytechnic Institute and State University
Department of Agricultural Engineering
Blacksburg, VA 24061-0303

Principal Investigators: J. Cundiff and L. Marsh

Telephone: (703) 231-7603

Contract Number: XAC-3-13277-04

Contract Period: 06/93-12/93

Contract Funding (Source):

FY 1993: \$34,289 (DOE)

Objective:

Determine the impact of proposed storage methodology on switchgrass for the biomass-to-ethanol and thermochemical fuels research. Determine impacts of rain damage on feedstock quality. Determine biomass loss due to harvesting and baling.

Approach/Background:

This subcontract is the last phase of a 3-phase study to determine storage and handling impacts on feedstock quality. The first phase involved using several different harvesting methods, the second phase focused on sources of variation in feedstock quality, and this phase will provide climatic information plus supplemental handling data.

Status/Accomplishments:

Switchgrass has been harvested and analysis of rain damage have been completed. The storage phase of the research is in its fifth month and switchgrass

samples from the first quarter-year of storage have been sent to NREL for analysis. Weather and bale environmental data are being collected and analyzed.

Major Project Reports:

Cundiff, J.; Marsh, L. (1993). *Effects of Ambient Environment on the Storage of Switchgrass for Biomass to Ethanol and Thermochemical Projects QA/QC Plan*. Golden, CO: National Renewable Energy Laboratory. Work performed by Virginia Polytechnic Institute and State University, Blacksburg, VA.

Summary Date: April 1994

Effects of Storage on Eucalyptus

Directing Organization:

U.S. Department of Energy (DOE)
through the

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

Project Manager: A. Wiselogel

Telephone: (303) 275-4466

Contractor:

Bioenergy Development Corporation
888 Kalanianoale Avenue
Hilo, HI 96720

Principal Investigator: T. Crabb

Telephone: (808) 961-0411

Contract Number: AA-2-11210

Contract Period: 01/93-05/93

Contract Funding (Source):

FY 1993: \$15,784 (DOE)

Objective:

Determine the impact of proposed storage methodology on eucalyptus for the biomass-to-ethanol and thermochemical fuels research.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time. The length of storage will be affected by the size of the conversion facility, its location, the number of feedstocks that it will use, and the production characteristics of the feedstocks. During storage, various feedstock may undergo both mass and compositional changes that impact the yield of the fuel produced. These changes, as a function of season, harvest method, and storage time, are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, as well as for testing of the pretreatability and suitability of the substrates for conversion to ethanol or thermochemical-derived fuels.

Status/Accomplishments:

The storage phase was completed on schedule and all reports, biomass samples, and data have been received.

Major Project Reports:

Crabb, T; Schubert, T. (1992). *Final Report: Effects of Storage on Eucalyptus*. Golden, CO: National Renewable Energy Laboratory. Work performed by Bioenergy Development Corporation, Hilo, HI.

Summary Date: April 1994

Effects of Storage on Hybrid Poplar (DN34)

Directing Organization:

U.S. Department of Energy (DOE)
through the

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

Project Manager: A. Wiselogle

Telephone: (303) 275-4466

Contractor:

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: 01/93-06/93

Contract Funding (Source):

FY 1993: \$24,288 (DOE)

Objective:

Determine the impact of proposed storage methodology on hybrid poplar for the biomass-to-ethanol and thermochemical fuels research.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time. The length of storage will be affected by the size of the conversion facility, its location, the number of feedstocks that it will use, and the production characteristics of the feedstocks. During storage, various feedstock may undergo both mass and compositional changes that impact the yield of the fuel produced. These changes, as a function of season, harvest method and storage time, are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, as well as for testing of the pretreatability and suitability of the substrates for conversion to ethanol or thermochemical-derived fuels.

Status/Accomplishments:

Completed storage phase on schedule and all reports, biomass samples, and data have been received.

Major Project Reports:

Johnson, W.; Wene, E.; Hanson, E. (1992). *Final Report: Effects of Storage on Hybrid Poplar*. Golden, CO: National Renewable Energy Laboratory. Work performed by the University of Minnesota, Minneapolis, MN.

Summary Date: April 1994

Effects of Storage on Switchgrass

Directing Organization:

U.S. Department of Energy (DOE)
through the

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

Project Manager: A. Wiselogle

Telephone: (303) 275-4466

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: 01/93-06/93

Contract Funding (Source):

FY 1991: \$14,526 (DOE)

FY 1992: \$ 5,000 (DOE)

Objective:

Determine the impact of proposed storage methodology on switchgrass for the biomass-to-ethanol and thermochemical fuels research.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time. The length of storage will be affected by the size of the conversion facility, its location, the number of feedstocks that it will use, and the production characteristics of the feedstocks. During storage, various feedstock may undergo both mass and compositional changes that impact the yield of the fuel produced. These changes, as a function of season, harvest method and storage time, are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, as well as for testing of the pretreatability and suitability of the substrates for conversion to ethanol or thermochemical-derived fuels.

Status/Accomplishments:

The storage phase was completed on schedule and all reports, biomass samples, and data have been received.

Major Project Reports:

Sanderson M. A.; Hussey M. A.; Wiselogle A. E. (1992). "Potential of Sustainable Biomass Production Systems in Texas." *Liquid Fuels from Renewable Resources*. ASAE. December.

Sanderson M. A. (1992). *Final Report: Effects of Storage on Alamo Switchgrass*. Golden, CO: National Renewable Energy Laboratory. Work performed by Texas A&M University Research Foundation, College Station, TX.

Summary Date: April 1994

Effects of Storage on Sericea Lespedeza

Directing Organization:

U.S. Department of Energy (DOE)
through the

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

Project Manager: A. Wiselogel

Telephone: (303) 275-4466

Contractor:

David Bransby
Department of Agronomy and Soils
Auburn University, AL 36849

Principal Investigator: D. Bransby

Telephone: (205) 844-3935

Contract Number: HW-1-11176-1

Contract Period: 01/93-03/93

Contract Funding (Source):

FY 1993: \$11,619 (DOE)
FY 1992: \$ 1,948 (DOE)

Objective:

Determine the impact of proposed storage methodology on sericea lespedeza for the biomass-to-ethanol and thermochemical fuels research.

Approach/Background:

Because biomass production is seasonal and regional, there will be a need to store feedstocks for a certain length of time. The length of storage will be affected by the size of the conversion facility, its location, the number of feedstocks that it will use, and the production characteristics of the feedstocks. During storage, various feedstock may undergo both mass and compositional changes that impact the yield of the fuel produced. These changes, as a function of season, harvest method and storage time, are being quantitatively assessed. Samples from these tests are being submitted to NREL for analysis, as well as for testing of the pretreatability and suitability of the substrates for conversion to ethanol or thermo-chemical-derived fuels.

Status/Accomplishments:

The storage phase was completed on schedule and all reports, biomass samples, and data have been received.

Major Project Reports:

Bransby, D. (1992). *Final Report: Effects of Storage on Sericea Lespedeza*. Golden, CO: National Renewable Energy Laboratory. Work performed by Auburn University, Department of Agronomy and Soils, Auburn University, AL.

Summary Date: April 1994

Evaluation of Feedstock Pretreatability And Saccharification/Fermentation

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: A. Wiselogle

Telephone: (303) 275-4466

Contractor:

Colorado State University
University Services Center
Fort Collins, CO 80523-36849

Principal Investigators: J. Linden and
H. Schroeder

Telephone: (303) 491-6122

Contract Number: XC-1-11142-1

Contract Period: 01/93-12/93

Contract Funding (Source):

FY 1991: \$189,246 (DOE)
FY 1992: \$189,216 (DOE)

Objective:

Determine the suitability of potential sources of biomass as lignocellulosic 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 under this subcontract includes corn stover storage and the use of ensilage as an enzymatic pretreatment.

Approach/Background:

NREL is evaluating a range of herbaceous and woody feedstock 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 of feedstocks; and the fermentability of feedstocks after pretreatment. Ensilage, which involves the use

of enzymes during the storage of silage to enhance fermentability, has been studied using cellulase enzymes.

Because corn stover production is seasonal, there will be a need to store feedstocks for a certain length of time. The length of storage will be affected by the size of the conversion facility, its location, the number of other feedstocks used, and the production characteristics of corn stover. During storage, corn stover may undergo both mass and compositional changes that impact the yield of the fuel produced. These changes, as a function of season, harvest method, and storage time, are being quantitatively assessed.

Status/Accomplishments:

The corn stover storage phase was completed on schedule and all reports, biomass samples, and data have been received.

Because of concerns about data quality, the pretreatment and simultaneous saccharification and fermentation (SSF) analysis has been stopped until the problems are corrected.

Major Project Reports:

Linden, J.; Schroeder, H. (1992). *Annual Report: Evaluation of Feedstock Pretreatability and Saccharification/Fermentation*. Golden, CO: National Renewable Energy Laboratory. Work performed by Colorado State University.

Lipovitch, M.L. (1993). *The Stability and Pretreatability of Corn Stover as a Substrate for Simultaneous Saccharification and Fermentation*. Masters Thesis. Fort Collins, CO: Colorado State University.

Summary Date: April 1994

Feedstock Assessment

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: A. Wiselogel

Telephone: (303) 275-4466

Contractor:

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

Principal Investigators: D.K. Johnson and
S. Deutch

Telephone: (303) 231-7633

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/93

Contract Funding (Source):

FY 1993: \$427,407 (DOE)

Objective:

Determine the importance of different sources of plant variability (genetic, environmental, physiology, and storage) on the composition of biomass feedstocks.

Approach/Background:

This project studied compositional variability in biomass feedstocks that are relevant to the biofuels program. In addition to measuring the compositions of different species, this work also assessed the compositional differences between clones and varieties of the same species, as well as compositional variation due to the conditions and climate under which the biomass was grown, harvested and stored. The goal of this work was to obtain summative analyses. Data will be correlated with data from biochemical and thermochemical conversion processes and will be used to generate models that predict the effect of external parameters, such as growth and storage conditions, on biomass quality. This will improve our ability to quantitatively assess the cost of feedstock production, leading to more-accurate assessments of the overall cost of biofuels production.

Status/Accomplishments:

Compositional data have been collected on four woody and six herbaceous species, four of which were harvested at different times. These feedstocks were also part of a storage experiment that has generated data on the change in composition of feedstocks subjected to unprotected storage for 26 to 52 weeks. Identified losses from the stored feedstocks were quite small, particularly for the structural components. The largest change observed across all species was loss of extractable materials (often more than 50%). The loss of extractives is an important issue for the use of these feedstocks for production of ethanol, particularly for herbaceous feedstocks where the extractives can contain a large fraction of fermentable non-structural carbohydrate. All hardwoods were quite similar in composition, and changes in composition were small. In contrast, the composition of herbaceous feedstocks varied substantially between harvests and species, and the effects of storage on composition were generally larger and more species-dependent. Descriptive modeling has been used to obtain a "global" view of the total biomass data base and for summarizing the multi-variable information collected. Predictive modeling has been used to demonstrate how the various measurements may be used to predict the yields of fuels obtained from thermochemical and biochemical conversion of the feedstocks.

Major Project Reports:

Johnson, D.K.; Adam, P.; Ashley, P.; Chum, H.; Deutch, S.; Fennell, J.; Wiselogel, A. (1993). *Study of Compositional Changes in Biomass Feedstocks upon Storage (Results)*. Golden, CO: National Renewable Energy Laboratory.

Meglen, R.; Johnson, D.K.; Deutch, S.; Agblevor, F.; Fennell, J.; Ashley, P.; Davis M.; Schroeder, H.; Linden J. (1993). *Milestone Report: Analytical Composition Model*. Golden, CO: National Renewable Energy Laboratory.

Summary Date: April 1994

Feedstock Interface

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: A. Wiselogel

Telephone: (303) 275-4466

Contractor:

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

Principal Investigator: A. Wiselogel

Telephone: (303) 275-4466

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92–09/93

Contract Funding (Source):

FY 1993: \$23,986 (DOE)

Objective:

Facilitate the interface between DOE-sponsored biomass feedstock development and conversion research efforts.

Approach/Background:

A biomass feedstock supply service has been developed to provide NREL conversion research efforts with well-defined biomass feedstocks. A network of reliable feedstock producers and suppliers has been established for obtaining samples of feedstock materials ranging in size from 1 kg to 300 tons. Information on the samples is maintained by the project leader. Dry- and freezer-storage facilities have been developed over the past 2 years and continues to expand as the direction of the NREL research effort expands and changes.

Activities to improve the working relationship with the Biofuels Feedstock Development Program at Oak Ridge National Laboratory (ORNL) have been outlined and initiated. By facilitating collaboration between the Biofuels Program at NREL and the Biofuels Feedstock Development Program at ORNL, overall improvements in the direction of the research efforts of both programs will be improved.

Developing formalized communications, collaboration on programmatic issues, and joint research efforts are all being used to promote a more coordinated research effort.

Status/Accomplishments:

The major users of the biomass feedstock supplying service have been the Alternate Pretreatment Subcontractors, NREL Chemical Analysis Testing Task, PDU vendor testing, assorted NREL thermochemical research efforts, and NREL pretreatment and fermentation researchers. Approximately 400 tons of biomass feedstocks were purchased and processed this year. Currently, 7 tons of switchgrass, 6 tons of corn stover, 5 tons of aspen, 2 tons of hybrid poplar, and 1 ton of paper have been obtained in anticipation of FY 1994 research activities.

During FY 1993, the Terrestrial Biomass Feedstock Interface Project and the Biofuels Feedstock Development Program have had representatives at their respective external reviews. This has given each group the opportunity to provide input to the other's operational and long-range planning efforts. Both have been involved in the development of the DOE Biofuels Systems Division strategic planning efforts. Technical interactions include completion of a Terrestrial Biomass Feedstock Interface Project subcontract on sycamore storage by ORNL, presentations by both at the other's reviews and work-shops, having the same contact at the DOE Biofuels Systems Division level, and developing a feedstock production modeling subcontract for FY 1994.

Major Project Reports: None

Summary Date: April 1994

Innovative Research for the Harvesting, Handling, Processing, and Storage of Sorghum for Use as a Cellulosic Feedstock for Conversion to Ethanol By Simultaneous Saccharification and Fermentation

Directing Organization:

U.S. Department of Energy (DOE)
through the
National Renewable Energy Laboratory (NREL)
1617 Cole Boulevard
Golden, CO 80401-3393

Project Manager: A. Wiseloge

Telephone: (303) 275-4466

Contractor:

Iowa State University
Agronomy Department
Ames, IA 50011

Principal Investigator: I. Anderson

Telephone: (515) 294-9651

Contract Number: XAC-3-13279-01

Contract Period: 06/93-12/93

Contract Funding (Source):

FY 1993: \$35,149 (DOE)

Objective:

Determine the impact of proposed storage methodology on sorghum for the biomass-to-ethanol research.

Approach/Background:

Sorghum produces more carbohydrates and biomass per unit area of land than most crops. However, the short harvest time and high moisture and sugar content of sorghum has resulted in the practice of storing sorghum as silage. Silage has several drawbacks as a suitable method of storage: anaerobic conditions need to be maintained; more than 70% of the weight is water; effluent disposal; and loss of carbohydrates. To make sorghum a more attractive carbohydrate and biomass feedstock for conversion to ethanol, alternative harvesting, handling, processing, and storage systems need to be developed. An experiment designed to analyze the feasibility of dry storage of sorghum and to minimize sugar loss will

provide some answers to the future feasibility of sorghum as an energy crop.

Status/Accomplishments:

Sorghum has been harvested using two different methods. The sorghum is being stored as piles of stocks and bales of "press cake." Sorghum and sorghum extract samples have been sent to NREL for analysis. Weather and storage environment variables are being measured and analyzed.

Major Project Reports:

Anderson, I. (1993). *Innovative Research for the Harvesting, Handling, Processing, and Storage of Sorghum for Use as a Cellulosic Feedstock for Conversion to Ethanol by Simultaneous Saccharification and Fermentation QA/QC Plan*. Golden, CO: National Renewable Energy Laboratory. Work performed by Iowa State University Agronomy Department, Ames, IA.

Summary Date: April 1994

Market Analysis on Near-Term Sources Of Biomass Feedstocks

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: A. Wiselogel

Telephone: (303) 275-4466

Contractor:

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

Principal Investigator: S. Tyson

Telephone: (303) 275-4466

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/93

Contract Funding (Source):

FY 1993: \$21,720 (DOE)

Objective:

Provide a market analysis (distribution, availability, and cost) for near-term biomass feedstocks identified by the Ethanol, Ethers, and Syngas Project leaders.

Approach/Background:

Develop a list of waste resources in conjunction with biofuel program managers and biofuel scientists. Prioritize the list based on near-term analytic needs of biofuels programs. Collect, evaluate, and summarize existing resource-assessment information describing wastes quantities, qualities, and values. Develop estimates for waste resources for the following sources: paper and pulp mills, crop residues, and mixed waste. Construct a data base of waste resources to determine favorable locations for near-term biofuel facilities. Identify information to assist strategic planning and cooperative research and development agreement (CRADA) partners with development of biofuels. Develop projections for future waste availability given competition within the energy markets and other markets for waste resources. Provide a report or a series of issue

papers describing opportunities for near-term biofuel development.

Status/Accomplishments:

Selected list of feedstocks through meetings with program leaders. Developed list of contacts made and delivered list to AMOCO. Arranged contact between AMOCO and interested municipal solid waste (MSW) power developer. Developed inventory of material collected to date. Began construction of electronic data base for resource assessment. Prepared list of ethanol facilities. Presented program design and status to DOE/EE Sector Offices and the Regional Biomass Energy Program. Developed contacts at a wide number of organizations for data collection and review. Developed draft report on supplies of paper and pulp mill residues; awaiting comments. Solicited comment on strategic plan for this project from program leaders.

Major Project Reports:

Tyson, S. (1994). "Report on Paper and Pulp Mill Residues, Preliminary Stage." Presented at Annual Meeting of Regional Biomass Energy Programs, Washington, D.C., 24-25 February.

Summary Date: April 1994

Thermochemical Conversion and Rapid Analysis

Directing Organization:

U.S. Department of Energy (DOE)
1000 Independence Avenue, SW
Washington, D.C. 20585

Project Manager: A. Wiseloge!

Telephone: (303) 275-4466

Contractor:

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

Principal Investigators: D.K. Johnson and
F. Agblevor

Telephone: (303) 231-7633

Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/93

Contract Funding (Source):

FY 1993: \$220,746 (DOE)

Objective:

Determine the importance of different sources of plant variability (genetic, environmental, physiology, and storage) on the thermochemical conversion of biomass feedstocks into fuel precursors.

Approach/Background:

Study the variability in biomass feedstocks using rapid pyrolysis. This is performed on two scales. At an analytical scale using pyrolysis/mass spectrometry (Py/MS), large numbers of biomass samples can be screened in a short time. Multivariate analysis of the mass spectrometric data is then employed to categorize the samples based on the chemical make-up of the pyrolysis products. Selected samples can then be pyrolyzed on a preparative scale in a fluidized-bed reactor, so that sufficient product can be collected for chemical characterization, to distinguish differences in the feedstocks based on their pyrolysis products.

A complementary effort is the development of rapid analysis techniques based on data-rich spectroscopic measurements, such as Py/MS, coupled with data reduction techniques such as multivariate analysis.

In addition, the establishment of standard materials and analytical methods for biomass is also being pursued.

Status/Accomplishments:

Samples from the feedstock storage study, including four woody and six herbaceous species, four of which were harvested at different times, have been examined using Py/MS coupled with multivariate analysis. Selected feedstocks were also pyrolyzed in the fluidized bed reactor. Chemical characterization of the oils showed that pyrolysis on the bench scale was very reproducible. Feedstock pyrolysis on both scales agreed that there were significant differences in the oils and chars produced from different species. However, there appeared to be little difference between the oils and chars from fresh and stored samples, when the stored material came from the major fraction of the stored biomass which had degraded to a limited extent. Analytical pyrolysis identified small fractions of more degraded material that did behave differently when pyrolyzed.

Two rapid-analysis methods have shown promise for quantifying the structural components in biomass. One method is based on the analytical pyrolysis method used in screening samples in the feedstock storage study. The other uses fourier transform infrared (FTIR) spectroscopy coupled with partial least squares (PLS) analysis. Four biomass feedstocks (a hardwood, a softwood, sugarcane bagasse, and wheat straw) were established as standards and can be obtained from the National Institute of Science and Technology (NIST).

Major Project Reports:

Agblevor, F.A.; Rejai, B.; Evans, R.J.; Johnson, K.D. (1992). "Pyrolytic Analysis and Catalytic Upgrading of Lignocellulosic Materials by Molecular-Beam Mass Spectrometry". Presented at Energy from Biomass and Wastes XVI, Orlando, FL, 2-6 March.

Milne, T.A.; Chum, H.L.; Agblevor F.; and Johnson, D.K. (1992). "Standardized Analytical Methods." *Biomass and Bioenergy*, (2:1-6); pp. 341-366.

Summary Date: April 1994

Biofuels Feedstock Development

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

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: V.R. Tolbert

Telephone: (615) 574-7377

Contractor:

National Audubon Society
950 Third Avenue
New York, NY 10022

Principal Investigators: J. Beyea, J.H. Cook,
and W. Hoffman

Telephone: (516) 224-3730

Contract Number: 1BX-SL237V

Contract Period: 06/92-05/94

Contract Funding (Source):

FY 1992: \$38,000 (DOE)
FY 1993: \$38,000 (DOE)

Objective:

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

Approach/Background:

The first effort involves field investigations of bird diversity in large short-rotation woody crop and switchgrass monocultures where access to relatively large stands may be gained from cooperators. Comparisons of bird diversity and habitat use are being made (1) between the edges and centers of larger plantings, and (2) between these plantings and surrounding habitats.

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. We are developing testable recommendations for the design and management of short-rotation tree plantations, with the goal of minimizing the environmental costs and maximizing the benefits.

Status/Accomplishments:

We have assessed bird usage of (1) short-rotation hybrid poplar plantations in Ontario, (2) short-rotation cottonwood plantations in western Oregon and Washington, and (3) switchgrass plantings in Iowa. The studies have provided some unifying principles for the ecology of energy plantations.

The perennial crops provide better bird habitat than most annual agricultural crops. However, native forest or prairie would probably be better. Young plantations had higher densities of birds whereas older plantations had more species diversity.

The habitat value appears to be enhanced by deviations from uniformity, whether contrasting habitat inclusions or weedy invasion. This implies that success at providing habitat on biomass farms will be related to variations in the monoculture.

Habitat value is also contingent on the landscape. Similar plantations may provide very different habitat values in different landscapes or in different places within a landscape.

Fall migration studies indicated that bird use of the plantations during fall was much less than in the breeding season. We suspect that the difference in use is a result of territoriality—or, rather, the lack of it. Most of the breeding songbirds defend relatively exclusive territories when nesting, but not at other seasons.

The bird censuses in Iowa switchgrass plantings established that the methods work and give useful data.

Major Project Reports:

Hoffman, W.A.; Cook, J.H.; Beyea, J. (1993). "Some Ecological Guidelines for Large-Scale Biomass Plantations." Presented at First Biomass Conference of the Americas, Burlington, VT, August 30-September 2.

Summary Date: March 1994

Biochemical and Molecular Bases Of Water Stress Tolerance of Poplar

Directing Organization:

U.S. Department of Energy (DOE)
through the
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Environmental Sciences Division
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Oak Ridge, TN 37831-6352

Project Manager: G.A. Tuskan

Telephone: (615) 576-6830

Contractor:

Oak Ridge National Laboratory (ORNL)
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Principal Investigator: T.J. Tschaplinski

Telephone: (615) 574-4597

Contract Number: N/A

Contract Period: 10/90-09/93

Contract Funding (Source):

FY 1991: \$227,000 (DOE)
FY 1992: \$227,000 (DOE)
FY 1993: \$125,000 (DOE)

Objective:

Identify biochemical/molecular traits that can be used during genetic selection to screen for water stress tolerance in a traditional breeding program or in a somaclonal selection program via tissue culture. The primary objectives of the proposed research are to use poplar (*Populus*) clones to determine genetic variability and the limits of water stress tolerance; characterize the biochemical and molecular bases of water stress tolerance; and develop biochemical and molecular screening procedures based on *in vivo* and/or *in vitro* materials to identify clones with maximum water stress tolerance.

Approach/Background:

Selected poplar clones are screened in outdoor pot trials using repeated water-stress cycles. The relative growth rate of stems under stress versus that of well-watered plants is the variable used to determine which clones maintained their growth rate

under stress. Degree of osmotic adjustment to water stress is determined and accumulating stress metabolites identified. Six clones were established in tissue culture. Osmotic stress was applied to determine whether the same stress metabolites accumulate in culture as in whole plants.

Status/Accomplishments:

The biochemical bases of water stress tolerance in a pedigree consisting of eastern cottonwood (*P. deltoides*) and black cottonwood (*P. trichocarpa*) parental clones and four hybrid progeny were investigated. Analysis of the major metabolites and ions in fully expanded leaves demonstrated that the greatest degree of osmotic adjustment was displayed by two hybrid and the eastern cottonwood parent.

Six clones of this pedigree were regenerated in tissue culture and subjected to water stress. Water stress tolerance was assessed by callus growth and solute accumulation under different stress levels. Clonal differences in relative growth rate under any of the stress levels were not related to concentrations of major carbon-based solutes. Therefore, results obtained from callus cultures growing under artificially-induced water-stress regimes do not extrapolate to those obtained from water-stress studies on whole plants.

Major Project Reports:

D'Surney, S.J.; Tschaplinski, T.J.; Edwards, N.T.; Shugart, L.R. (1993). Biological Responses of Two Soybean Cultivars Exposed to Enhanced UVB Radiation." *Environ and Exp. Bot.*; Vol. 33, pp. 1-10.

Tschaplinski, T.J.; Norby, R.J. (1993). "Physiological Indicators of Nitrogen Response in Short-Rotation Sycamore Plantations." *Can. J. Bot.*; Vol. 71, pp. 841-847.

Tschaplinski, T.J.; Norby, R.J.; Wulschleger, S.D. (1993). "Responses of Loblolly Pine Seedlings to Elevated CO₂ and Fluctuating Water Supply." *Tree Physiol.*; Vol. 13, pp. 283-296.

Summary Date: March 1994

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

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
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Oak Ridge, TN 37831-6352

Project Manager: S.B. McLaughlin

Telephone: (615) 574-7358

Contractor:

University of Tennessee
Agricultural Experiment Station
Knoxville, TN 37901-1071

Principal Investigator: B.V. Conger

Telephone: (615) 974-8833

Contract Number: 19X-SL129C

Contract Period: 07/92-06/94

Contract Funding (Source):

FY 1992: \$78,000 (DOE)

FY 1993: \$91,000 (DOE)

Objective:

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

Approach/Background:

Research efforts to improve biomass production of switchgrass through breeding, genetics, and physiology should logically include the development of systems that can be used in the potential applications of biotechnology. Most, if not all, of these applications require the regeneration of whole plants from cells or tissues cultured *in vitro*. 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:

Our efforts during 1993 were concentrated on establishment of cultures, initiation of callus, and regeneration of plants from two explant sources, mature caryopses, and young leaf tissue. Two cultivars, "Alamo" and "Cave-in-Rock" were used in our experiments. Compared to Cave-in-Rock, plants of Alamo were easier to establish and maintain in the greenhouse. *In vitro* responses were also superior from Alamo.

Murashige and Skoog (MS) medium solidified with 0.8% agar and containing 22.5 μ M 2,4-dichlorophenoxyacetic acid (2,4-D) and either 45 μ M (mature caryopses) or 5.0 μ M (young leaf explants) 6-benzylaminopurine (BAP) was used for callus initiation. After 4-week incubation at 20°C, resultant calli were transferred to MS medium without growth regulators and incubated in the light at 29°C. After another 4 weeks, shoot regeneration and somatic embryogenesis were observed.

Thousands of plantlets were regenerated during the course of the studies mentioned above. We established 1000 plants in the field on 4-ft centers during the late spring and early summer. These consist of 983 regenerants of Alamo and 27 of Cave-in-Rock. We obtained dry weights from more than 300 plants transplanted on May 18. Individual plant weights ranged from less than 0.5 lb. to more than 4 lb. Individual plants will be observed and notes will be taken on them during the 1994 growing season. Individual plants that exhibit outstanding biomass production will be used for further tissue culture studies and made available to other investigators.

Major Project Reports:

Denchev, P.D.; Conger, B.V.. (1993).

"Development of *In Vitro* Regeneration Systems for Switchgrass." *In Vitro Cell Dev. Bio.*; 29A, No. 3 Part II:63A.

Summary Date: March 1994

Early Selection Criteria and Clonal Propagation Methods For Increased Productivity of Sycamore In Short-Rotation Energy Systems

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: G.A. Tuskan

Telephone: (615) 576-8141

Contractor:

Mississippi Agricultural & Forestry
Experiment Station
Mississippi State, MS 39762

Principal Investigator: S.B. Land, Jr.

Telephone: (601) 325-2786

Contract Number: 19X-95902C

Contract Period: 04/91-09/94

Contract Funding (Source):

FY 1991: \$77,000 (DOE)
FY 1992: \$85,000 (DOE)

Objective:

Provide basic information on characteristics of American sycamore (*Platanus occidentalis* L.) reproductive materials; determine magnitudes of genetic and common-environmental (maternal) effects; estimate sizes of juvenile-"mature" genetic correlations; develop "hedging" techniques for production of rooted cuttings; determine tissue-culture techniques for production of plantings; and determine if apomixis occurs in sycamore (as an alternative to cuttings and tissue culture for asexual reproduction).

Approach/Background:

Seed and seedling characteristics should affect the performance of American sycamore in short-rotation plantations. These characteristics are subject to genetic and environmental effects that can be managed to increase productivity. The use of vegetative

propagules rather than seedlings can allow greater use of genetic improvement in productivity.

Status/Accomplishments:

Fifty-one traits on seed germination and early seedling development have been collected and correlated to determine if they can be used for quick, "juvenile" selection to genetically improve sycamore for biomass production in short-rotation energy plantations.

One-year sprouts for cuttings on 11-year-old grafts (19-year-old scions) were increased by cutting stems at mid crown. Partial girdling of the stem increased the number of epicormic sprouts. Only 2% of the cuttings from the hedged trees had rooted and were alive by 3 months after planting, whereas 73% of the cuttings from the 24-month-old seedlings had rooted.

DNA from 5 mother trees, from 131 seedlings of self-pollination origin, and from 128 seedlings of putative apomictic origin has been purified and quantified.

Measurements on 32-month-old trees (from time of seed germination) that were grown identically for 4 months and then (a) planted as 4-month-old containerized seedlings in July or (b) planted in a nursery and then outplanted as 8-month-old bare-root seedlings on the same site in November indicated that aboveground dry biomass (stem + limbs) was three times larger for (a) than (b). This difference may reflect the importance of drip irrigation, used only in the first growing season of (a).

Major Project Reports:

Tang, Z.; Land, S.B., Jr. (in press). "Genetic and Maternal Effects on First-Year Development of American Sycamore Seedlings." *Canadian J. of Forest Research*.

Tang, Z.; Land, S.B. Jr. (1993). *Leaf Area Development, Tree Growth, and Dry Weight Production of Four American Sycamore Clones*. Oak Ridge, TN: Oak Ridge National Laboratory. Work performed by the Mississippi Agricultural & Forestry Experiment Station.

Summary Date: March 1994

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

Directing Organization:

U.S. Department of Energy (DOE)
through the
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Project Manager: R.L. Graham

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Contractor:

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

Principal Investigator: B.C. English

Telephone: (615) 974-7486

Contract Number: 11B-99732C S19

Contract Period: 11/90-09/94

Contract Funding (Source):

FY 1991: \$33,000 (DOE)
FY 1992: \$33,000 (DOE)
FY 1993: \$33,000 (DOE)

Objective:

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

Approach/Background:

For each objective, a review of literature is conducted to identify a suitable analytical method and data source. Once an approach is selected for a given objective, the analysis commences. The data sources include DOE annual reports, the Fertilizer Institute's manufacturers survey data, U.S. Department of Agriculture's Marketing Service data on trucking rates, and other published reports.

Status/Accomplishments:

The weighted-average energy uses in production of nitrogen and phosphorous nutrients under 1987 technology were estimated as 51.93 and 10.29 GJ/mt, respectively. Under 1985 technology, energy requirement of potash was 2.88 GJ/mt. Energy requirements for nitrogen and potash nutrients have substantially declined during the 1979-1987 period, causing a significant reduction in agriculture's dependence on nonrenewable fossil resources.

Emphasis is currently focused on revising cost estimates for biomass harvesting operations. A methodology is being developed to evaluate the trade-offs between increasing biomass yields, the number of cuttings per year, and harvesting costs.

The cost of transporting biomass feedstocks in various regions of the United States is being developed using weekly truck-rate information for more than 25 major routes. A regression model is being developed to estimate the unit transportation cost of biomass feedstock as a function of transportation distance.

Analyses of the 35 major Iowa soils in several different conventional and biomass crop rotations are planned. Previous analysis of six Iowa farm sites indicated that although sorghum for biomass appears to be more economical, it is also more erosive than switchgrass.

Major Project Reports: None

Summary Date: March 1994

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

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
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Oak Ridge, TN 37831-6352

Project Manager: G.A. Tuskan

Telephone: (615) 576-8141

Contractor:

Southern Illinois University
Carbondale, IL 62901

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

Telephone: (618) 453-1796

Contract Number: 86X95908C

Contract Period: 12/86-06/94

Contract Funding (Source):

FY 1991: \$80,000 (DOE)
FY 1992: \$81,000 (DOE)
FY 1993: \$82,000 (DOE)

Objective:

Select and test outstanding clones of silver maple (*Acer saccharinum* L.) from different provenances collected throughout the eastern United States and southeast Canada during 1987.

Approach/Background:

This project uses 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 spring 1992. Ten blocks of 60 clones, (4 clones each from 15 provenances with

3 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 bottom-land plantations, respectively. Survival rates in Kansas and Minnesota at the end of the 1992 growing season were greater than 85%. The mulch resulted in negligible soil erosion, conserved soil moisture, and facilitated weed control.

Trees in the Illinois plantation at least doubled their heights and nearly doubled their basal caliper during the 1993 growing season. This implies that the trees had entered into the logarithmic phase of growth in the third growing season.

The Kansas site is situated on a flood plain below a reservoir dam that released excess flood water during the summer of 1993, resulting in 30-40 cm of standing water on the plantation from July 21 until August 12. The trees doubled or tripled their heights during the 1993 growing season and had greater calibers than either of the two Illinois plantations. This indicates that silver maples can thrive under flooded conditions and that the soil in Kansas is excellent.

Major Project Reports:

Ashby, W.C.; Bresnan, D.F.; Roth, P.L.; Preece, J.E.; Huetteman, C.A. (1992). "Nursery Establishment, Phenology and Growth of Silver Maple Related to Provenance." *Biomass and Bioenergy*; Vol. 3, pp. 1-7.

Huetteman, C.A.; Preece, J.E. (1993) "Thidiazuron: A Potent Cytokinin for Woody Plant Tissue Culture." *Plant Cell Tissue and Organ Culture*; Vol. 33, pp. 105-119.

Preece, J.E.; Huetteman, C.A.; Ashby, W.C.; Roth, P.L. (1993). "Clonal Propagation of Biofuel Trees with Emphasis on Silver Maple." *Comb. Proc. Intl. Plant Prop. Soc.*; Vol. 42, pp. 74-78.

Summary Date: March 1994

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

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
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Oak Ridge, TN 37831-6352

Project Manager: G.A. Tuskan

Telephone: (615) 576-8141

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. Hinkley, and
H.D. Bradshaw, Jr.

Telephone: (206) 543-2723

Contract Number: 19X43382C

Contract Period: 09/89-04/94

Contract Funding (Source):

FY 1991: \$218,000 (DOE)
FY 1992: \$222,000 (DOE)
FY 1993: \$200,000 (DOE)

Objective:

Genetically improve black cottonwood (*Populus trichocarpa*) for biomass production; define critical components of productivity and how they can be manipulated genetically and environmentally; and encourage industrial poplar culture in the Northwest.

Approach/Background:

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

Status/Accomplishments:

The hybrid material developed in the program is now being grown by forestry industry and farmland owners on more than 7000 ha. More than 2000 selected clones are under testing in 39 field trials

located between British Columbia and northern California on both sides of the Cascades.

Rapid advances were made in the molecular analysis of a 3-generation *P. trichocarpa* x *P. deltoides* hybrid pedigree. The *Populus* linkage map contains 353 RFLP, STS, and RAPD markers covering approximately 90% of the genome. The map was used to locate quantitative trait loci (QTLs) involved in volume growth, stem and branch form, spring phenology, and disease resistance. In all these traits, as few as 1-5 QTLs explain 25%-96% of all genetic variance. Several growth-controlling loci clustered with QTLs, controlling branch and leaf traits and confirming trait correlations identified in earlier physiological studies.

Nine new plantations were installed in 1993. Two will test 3-generation hybrid pedigrees in two contrasting environments in Oregon. Containing more than 8000 plants, they will expand the QTL analysis to a larger material of two related F₂ families. They will be the focus of collaborative studies, linking physiological and pathological research with molecular/genetic analysis. The other seven plantations are field trials for the selection of new hybrids for commercial production.

Physiological research in 1993 was focused on root studies. Two field studies compared *P. trichocarpa* x *P. deltoides* hybrids with the two parental species. A third study investigated the degree to which root genotype may limit shoot growth.

Major Project Reports:

Bradshaw, H.D. Jr.; Villar, M.; Watson, B.D.; Otto, K.G.; Stewart, S.; Stettler, R.F. (in review). "Molecular Genetics of Growth and Development in *Populus*." *Theo. Appl. Gen.*

Heilman, P.E.; Xie, F.-G. (in press). "Influence of Nitrogen on Growth and Productivity of Short-Rotation *Populus trichocarpa* x *P. deltoides* hybrids." *Can. J. For. Res.*

Summary Date: March 1994

Genetic Variation Among Switchgrasses For Agronomic Traits, Forage Quality, And Biomass Fuel Production

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: S.B. McLaughlin

Telephone: (615) 574-7358

Contractor:

USDA, Agricultural Research Service
Lincoln, NE 68583-0910

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

Telephone: (402) 472-1564

Contract Number: DE-AI05-900R21954

Contract Period: 09/90–09/97

Contract Funding (Source):

FY 1991: \$ 18,000 (DOE)
FY 1992: \$ 44,000 (DOE)
FY 1993: \$110,000 (DOE)

Objective:

Determine magnitude of genetic variability for agronomic and biomass fuel production traits and the stability of those traits across the midwestern states for: (1) one "raw" germ plasm collected from remnant prairie sites in the Midwest and (2) "elite" germ plasm and released cultivars and experimental strains of switchgrass.

Approach/Background:

Two separate experiments were conducted at each of three sites: Mead, Nebraska; Ames, Iowa; and West Lafayette, Indiana. In 1991 and 1992, the experiments were harvested for biomass yield. Forage samples were collected for forage quality analyses and cellulose and hemicellulose concentrations.

Status/Accomplishments:

Experiment 1: Germ plasm trials—A total of 23 accessions collected from midwestern prairies and

five check strains were evaluated in space-planted nurseries. Forage composition and *in vitro* dry-matter digestibility (IVDMD) was determined using near-infrared reflectance spectroscopy. Across locations and years, significant variation among accessions was observed for forage yield at heading, vegetative stage IVDMD, and heading date. Except for disease rating, genotype by environmental (G×E) interactions were important for all traits, indicating that switchgrass germ plasm needs to be evaluated in several environments to identify broadly adapted accessions.

Experiment 2: Elite trials—Objectives of this research were to determine genetic differences between switchgrass populations adapted to the midwestern states for agronomic, forage quality, and biofuel traits, and to determine if the relative ranking of populations varied at different locations due to environmental effects (genotype × environment interaction effects). Twenty "elite" switchgrass populations were planted and evaluated during 1991 to 1992. Despite large genotype × environment interaction effects, some populations consistently ranked high in forage and holocellulose yield. Populations also were identified that consistently ranked high in forage digestibility at all locations. Assuming a conversion efficiency of 75%, these strains have the capability to produce over 5000 L/ha (500 gal/acre) ethanol. Results also indicated multiple-location, multiple-year trials are required to develop and identify improved switchgrasses that are broadly adapted to the Midwest.

Major Project Reports:

Hopkins, A.A.; Vogel, K.P.; Moore, K.J.; Johnson, K.D.; Carlson, I.T. (submitted). "Genotype and Genotype × Environment Interaction Effects on Forage Yield of Switchgrass in the Midwestern States." *Crop Science*.
Hopkins, A.A.; Vogel, K.P.; Moore, K.J.; Johnson, K.D.; Carlson, I.T. (1993). "Genetic Differences Between Elite Switchgrass Strains for Agronomic, Biofuel, and Forage Quality Traits." *Agronomy Abstracts*; p. 90.

Summary Date: March 1994

Herbaceous Biomass Potential in the Eastern United States

Directing Organization:

U.S. Department of Energy (DOE)
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Project Manager: R.L. Graham

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Contractor:

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Knoxville, TN 37901-1071

Principal Investigator: B.C. English

Telephone: (615) 974-7486

Contract Number: 11B-99732C-S60

Contract Period: 05/91-09/94

Contract Funding (Source):

FY 1991: \$65,000 (DOE)
FY 1992: \$62,000 (DOE)
FY 1993: \$68,000 (DOE)

Objective:

Evaluate herbaceous biomass potential in the eastern United States with regard to the agricultural sector. Appraisal will include estimates on environmental impacts as well as estimates on economic feasibility on a regional basis. A second objective was added to the project this past year. A National Energy Modeling Component for ethanol is to be developed—derived first from corn, and then (pending funding) from Biomass resources.

Approach/Background:

The project employs ARIMS, the Agricultural Resource Interregional Modeling System. 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 3 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. Two sets of biomass activities were constructed and the demand for biomass was 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 sub-models for seven major crops and seven livestock categories. Incorporated into POLYSIM is an ethanol model that is capable of determining the impacts 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, as well as the agriculture sector impacts of introducing large-scale ethanol/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. POLYSIM model is up and running, with the ethanol sector in place.

Major Project Reports: None

Summary Date: March 1994

Herbaceous Biomass Crops in the Northern Great Plains

Directing Organization:

U.S. Department of Energy (DOE)
through the Oak Ridge National Laboratory
(ORNL)
Environmental Sciences Division
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Oak Ridge, TN 37831-6352

Project Manager: S.B. McLaughlin

Telephone: (615) 576-8144

Contractor:

North Dakota State University
Fargo, ND 58105-5051

Principal Investigators: D.W. Meyer,
D.O. Erickson, and R.G. Johnson

Telephone: (701) 237-8154

Contract Number: 19XSB844C

Contract Period: 04/88–03/93

Contract Funding (Source):

FY 1991: \$78,000 (DOE)
FY 1992: \$79,000 (DOE)

Objective:

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 crop alternatives in 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 three good, two marginal, and one irrigated site during 1988–1992. Maximum biomass yields were obtained with forage sorghum at Carrington (irrigated) averaging 16.9 Mg/ha⁻¹. Likewise, forage sorghum has been the highest yielding annual species on dryland averaging 16.2 Mg/ha⁻¹ at Prosper. Sorghum ×

sudan has been only slightly lower-yielding than forage sorghum at these high-moisture sites, and higher-yielding at Hettinger and Glenfield Poor, the droughty sites. Foxtail millet generally has produced less biomass than the other species but may have a place for a short-season crop. Kochia, properly managed, was highest yielding in droughty environments and often competitive under productive environments. More research is needed on kochia as a biomass species. Switchgrass produced the highest biomass yields of tested perennials at the two sites evaluated. Reed canarygrass has been the second-most productive species. Intermediate wheatgrass or mixtures with intermediate have been the most consistent biomass-producing species of cool-season perennials across years.

The economic feasibility of producing herbaceous biomass crops were evaluated for three regions of North Dakota; eastern (Cass County), central (Foster County), and western (Adams County). At a \$35/ton price, biomass crops were included in profit-maximizing farm plans in all three areas. Kochia was the biomass crop included in all regions. When kochia was excluded, sudan/sorghum (Adams), forage sorghum (Cass), and sudan/sorghum (Foster) were the most profitable biomass crops.

Major Project Reports:

Johnson, R.G.; Sell, R.S.; Meyer, D.W. (1993). *Economic Evaluation of Herbaceous Biomass Crops in the Northern Great Plains*. Oak Ridge, TN: Oak Ridge National Laboratory. Work performed by North Dakota State University.

Meyer, D.W.; Norby, W.E.; Erickson, D.O.; Johnson, R.G. (1993). *Evaluation of Herbaceous Biomass Crops in the Northern Great Plains—Annual Report-1992*. Oak Ridge, TN: Oak Ridge National Laboratory. Work performed by North Dakota State University.

Summary Date: March 1994

Increasing Yields of Poplar Energy Plantations

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
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Oak Ridge, TN 37831-6352

Project Manager: G.A. Tuskan

Telephone: (615) 576-8141

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: 05/91–07/93

Contract Funding (Source):

FY 1991: \$117,400 (DOE)
FY 1992: \$148,000 (DOE)
plus \$50K/capital equipment
FY 1993: \$121,000 (DOE)

Objective:

Improve poplar (*Populus* spp.) biofuels yield from biomass through selection based on physiology research and by improving critical plant traits through biotechnology.

Approach/Background:

Research studies performed under this interagency agreement are part of an interdisciplinary program jointly funded by the USDA Forest Service. Scientific information is being developed for increasing plantation for biofuels yields with selected studies on tree physiology, biotechnology, process modeling, and clonal screening.

Status/Accomplishments:

Breeding nurseries of selected somaclonally derived poplar clones with known tolerance to the herbicides glyphosate and oust were established at two locations in northern Wisconsin near Rhinelander

and Langlade. The materials were archived for future applications should they arise. The process for somaclonal production of herbicide-tolerant poplars was patented by station scientists.

Genetic variation among populations of balsam poplar from the Lake States region was studied using principal component analysis. Tree height, phenology, and sylleptic branching varied according to geographical origin. Populations from the south-east part of the region grew taller, produced more late-season leaves, and set bud later than populations from other parts of the region. Such patterns of variation in traits such as phenology should be considered when establishing breeding populations.

Field data from plantations of five poplar clones in Michigan, Washington, and Wisconsin were used to validate ECOPHYS, a whole-tree process model of poplar. The model predicted height growth within 1 standard deviation of the plantation means. Clonal parameters such as photosynthetic rate, specific leaf area, and budset were primary factors in predicting differences in growth. The model is currently being used to scale from the tree to the stand level.

Major Project Reports:

Haissig, B.E.; Riemenschneider, D.E. (1993). "Strategic Planning for Applying Biotechnology to Woody Plant Genetics and Breeding." *Micropropagation of Woody Plants*. Edited by M.R. Ahuja. Dordrecht, The Netherlands: Kluwer Academic Publishers; pp. 485–500.

Michler, C.H. (1993). "In Vitro Genetic Selection for Woody Plant Improvement." *Micropropagation of Woody Plants*. Edited by M.R. Ahuja. Dordrecht, The Netherlands: Kluwer Academic Publishers; pp. 443–455.

Riemenschneider, D.E.; McMahon, B.G. (1993). "Genetic Variation Among Lake States Balsam Poplars is Associated with Geographic Origin." *For. Sci.*; Vol. 39, pp. 130–136.

Summary Date: March 1994

Optimal Establishment and Cultural Practices For Switchgrass as an Energy Crop In the Southeastern United States

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: S.B. McLaughlin

Telephone: (615) 574-7358

Contractor:

Auburn University
Auburn, AL 36849-4109

Principal Investigator: D.I. Bransby

Telephone: (205) 844-3935

Contract Number: 19X-SL227C

Contract Period: 06/93-04/94

Contract Funding (Source):

FY 1992: \$72,250 (DOE)
FY 1993: \$85,000 (DOE)

Objective:

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:

All plot studies were seeded and good stands were obtained. "Alamo" continues to be the best variety at all locations in Alabama. A wide row spacing (0.82 m) gave slightly higher yields than the narrow (0.21 m) row spacing. Yield increased with N

fertilization up to 200 kg N/ha. Subsoiling and companion cropping did not increase yield. Based on five locations, sandy (Coastal Plain and Sand Mountain) soils provided lower yields than soils with a high clay content. Yields of "Alamo" switchgrass established in south-central Alabama in 1988 were 23 kg/ha. Yields of plots established in 1992 averaged 17 kg/ha in 1993.

Major Project Reports: None

Summary Date: March 1994

Productivity Research of Biomass Plantations Of Alder and Cottonwood in the Pacific Northwest

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: G.A. Tuskan

Telephone: (615) 576-8141

Contractor:

USDA Forest Service
Pacific Northwest Research Station
3625 93rd Avenue S.W.
Olympia, WA 98512

Principal Investigator: D.S. DeBell

Telephone: (206) 956-2345

Contract Number: DE-AI05-81OR20914

Contract Period: 02/90-02/94

Contract Funding (Source):

FY 1991: \$132,000 (DOE)
FY 1992: \$ 89,000 (DOE)
FY 1993: \$ 15,000 (DOE)

Objective:

Develop guidelines for crop management and identify morphological and physiological traits useful 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 most suitable for short-rotation bioenergy plantations in Oregon and Washington. Biological information is needed to provide guidelines for growing productive, cost-effective crops. Three research plantations are providing information on the effects of several short-rotation intensive culture practices. The practices include spacing, fertilizers, irrigation, and weed-control treatments.

Status/Accomplishments:

Red alder plots in Plantation I were remeasured at the end of the seventh (1992) growing season. Data from this study were combined with other data to develop and publish a density management guide for red alder.

At the end of the seventh (1992) 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. Only 20% 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, 1.5 m), has excellent growth. Results from a harvest after three growing seasons (1992) 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.

A major windstorm in January 1993 caused substantial damage in Plantations I and III. No damage occurred in plots of red alder or *Populus* hybrid clone D-01, but *Populus* hybrid clone 11-11 in Plantations I and III and clone 49-177 in Plantation III sustained heavy damage in some plots. The native *Populus* clones (7-75, 8-81, and Capitol Lake) and hybrid clone 47-174 suffered little or no damage.

Major Project Reports:

DeBell, D.S.; Clendenen, G.W.; Zasada, J.W. (1993). "Growing *Populus* Biomass: Comparison of Woodgrass Versus Wider-Spaced Short-Rotation Systems." *Biomass and BioEnergy*; Vol. 4, pp. 305-313.

DeBell, D.S.; Harrington, C.A. (1993). "Deploying Genotypes in Short-Rotation Plantations: Mixtures and Pure Cultures of Clones and Species." *Forestry Chronicle*; (69:6); pp. 705-713.

Radwan, M.A.; Tanaka, Y.; Dobkowski, A.; Fangen, W. (1992). *Production and Assessment of Red Alder Planting Stock*. USDA Forest Service Research Paper PNW-RO-450. Olympia, WA: Pacific Northwest Research Station.

Summary Date: March 1994

Productivity Research of Switchgrass (*Panicum virgatum*) As a Biofuels Crop

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: S.B. McLaughlin

Telephone: (615) 574-7358

Contractor:

Oak Ridge National Laboratory (ORNL)
P.O. Box 2008
Oak Ridge, TN 37831

Principal Investigators: S.D. Wulschleger,
T.J. Tschaplinski, and P.J. Hanson

Telephone: (615) 574-4597

Contract Number: N/A

Contract Period: 10/92–09/93

Contract Funding (Source):

FY 1991: \$ -0- (DOE)
FY 1992: \$50,000 (DOE)
FY 1993: \$90,000 (DOE)

Objective:

Support the development of switchgrass as a herbaceous energy crop by better understanding the linkages that exist between physiology and genetics in the species. Research focuses on defining the importance of genetic variation relative to carbon acquisition, patterns of carbon allocation, limited-resource utilization, and stress-tolerance in both lowland and upland ecotypes of switchgrass.

Approach/Background:

Growth-analysis techniques combined with physiological measures are used to investigate the linkages between physiology and genetics within switchgrass genotypes. Once the processes are documented, they can be incorporated into productive plant-breeding programs.

Status/Accomplishments:

Initial greenhouse studies focused on photosynthetic differences between lowland and upland ecotypes of switchgrass. These studies were expanded to the field, where we used two regional variety trials (Knoxville, Tennessee and Blacksburg, Virginia) for our experimental studies. A third field site was added (Stephenville, Texas).

Differences in photosynthesis were observed among switchgrass varieties at all sites. Although significant, these differences were not large. At all sampling times, however, these differences did indicate a higher rate of photosynthesis for lowland ecotypes. Lowland ecotypes had higher rates of photosynthesis than did upland varieties early in the season; however, photosynthetic rates of upland ecotypes later surpassed those of the lowland varieties. This transition coincided with a lack of precipitation which caused rates of photosynthesis to decline in both ecotypes. Thereafter, upland varieties had photosynthetic rates higher than those of lowland ecotypes—a pattern that was highly significant and one that continued well into the reproductive stages of crop development.

The physiological data are being correlated with information on the nuclear DNA content (i.e., ploidy) of these same switchgrass varieties.

Major Project Reports:

Wulschleger, S.D.; Sanderson, M.A. (1993). "Ecotypic Differences in the Photosynthetic Rates of Switchgrass." *Agronomy Abstracts*; p. 127.

Wulschleger, S.D. (1993). *Physiological Studies Support the Development of Switchgrass as a Herbaceous Energy Crop*. Oak Ridge, TN: Oak Ridge National Laboratory.

Summary Date: March 1994

Regional Supply of Biomass in Three Midwest Sites

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
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Project Manager: R.L. Graham

Telephone: (615) 576-7756

Contractor:

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

Principal Investigator: B.C. English

Telephone: (615) 974-7480

Contract Number: 11B-99732C-S60

Contract Period: 04/92-09/94

Contract Funding (Source):

FY 1992: \$68,000 (DOE)
FY 1993: \$68,000 (DOE)

Objective:

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 (LP) models for each of the three sites. Using the baseline LP model, incorporate biomass production and develop a regional supply curve for biomass. Provide impacts on the agricultural sector in each of the three regions along with 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 have 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. The model has been run for the Cass County region without livestock and a regional supply curve generated. Data for the other two regions have been developed. Modifications to the linear programming model that allow livestock have been made.

Major Project Reports: None

Summary Date: March 1994

Selection and Breeding Of New Switchgrass (*Panicum virgatum*) Varieties for Increased Biomass Production

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
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Project Manager: S.B. McLaughlin

Telephone: (615) 574-7358

Contractor:

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

Principal Investigator: C.M. Taliaferro

Telephone: (405) 744-6410

Contract Number: 19X-SL127C

Contract Period: 05/92-04/97

Contract Funding (Source):

FY 1992: \$78,000 (DOE)
FY 1993: \$91,000 (DOE)

Objective:

Increase biomass yield potential in four switchgrass breeding populations synthesized from germ plasms of (a) lowland tetraploid southern types, (b) lowland tetraploid northern types, (c) upland hexaploid southern types, and (d) upland hexaploid northern types.

Approach/Background:

The reproductive characteristics of switchgrasses and the inheritance of the selection trait (biomass yield) dictate use of a breeding procedure that will effect population improvement over time. Recurrent Restricted Phenotypic Selection (RRPS) is a simple but powerful means of increasing the frequency of the quantitative genes controlling biomass production. RRPS will be practiced within each of the four synthesized breeding populations

mentioned above. Germ plasm identified as having superior genetic value will be incorporated into the breeding populations as the project progresses.

Status/Accomplishments:

The initial cycle (C_0) of RRPS initiated in 1992 was completed for each of the four populations and a second cycle was initiated in 1993. Final C_1 plant selections were based on biomass yields taken in late 1993, and the selected plants are being intercrossed in the greenhouse to generate plants for the C_2 nurseries to be established in spring 1994.

Individual plants in C_0 nurseries were harvested in fall 1993 to determine correlations between biomass yield in the establishment year (1992) and the subsequent year (1993). The data suggest that maximizing growth of plants in the establishment year via early planting and maintenance of good growing conditions will improve the probability of selecting genetically superior plants in the establishment year.

A diverse switchgrass germ-plasm collection of over 100 accessions from commercial, as well as native, stand has been accumulated. Somatic chromosome numbers have been determined for about 20 accessions. Research was initiated to adapt flow cytometry techniques to switchgrass to facilitate determination of ploidy level.

Major Project Reports: None

Summary Date: March 1994

Selection and Breeding of Pest-Resistant Clones Of *Populus* for Biomass Energy Production In the North Central United States

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: G.A. Tuskan

Telephone: (615) 576-8141

Contractor:

Iowa State University
Ames, IA

Principal Investigators: R.B. Hall, E.R. Hart,
H.S. McNabb, Jr., and R.C. Schultz

Telephone: (515) 294-1453

Contract Number: 19X43391C

Contract Period: 07/89-03/94

Contract Funding (Source):

FY 1991: \$141,000 (DOE)
FY 1992: \$142,000 (DOE)
FY 1993: \$150,000 (DOE)

Objective:

Develop new clones of *Populus* for use in the production of biomass energy in the north central United States.

Approach/Background:

Emphasis is on selection and breeding for pest resistance, dry weight yield potential, and ease of propagation; and screening for pest resistance in hybrid *Populus* populations.

Status/Accomplishments:

New *P. deltoides* germ plasm was introduced by crossing 20 parents from Iowa, Minnesota, and Missouri; obtaining 27 clones from the University of Minnesota; and by cloning 289 trees representing 30 natural populations in southern Nebraska and along the Des Moines, Illinois, Minnesota, and

Missouri Rivers. Twenty-one control-pollinated *P. alba* families came from a private breeder in Minnesota. Twelve clonal advancement and screening trials were carried out in the nursery. Twenty-nine clones were distributed for field tests.

Record rainfall and flooding destroyed two field experiments and hampered use of several others.

A study was completed on the population structure of *Melampsora* spp., the causal agent of leaf rusts, on poplars in central Iowa. Three field trails were planted in Iowa, Minnesota, and Wisconsin to study variations in the *Melampsora* population across the region. Wet weather stimulated Septoria stem canker development and allowed us to develop a canker ranking scale that shows promise of differentiating levels and mechanisms of host-plant resistance. Technique development was completed for studying the protein metabolism of the *Septoria* pathogen *in vitro*.

Cottonwood leaf beetle (*Chrysomela scripta*) damage was monitored in a field plantation across two years and seven generations. Two studies were completed on the impact that simulated leaf-beetle damage has on clonal performance.

Major Project Reports:

Hart, E.R.; McNabb, H.S. Jr.; Hall, R.B. (1993). "The Role of Host Genetics in the Biological Control of Forest Insect Pests." Chapter in *Biological Control of Forest Pests in the Great Plains: Status and Needs—a Symposium*. Great Plains Agricultural Council, publication # 145, pp. 94-101.

Klopfenstein, N.B.; McNabb, H.S. Jr.; Hart, E.R.; Hall, R.B.; Hanna, R.D.; Heuchelin, S.A.; Allen, K.K.; Shi, N.-Q.; Thornburg, R.W. (1993). "Transformation of *Populus* Hybrids to Study and Improve Pest Resist." *Silvae-Genetica*, Vol. 42, pp. 86-90.

Tabor, G. M. (1993). *Population Structure of Melampsora medusae in Central Iowa*. Master's Thesis. Ames, IA: Iowa State University.

Summary Date: March 1994

Short-Rotation Forest Plantations in Brazil

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: L.L. Wright

Telephone: (615) 574-7378

Contractor:

Colorado State University
College of Natural Resources
Fort Collins, CO 80523

Principal Investigator: D.R. Betters

Telephone: (393) 491-6911

Contract Number: 19X-SM842-V

Contract Period: 04/93-03/94

Contract Funding (Source):

FY 1993: \$20,000 (DOE)

Objective:

Describe real or perceived environmental problems and benefits associated with large, short-rotation plantations in Brazil; document and describe existing and proposed environmental regulations in Brazil that affect or may affect how short-rotation plantations are managed; describe how problems, if any, are being dealt with by leading companies in terms of self-imposed changes in management approaches, externally imposed regulations, and high-priority research areas; and provide qualitative and quantitative information and facilitate data exchange on short-rotation plantation costs, yields, and effects on attributes such as biodiversity, nutrients in soils, landscape changes, and carbon storage in wood or soil.

Approach/Background:

An extensive literature review has been done. Personal contact with appropriate Brazilian company and governmental organizations by Dr. Laircio Couto, former Head of the Department of Forestry, Federal University of Vicosa

and currently Visiting Professor, Colorado State University. To meet the objectives, Dr. Couto traveled to Brazil to arrange for data exchange and to collect pertinent information.

Status/Accomplishments:

During this time period, work began on a report entitled "Short Rotation Eucalypt Plantations in Brazil: Social and Environmental Issues." Data were collected from several Brazilian companies concerning plantation costs, yields, soils, and climatic variables. These data are being entered into a data base system.

Major Project Reports:

Betters, D.R.; Wright, L.L.; Couto, L. (1992). "Short-Rotation Woody Plantations in Brazil and the United States." *Biomass and Bioenergy* 1(6): pp. 305-316.

Couto, L.; Graca, L.R.; Betters, D.R. (1993). "Energy from Wood Biomass: The Experience of the Brazilian Forest Sector." Presented at First Biomass Conference of the Americas, Burlington, VT. August 30-September 2.

Ferrell, J.; Tariff, M.L.; Couto, L.; Graca, L.R.; Betters, D.R.; Ashworth, J. (1993). "Overview of Feedstock Research in the United States, Canada and Brazil." Presented at First Biomass Conference of the Americas, Burlington, VT. August 30-September 2.

Summary Date: March 1994

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Short-Rotation, Woody Crop Trials For Energy Production in the North Central United States

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: G.A. Tuskan

Telephone: (615) 576-8141

Contractor:

USDA Forest Service
North Central Forest Experiment Station
Grand Rapids, Minnesota 55744

Principal Investigators: E.A. Hansen

Telephone: (218) 326-7109

Contract Number: DE-AI05-89OR21871

Contract Period: 03/93-03/94

Contract Funding (Source):

FY 1991: \$120,000 (DOE)
FY 1992: \$149,000 (DOE)
FY 1993: \$120,000 (DOE)

Objective:

Establish 8-ha plantations to estimate biomass yields and production costs of wood energy in the north-central United States.

Approach/Background:

In a project jointly funded by DOE, the USDA Forest Service, and the Electric Power Research Institute, information on growing high yields of some woody species, particularly *Populus*, is being tested in large commercial-sized field plantings.

Status/Accomplishments:

Weed control by disking or herbicides was done on plantations at eight sites from the eastern Dakotas into western Wisconsin. Data collection and analyses from the 1993 season were completed showing biomass production up to 10.5 Mg/ha in the 7-year-old plantations. The best 7-year-old

clone in the small test plots of the clonal trials exceeded 13.0 Mg/ha/yr. Greatest yields were on high watertable lands in east central Minnesota and on deep loess soils in western Wisconsin. Plantations in the more arid areas of western Minnesota had slower growth.

Data from 6- and 7-year-old hybrid trials were analyzed to identify the best-performing clones. Performance of individual clones tended to be uniform across the region. The six best clones of the 150 tested were released to commercial nurseries for general distribution. Soils data collected beneath older hybrid poplar plantations and adjacent cropland showed that hybrid poplar plantations sequester soil carbon in addition to the aboveground carbon stored in the biomass. Trials across the region indicate that fertilizing with nitrogen increases leaf-tissue nitrogen content, but has little or no effect on tree growth on the plantation's agricultural soils. Nitrogen fertilization may not be required more than once (if that) during a 10-year rotation of hybrid poplar.

Major Project Reports:

Hansen, E.A. (1992). *Mid-Rotation Yields of Biomass Plantations in the North Central United States*. USDA Forest Research Paper NC-309. Grand Rapids, MN: North Central Forest Experiment Station.

Hansen, E.A.; Netzer, D.A.; Tolsted, D.N. (1993). *Guidelines for Establishing Poplar Plantations in the North-Central United States*. USDA Forest Service Research Paper NC-363. Grand Rapids, MN: North Central Forest Experiment Station.

Ostry, M.; Hackett, W.; Michler, C.; Serres, R.; McCown, B. (Accepted for publication). "Influence of Regeneration Method and Tissue Source on the Frequency of Somatic Variation in *Populus* to Infection by *Septoria musiva*." *Plant Science*.

Summary Date: March 1994

Strategy for Establishment of Hybrid Willow Farms In Northeastern United States

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: G.A. Tuskan

Telephone: (615) 576-8141

Contractor:

The Research Foundation of the State University
of New York
1 Forestry Drive
Syracuse, NY 13210

Principal Investigators: E.H. White and
L.P. Abrahamson

Telephone: (315) 470-6777

Contract Number: 19X-SK934V

Contract Period: 04/93–03/94

Contract Funding (Source):

FY 1992: \$12,000 (DOE)
FY 1993: \$15,000 (DOE)

Objective:

Establish cutting orchards for nursery propagation of genetically improved willow clones for future scale-up. 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 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 plantations fit into the overall energy supply of the northeastern United States.

Status/Accomplishments:

Nursery stool beds at Orono, Ontario, grew vigorously and will be able to supply cuttings from approximately 15 genetically improved willow clones in 1995. Cutting orchards containing 16 willow clones were established in Syracuse and Tully, New York, in spring 1993. Clone-site trials were planted in spring 1993 in Massena, New York, and in Tully, New York.

Willow cutting survival in the Syracuse and Tully cutting orchards averaged 67% and 78%, respectively. Survival in clone-site trials at Massena and Tully, was measured in June and August. In June, survival averaged 93% and 80% at Massena and Tully, respectively. Fresh cuttings were planted approximately 6 weeks after initial planting at Massena and Tully to replace cuttings that failed to grow. In August, survival averaged 89% and 83% at Massena and Tully, respectively.

The Massena and Tully clone-site trials were surveyed during late August and early September 1993 for disease, insect pests, and mammal browsing damage. Disease samples were collected and identified by a pathologist. Growth of all plantings was typical for first-year willow plantings. Weed competition in the no-till Massena clone-site trial was severe in early summer but was controlled by spot-spraying with glyphosate.

Major Project Reports:

Kopp, R.F.; Bickelhaupt, D.H.; White, E.H.; Abrahamson, L.P. (1993). "Effect of Fertilization on Willow Biomass Production and Nutrient Removal in a Bioenergy System." *Agronomy Abstracts*; p. 337.

Kopp, R.F.; White, E.H.; Abrahamson, L.P.; Nowak, C.A.; Zsuffa, L.; Burns, K.F. (1993). "Willow Biomass Trials in Central New York State." *Biomass and Bioenergy* (5:2); pp. 179–187.

White, E.H.; Abrahamson, L.P.; Kopp, R.F.; Nowak, C.A. (1993). "Willow Bioenergy Plantation Research in the Northeast." Presented at First Biomass Conference of the Americas, Burlington, VT, August 30–September 2.

Summary Date: March 1994

Switchgrass Cultivars and Cultural Methods For Biomass Production in the South Central United States

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: S.B. McLaughlin

Telephone: (615) 574-7358

Contractor:

Texas A&M University
Texas Agricultural Experiment Station
Stephenville, TX 76401

Principal Investigators: M.A. Sanderson,
J.C. Read, W.R. Ocumpaugh, M.A. Hussey,
C. Tischler

Telephone: (817) 968-4144

Contract Number: 19X-SL128C

Contract Period: 05/92-04/97

Contract Funding (Source):

FY 1992: \$93,000 (DOE)
FY 1993: \$93,000 (DOE)

Objective:

Obtain data on adaptation of switchgrass (*Panicum virgatum* L.) cultivars in the south central United States; develop guidelines for optimum germination and storage of switchgrass seed; develop germ plasm with reduced seed dormancy; develop germ plasm with improved establishment; and 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 establishment of switchgrass as a biomass crop.

Status/Accomplishments:

Yield data were collected on nine switchgrass entries at six sites in Texas during 1993. Alamo switchgrass was among the highest-yielding entries at each site. Averaged for all locations, Alamo and PMT-785 were the highest-yielding entries under a single harvest system. Alamo was also the highest-yielding entry under the two-harvest system. The highest biomass yield was 16 112 kg of dry biomass per hectare obtained with Alamo in a single harvest at College Station. First-year production yields were reduced at several locations because of drought during June to August in 1993.

A simple, rapid technique for reducing post-harvest seed dormancy in small quantities of switchgrass was developed. A 10-minute sulfuric acid treatment followed by extensive water rinsing optimized germination in freshly harvested switchgrass seed. Progress toward selecting switchgrass populations for reduced post-harvest seed dormancy and altered crown node elevation has been excellent. A third cycle of selection for each trait will be completed in 1994.

Switchgrass morphological development has been measured at Stephenville and Blacksburg, Virginia, for several years. When developmental rate was plotted against day of the year, vegetative development varied considerably from year to year. However, when plotted against cumulative growing degree days (base 10°C) after burning, development followed a common line each year. This indicates that a simple biometeorological indicator (growing degree days) can be used to predict development of switchgrass.

Major Project Reports: None

Summary Date: March 1994

Variety Trials and Cultural Improvements of Switchgrass As A Biofuels Crop for the Upper Southeastern United States

Directing Organization:

U.S. Department of Energy (DOE)
through the
Oak Ridge National Laboratory (ORNL)
Environmental Sciences Division
P.O. Box 2008
Oak Ridge, TN 37831-6352

Project Manager: S.B. McLaughlin

Telephone: (615) 574-7358

Contractor:

Virginia Polytechnical Institute and State University
Blacksburg, VA 24061

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

Telephone: (703) 231-9778

Contract Number: 19X-SK098C

Contract Period: 05/92-04/97

Contract Funding (Source):

FY 1992: \$77,000 (DOE)
FY 1993: \$85,000 (DOE)

Objective:

Evaluate the productivity of switchgrass varieties adapted to the upper southeast United States and managed under two harvest regimes; refine no-till establishment practices for switchgrass that will include considerations of insecticides, planting dates, and seed quality (dormancy); and examine the over-winter losses of standing switchgrass biomass and model a biofuels cropping system.

Approach/Background:

Switchgrass has been shown to have good potential as a biofuels feedstock, but more information is needed on its establishment and management to optimize productivity regionally. Several field and laboratory studies have been undertaken to assess yield and other responses to management. The largest study is a five-state, six-variety, cutting-management, and maximum-yield trial. Other work involves studies to consistently obtain success in establishment under a variety of conditions.

Status/Accomplishments:

Virginia Tech has established a regional variety-screening study at eight sites in Kentucky, North Carolina, Tennessee, Virginia, and West Virginia. Yields from the 1992 growing season (establishment year) ranged from 0 to 8 Mg/ha. In 1993, the yields ranged up to 19 Mg/ha for the best variety at one of the sites. Average yields within a site varied from 5.6 to 14.6 Mg/ha. The two lowest-yielding sites experienced much-drier-than-average growing seasons. When averaged across sites and cutting managements, "Alamo" and "Kanlow" were somewhat more productive than the other four varieties. Two harvests (late June and early November) provided more total biomass than did a single harvest (in early November).

Laboratory studies revealed that neoteric (recently harvested) switchgrass seeds are often highly dormant. Germination levels may be 10% or less when seeds are germinated under warm, moist conditions. The dormancy may be broken by several treatments: stratification (exposure of wet seeds to 10°C for 14 to 35 days), scarification (mechanical or chemical disruption of the seed covering), after-ripening (dry storage at warm temperatures), and submersion in various aqueous solutions. Brief periods (3 to 7 days) of exposure of dry seeds to 50° to 60°C may be one of the more-feasible ways to relieve much of the dormancy.

Major Project Reports:

Parrish, D.J.; Wolf, D.D. (1993). "Establishing Switchgrass as a Biofuels Crop for the Upper Southeast." Presented at First Biomass Conference of the Americas, Burlington, VT, August 30-September 2.

Parrish, D.J.; Wolf, D.D.; Dafang, B. (1993). "Dormancy of Switchgrass Seeds." Presented at Plant Growth Regulator Society of America.

Wolf, D.D.; Parrish, D.J.; Reynolds, J.H.; Balasko, J. A.; Greene, J.T. (1993). *Annual Report: Switchgrass as a Biofuels Crop for the Upper Southeast*. Oak Ridge, TN: Oak Ridge National Laboratory. Work performed by Virginia Polytechnic Institute and State University.

Summary Date: March 1994

Technical Evaluation and Planning

Analysis and Assessment

Directing Organization:

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Principal Investigator: C.J. Wallace

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Contract Number: DE-AC04-83CH10093

Contract Period: 10/92-09/93

Contract Funding (Source):

FY 1993: \$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 being conducted in other countries, which helps the nation to leverage its research dollars. At present, the United States pays only about 10% of the total costs of this work.

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 plus the European Union. The cooperation consists of specific programs, called Tasks, in four different areas. Task VIII deals with biomass production, Task IX with biomass harvesting and supply, Task X with biomass utilization, and Task XI with municipal solid waste (MSW) 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 leads Task X. As the Operating Agent, the U.S. leads the technical program and administers the funds provided by the participants. NREL performs the Operating Agent function for Task X, effective January 1, 1993.

Status/Accomplishments:

These funds are used as follows: (1) pay 1993 U.S. contributions to the IEA/BA, (2) sponsor joint workshops, and (3) make IEA payments for Task IX, as well as participate in long-range planning exercises.

Major Project Reports: None

Summary Date: March 1994

Analysis of Highest Value Uses of Biomass-Derived Products: Technical Options Report

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Principal Investigator: R.M. Tshiteya

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Contract Number: YAS-3-13222-01-106457

Contract Period: 01/13/93–12/31/93

Contract Funding (Source):

FY 1993: \$75,000 (DOE)

Objective:

This report fills the need to understand, in greater detail, the range of cellulosic-based products that can be produced, their characteristics, and their approximate values. The major objectives are to determine the extent to which chemicals from biomass can lessen the dependency on petroleum and natural gas resources (especially imported petroleum) and to identify research and development activities that will encourage increased production of fuels and chemicals from biomass.

Approach/Background:

The document is based on primary data collection and analysis. It begins with a summary evaluation of the biomass resource base available in the United States for conversion to fuels and commodity chemicals. In a second step, an attempt is made to identify the most promising and useful fuels and chemicals that have the potential to be produced from biomass. Evaluation criteria are developed as a

potential methodology that can be applied in selecting conversion processes and slates of products. Finally, given the transportation focus of the Biofuels Program, the analysis is narrowed to the conversion processes and products with the highest potential value in transportation, especially those that can best help implementing the Clean Air Act Amendments (CAAA) of 1990.

Status/Accomplishments:

Throughout the report, state-of-the-art technical options are evaluated to clearly examine the range of end-products that can be commercially produced from biomass. The analysis also examines requirements made by the CAAA of 1990 and points out how each biomass-based product selected contributes to meet the mandates for 1995, 2000, 2005, and 2010. The final document was published in July 1993.

Major Project Reports:

Tshiteya, R.M. (1993). *Technical Options Report: Analysis of Highest-Value Uses of Biomass-Derived Products*. Golden, CO: National Renewable Energy Laboratory. Work performed by Meridian Corporation, Alexandria, VA.

Summary Date: December 1993

Analysis of the Role of ETBE In Supplying Reformulated Gasoline to Regional Markets

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Project Manager: C.J. Wallace

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Principal Investigator: J.H. Ashworth

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Contract Number: YAS-3-13222-01-106457

Contract Period: 01/13/93-12/31/93

Contract Funding (Source):

FY 1993: \$72,000 (DOE)

Objective:

This study will help NREL and DOE's Biofuels Program analyze the potential role of ethanol and ETBE in meeting the standards for reformulating both summertime and wintertime gasoline imposed by the Clean Air Act Amendments (CAAA) of 1990.

Approach/Background:

For the analysis, further development of the REFORMGAS Model included the creation of modules for Petroleum Administration for Defense Districts (PADDs) 2 and 3, the addition of EPA's "complex" model gasoline standards (REFORMGAS already uses EPA's "simple" model), options for refinery improvements, and the production of amyl ethers. The key overall trends in gasoline refining (1994-2000) are addressed for summer and winter gasolines. The role of ethanol-based ethers (butyl and amyl) in helping refiners is examined and recommendations are made for future analysis and model development.

Status/Accomplishments:

This work will enable the agencies to estimate the national market for ETBE and other oxygenates, and to determine regional production of oxygenates to meet these national requirements. Regional modules are developed for the REFORMGAS Model for PADDs 2 and 3, which currently serve as suppliers of finished gasoline and other petroleum products for PADDs 1 and 5. These new modules are then linked to the existing modules for PADDs 1 and 5. Further detail is being added to the models so that they can differentiate between foreign sources (e.g., Venezuela) and movements from other PADDs. The "import" and "export" links between the various PADDs are being made more explicit and detailed. Distinguishing between internal regional trade and foreign shipments will be important because of the large investment that key foreign suppliers are currently making to export finished oil products to the United States in the next decade. In addition, the potential fate of smaller refiners is being examined under the new ground rules established by the CAAA of 1990. There has been much concern about the possible closure of many small refineries, especially in PADD 3 (the Gulf Coast). Possible configurations are being evaluated, using ETBE and other oxygenates, that will allow small refiners to continue operating as producers of final products. This report was published in November 1993.

Major Project Reports:

Ashworth, J.H., et al. (1993). *Analysis of the Role of ETBE in Supplying Reformulated Gasoline to Regional Markets*. Golden, CO: National Renewable Energy Laboratory. Work performed by Meridian Corporation, Alexandria, VA.

Summary Date: December 1993

Biomass-to-Ethanol Conversion Technology Characterization

Directing Organization:

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Project Manager: C.J. Wallace

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Contractor:

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Principal Investigator: J.W. Onstad

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Contract Number: YS-2-12079-1

Contract Period: 01/13/93–12/31/93

Contract Funding (Source):

FY 1993: \$25,000 (DOE)

Objective:

Characterize the biomass-to-ethanol conversion process from 1990 to 2030, based on a number of significant parameters.

Approach/Background:

This characterization is based on a format developed by NREL for DOE/EE's Office of Planning and Analysis and additional DOE guidance. Because the analysis uses four biomass feedstock classes for the biomass-to-ethanol conversion process, a multi-spreadsheet model was developed to undertake this characterization.

Status/Accomplishments:

For the biomass-to-ethanol conversion, all process inputs and outputs, systems descriptions, and significant R&D achievements were identified. The characterization required a representation of the technology and detailed estimates on energy requirements, emissions, equipment and labor costs, and expected end-product markets in a consistent

manner from 1990 to 2030. The biomass-to-ethanol conversion process is expected to use four biomass feedstock classes (agricultural residues, municipal solid wastes, herbaceous energy crops, and short-rotation woody crops) during this timeframe. As a result, data and characterizations were developed for each biomass class. This analysis was published in April 1993.

Major Project Reports:

Onstad, J.W., et al. (1993). *Biomass-to-Ethanol Conversion Technology Characterization*. Golden, CO: National Renewable Energy Laboratory. Work performed by Meridian Corporation, Alexandria, VA.

Summary Date: December 1993

Economic Evaluation of Biomass-Based Fuels And Chemicals for Transportation

Directing Organization:

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Contract Number: YAS-3-13222-01-106457

Contract Period: 01/13/93-12/31/93

Contract Funding (Source):

FY 1993: \$70,000 (DOE)

Objective:

This study focuses on the cost-competitiveness of biomass-derived fuels and chemicals compared to the same fuels and chemicals from conventional sources that have the potential of being used for the implementation of oxygenates mandates and/or for helping to meet the standards imposed for reformulated gasoline in both Phases I and II of the Clean Air Act Amendments (CAAA) of 1990.

Approach/Background:

The effort was researched using primary data collection and analysis. It begins with a review of the CAAA of 1990 and their impacts on transportation fuels and chemicals. A look is also given to the demand for new transportation fuels and chemicals, as well as oxygenate supply, production costs, and prices. Finally, the REFORMGAS Model is employed to determine the economic value of ethers and gasoline additives under the CAAA of 1990.

Status/Accomplishments:

The document provides an economic evaluation of biomass-derived fuels and chemicals that can be used in the transportation sector to meet the requirements of the CAAA of 1990. The analysis concentrates on the fuels and chemicals that have the potential of being used for the implementation of any oxygenates mandates and/or for helping to meet the standards imposed for reformulated gasoline in both Phases I and II. The impact of the availability and price of the feedstock needed by each technology to produce the fuel or chemical is addressed. Special attention is devoted to the detailed cost of production. Cost estimates for biomass-derived fuels and chemicals are presented for 1995 and 2000. The REFORMGAS Model is used to identify the demand for ethers and other oxygenates, and to determine whether the future demand for these products could justify significant investments in their production. The model computed the shadow prices of the most-promising oxygenates. The shadow price, which represents the change in gasoline prices that would take place at a point in time if less of the commodity were available, was used by REFORMGAS to calculate the netback value for the alcohol feed, the olefin feed, and the process equipment. This report was published in September 1993.

Major Project Reports:

Hertzmark, D.I., et al. (1993). *Economic Evaluation of Biomass-Based Fuels and Chemicals for Transportation*. Golden, CO: National Renewable Energy Laboratory. Work performed by Meridian Corporation, Alexandria, VA.

Summary Date: December 1993

The Energy Policy Act (EPACT) of 1992— Impacts for the Biofuels Systems Division

Directing Organization:

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Contract Number: YS-2-12079-1

Contract Period: 01/01/92–01/31/93

Contract Funding (Source):

FY 1993: \$10,000 (DOE)

Objective:

This work examines both the direct and indirect impacts that the Energy Policy Act (EPACT) of 1992 will have on the U.S. Department of Energy's (DOE's) Biofuels Systems Division (BSD).

Approach/Background:

A brief summary is given of all EPACT titles and sections that affect BSD in various degrees. The analysis of each title includes any due dates and appropriations/authorizations mentioned in EPACT. In addition, each section of the analysis includes recommendations and observations regarding possible response activities for BSD.

Status/Accomplishments:

Out of the 30 titles within EPACT, nine either directly influence the Biofuels Program or provide significant potential for increasing biofuels-related activities and thus, giving the Biofuels Program access to additional funds. The biofuels that qualify as either "alternative fuels" or "clean fuels" under

the terms of EPACT are biomass-based ethanol, methanol, and diesel fuels. It was suggested that BSD become highly involved with other parts of DOE in the planning stages of several of these programs before many of the multi-year program plans are due to Congress (April 1993). Playing such an integral role in other EPACT-mandated programs could potentially allow the BSD to leverage funds available to other DOE programs and increase the technological and commercial research undertaken on biofuels technologies. This report was published in January 1993.

Major Project Reports:

Onstad, J.W., et al. (1993). *The Energy Policy Act (EPACT) of 1992—Impacts for the Biofuels Systems Division*. Golden, CO: National Renewable Energy Laboratory. Work performed by Meridian Corporation, Alexandria, VA.

Summary Date: December 1993

Regional Biomass Energy Program: Review Technical Projects

Directing Organization:

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Project Manager: C.J. Wallace

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Contractor:

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Principal Investigator: P. Lusk

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Contract Number: CAE-3-13383-01

Contract Period: 07/93–02/94

Contract Funding (Source):

FY 1993: \$6,700 (DOE)

Objective:

Provide greater continuity and integration of the Regional Biomass Energy Program (RBEP) to the broader objectives of DOE's Office of National Programs, and also, to lend greater visibility to the accomplishments of the RBEP.

Approach/Background:

The RBEP is a federally supported program located in five regions of the continental United States with the specific goal to increase the production and use of biomass energy resources. The RBEP objectives are to (1) improve state and local government and industry capabilities and effectiveness in the production and use of biomass energy resources; (2) support planning efforts, particularly with regard to assessments of current and future resource availability, utilization, and applied research needs; (3) encourage economic development through public and private investment in biomass energy technologies; (4) perform applied research and engage in projects to demonstrate on a cost-shared basis the application of biomass energy technologies, reduce

or eliminate market barriers, understand economic and environmental costs and risks, and accelerate market acceptance of biomass energy technologies. The RBEP carries out activities related to technology transfer, industry support, and matching local resources to conversion technologies. Its major focus is the transfer of current, reliable information to potential biomass users with emphasis placed on technologies best-suited to near-term use and includes economic, as well as technical information.

Status/Accomplishments:

The *Review Report of the RBEP Technical Projects* provides a narrative of 15 of the technical projects being directly conducted by the five regional programs. For its projects, each RBEP seeks active cooperation and cost-sharing from the participating states, private industry, universities, and other federal agencies.

One section provided a narrative of special focus issues on convergent RBEP efforts in the areas of assessing the economic impacts of biomass energy use and their initiatives in alternative liquid fuels produced from biomass. Because there are 75 listings in the current RBEP technical project portfolio, the report did not have the opportunity to include many vital projects in the areas of stakeholder relationships, technology transfer, infrastructure development and industry support, or technology development and demonstration.

Moreover, it did not include any activities conducted under the state grants component of the regional programs, which provides states with an opportunity to strengthen and integrate the work of energy, forestry, air quality, and other relevant offices in promoting biomass energy use. For some regions, the state grant component is the primary method for conducting development and demonstration projects.

Major Project Reports:

Lusk, P. (1994). *Review Report of the Regional Biomass Energy Program Technical Projects*. Golden, CO: National Renewable Energy Laboratory. Work performed by Resource Development Associates, Washington, D.C.

Summary Date: March 1994

Regional Biomass Energy Program: Review and Categorize Resource Assessment And Information System Needs

Directing Organization:

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Principal Investigator: P. Lusk

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Contract Number: CAE-3-13383-01

Contract Period: 07/93-02/94

Contract Funding (Source):

FY 1993: \$13,400 (DOE)

Objective:

Provide greater continuity and integration of the Regional Biomass Energy Program (RBEP) to the broader objectives of DOE's Office of National Programs, and also, to lend greater visibility to the accomplishments of the RBEP.

Approach/Background:

The RBEP is a federally supported program located in five regions of the continental United States with the goal to increase the production and use of biomass energy resources.

Status/Accomplishments:

A second project initiative was to review and categorize the regional biomass energy program's resource assessment and information system needs. This report comprises three major subsections: (1) evaluation of the current RBEP resource assessment and information system needs; (2) scoping interviews with biomass users in RBEP regions to

determine market-based needs for resource assessment and information system activities; (3) evaluation of the potential for geographic information system (GIS) technology to meet RBEP and biomass-user resource assessment and information system needs.

Summary conclusions and recommendations arising from the information gathered were also provided. Among others, these included (1) one of the RBEP stated objectives is to support planning efforts, particularly with regard to assessments of current and future resource availability and utilization. Therefore, it is fitting that the RBEP be involved in GIS-related activities; (2) biomass users view the RBEP resource assessments and feedstock characterization efforts as being useful, especially with respect to low- and negative-valued resources; (3) an entity should fully inventory RBEP resource assessment and feedstock characterization efforts to produce an accurate record of the work completed to date; (4) with the input of biomass researchers and market users, the RBEP should develop a common protocol for future resource assessment and feedstock characterization efforts so that consistent conjectures may be made regionally and nationally; (5) the RBEP could initiate the development of this protocol by using market-based biomass-user focus groups to determine what particular information characteristics users require when making facility-siting and related decisions; (6) individual RBEP GIS-related activities are carried out on an incremental basis by each of the five regions for selected resources. Therefore, it is important that a national GIS protocol be developed so that these incremental RBEP efforts can eventually be aggregated on a national basis.

Major Project Reports:

Lusk, P. (1993). *Review and Categorization of the Regional Biomass Energy Program's Resource Assessment and Information Systems Needs*. Golden, CO: National Renewable Energy Laboratory. Work performed by Resource Development Associates, Washington, D.C.

Summary Date: March 1994

Regional Biomass Energy Program: Evaluate Current Opportunities for Methane Recovery from Animal Manures

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Contractor:

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Principal Investigator: P. Lusk

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Contract Number: CAE-3-13383-01

Contract Period: 07/93-02/94

Contract Funding (Source):

FY 1993: \$19,585 (DOE)

Objective:

Provide greater continuity and integration of the Regional Biomass Energy Program (RBEP) to the broader objectives of DOE's Office of National Programs, and also to lend greater visibility to the accomplishments of the RBEP.

Approach/Background:

The RBEP is a federally-supported program located in five regions of the continental United States with the goal to increase the production and use of biomass energy resources.

Status/Accomplishments:

Produced a casebook that examines some existing opportunities for recovery of methane gas from anaerobic digestion of animal manures. Volume I introduces the types of anaerobic digesters currently operating on livestock production facilities and some of the end-use applications for the resulting methane gas. Following this introduction is a series of *pro forma* economic evaluations of three types of anaerobic digesters found on U.S. farms. The selected technologies include covered lagoon, plug flow, and complete mix anaerobic digesters. The evaluations are based on engineering studies of digesters that generate electricity as the end-use

application on dairy and swine farms with differing herd sizes. Regression models, which can be used to estimate digester cost and internal rate of return as a function of herd size, are developed from the evaluations. To provide a reality base, a number of operating and non-operating anaerobic digestion systems are presented as case studies. Information on actual project and maintenance histories, and on the operator's "lessons learned," is provided. Note that the anaerobic digestion process is biologically based, and therefore, must be evaluated and implemented on a site-specific basis. As a result, few meaningful generalizations can be made.

The conversion of agricultural wastes, animal manures in particular, into an alternative energy resource has been the focus of intensive research for over two decades. Much has been learned about how manure can be used as an energy and nutrient source. However, the American farmer has not been motivated to adopt these new practices. More cost-effective and easily-managed manure management techniques are needed to encourage farmers to use their animals' waste for conversion into energy and nutrients. Not only will farmers benefit monetarily, the use of anaerobic digestion will also help mitigate animal manure's contribution to air, surface water, and groundwater pollution. Promising future waste-to-profit activities may enhance the economic performance of the overall farm manure management system. New end-use applications that can provide added value include fuel cells for the generation of electricity and process heat, greenhouses, aquaculture, and algae production. Extension of the anaerobic digestion process with methane recovery has considerable potential for industries with a waste stream similar to livestock manures. Among others, example industries include milk, livestock, food, fiber, and pharmaceutical processing. Some of these industries already operate anaerobic digestion facilities and recover methane for energy.

Major Project Reports:

Lusk, P. (1993). *Methane Recovery from Animal Manures: A Current Opportunities Casebook*. Golden, CO: National Renewable Energy Laboratory. Work performed by Resource Development Associates, Washington, D.C.

Summary Date: March 1994

Strategic Planning Workshops For the U.S. DOE Biofuels Program

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Contract Number: YAS-3-13222-01-106457

Contract Period: 01/13/93-12/31/93

Contract Funding (Source):

FY 1993: \$55,000 (DOE)

Objective:

The U.S. Department of Energy (DOE) is in the process of refocusing its research from strictly a long-term focus to one that focuses on commercialization of technologies that benefit the nation. Additionally, Congress has passed legislation that has become law requiring strategic planning for all government programs. This activity is designed to assist the DOE's Biofuels Systems Division (BSD) in developing a multi-year strategic plan, including a total reevaluation of program direction and interactions with industry.

Approach/Background:

A series of workshops and meetings are being held between BSD program managers and program managers from NREL and Oak Ridge National Laboratory (ORNL). A facilitator versed in strategic planning is being used to guide the process during the workshops and meetings and to document results.

Status/Accomplishments:

This is an ongoing effort, for which activities leading to a preliminary draft strategic plan for the Biofuels Program are 75% complete. To date, three workshops and several meetings have been conducted during which draft vision, mission, goals, and objectives have been developed. A situation analysis examining the program's strengths, weaknesses, threats, and opportunities has been completed. Implementation strategies and action plans are currently being developed.

Major Project Reports: None

Summary Date: December 1993

Current Publications

Ethanol

Adney, W.S.; Ehrman, C.I.; Baker, J.O.; Thomas, S.R.; Himmel, M.E. (1993). *Cellulase Assays. Methods from Empirical Mathematical Models*. Golden, CO: National Renewable Energy Laboratory.

Boynton, B.L.; McMillan, J.D. (in press). "Oxygen-Limited Xylose Fermentation by *P. stipitis*: Shake Flask Studies." *Biotechnol. Prog.*

Boynton, B.L.; McMillan, J.D. (in press). "High Yield Shake Flask Fermentation of Xylose to Ethanol." *Appl. Biochem. Biotech*; Vol. 45/46.

Boynton, B.L.; Newman, M.M.; McMillan, J.D. (1993). *Comparative Evaluation of Candidate Xylose-Fermenting Microorganisms on Hybrid Poplar DN34 Hemicellulose Hydrolyzate*. Golden, CO: National Renewable Energy Laboratory.

Boynton, B.L.; Newman, M.M.; McMillan, J.D. (1993). *July 1993 P-Level Milestone Report: Comparative Evaluation of Candidate Xylose-Fermenting Microorganisms on Hybrid Poplar DN34 Hemicellulose Hydrolyzate*. Golden, CO: National Renewable Energy Laboratory.

Brigham, J.S.; Ehrman, C.I.; Templeton, D.W.; Ruiz, R.O. (1993). *Ethanol Project Quality Assurance Plan*. Golden, CO: National Renewable Energy Laboratory.

Brink, D.L.; Merriman, M.M.; Herrmann, C.C. (1993). *Dilute Nitric Acid Pretreatment and Enzymic Hydrolysis of Lignocellulosic Materials*. Golden, CO: National Renewable Energy Laboratory. Work performed by Forest Products Laboratory, Richmond, VA.

Davis, B.P.; Dahn, K.; Kenealy, W.R.; Pittman, P.; Sreenath, H.K.; Jeffries, T.W. (1994). "Genetic Engineering of the Xylose-Fermenting Yeasts *Pichia stipitis* and *Candida shehatae* for Improved Ethanol Production." Presented at the 207th American Chemical Society National Meeting, San Diego, CA, March 13-17.

Deanda, K.; Zhang, M.; Eddy, C.; Franden, M.A.; Picataggio, S. (1994). "Metabolic Engineering of a

Biocatalyst for Ethanol Production from Lignocellulosic Hydrolysates." Presented at the American Institute of Chemical Engineers Annual Meeting, San Francisco, CA, November 13-18.

Donner, T.R.; Evans, B.R.; Affholter, K.A.; Woodward, J. (in press). "The Role of the Cellulose-Binding Domain of Cellobiohydrolase I in Cellulose Hydrolysis," Chapter in *Bioconversion of Fuels*. Edited by M.E. Himmel and J.O. Baber. Washington, D.C.: ACS Symposium Series.

Ehrman, C.I.; Himmel, M.E. (1993). *Simultaneous Saccharification and Fermentation of Pretreated Biomass: Improving Mass Balance Closure*. Golden, CO: National Renewable Energy Laboratory.

Ehrman, C.I.; Templeton, D.W.; Ruiz, R.O.; Brigham, J.S. (1993). *Chemical Analysis and Testing Task Laboratory Analytical Procedures Manual*. Golden, CO: National Renewable Energy Laboratory.

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