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Biofuels Program Quarterly Report

Second Quarter Fiscal Year 1996

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Contents

Preface

This report describes technical progress for the Biofuels Program at NREL for the period January 1 to March 31, 1996. The DOE Office of Fuels Development has agreed to permit the publication of this document on a semiannual schedule, covering the periods October 1 to March 31, and April 1 to September 30. Because agreement to change this schedule occurred after the first FY 1996 quarterly report was completed, the new document schedule will begin April 1, 1996. The next Biofuels technical progress document will be prepared for the period April 1 to September 30, 1996 and be available to DOE in late October 1996.

Advanced Biochemical Conversions

Project Management—General

All three technical focus groups of ABC2000 have been significantly affected by the recent downsizing at NREL. Furthermore, the Cellulase Development and Metabolic Engineering staffs have moved their research laboratories to another site and are just becoming fully operational. Ongoing NREL-wide reorganizational efforts dictate that ABC2000 be dissolved into three natural work teams. R. Elander, M. Himmel, and M. Finkelstein will be team leaders for the previous Pretreatment, Cellulase Development, and Metabolic Engineering areas, respectively. Despite expected delays in work package and milestone completions, significant progress was made this quarter.

Summary of Technical Achievements or Results

ABC Pretreatment Team

The major emphasis for pretreatment focused on continued testing of a complete hydrolysis process that will allow the hemicellulose fraction of hardwood yellow poplar sawdust and a significant portion of the cellulose fraction to be hydrolyzed. It would use a countercurrent reaction mode to minimize the formation of degradation products and maximize sugar yields. Converting cellulose and hemicellulose fractions of biomass in pretreatment may radically alter the quantities and use of enzymes to improve the overall economics of the process. Key issues, such as actual pretreatment yields, detoxification of prehydrolyzate liquor, and large-scale reactor design, are being addressed.

Work continued on developing the following:

- Countercurrent prehydrolysis pretreatments in terms of liquor fermentability and toxicity
- A "shrinking bed" reactor system to minimize liquor volumes
- ASPEN-based process engineering models that include various pretreatment options, hot-water pretreatment development, and alternate pretreatment process economic evaluations.

ABC Cellulase Development Team

NREL received the 2.0-Å resolution x-ray crystallographic structure of the EI endoglucanase with a cellotetraose molecule docked in the active site from Cornell University. It will be used for enzyme engineering studies.

Diafiltration-saccharification studies are under way to test the performance of commercial cellulases, NREL-grown *Trichoderma reesei* preparations, and synthetic mixtures of enzymes against a real substrate (dilute-acid-pretreated yellow poplar). Enzyme loadings for this series are 20 FPU/g cellulose. The temperature is 37°C, which simulates current saccharification conditions more closely than did previous studies at 50°C, the optimum for *T. reesei* cellulases.

The genetically engineered EI endoglucanase catalytic domain has been produced in a heterologous *Streptomyces lividans* host in sufficient quantities for crystallization. Subsequent x-ray structure analysis of this domain at Cornell University should confirm assumptions about the tertiary structure of this genetically derived domain.

ABC Metabolic Engineering Team

Initial analysis of tetracycline-resistant *Zymomonas mobilis* transconjugates by mini Tn5 showed the transposon is integrated into its chromosome. Total DNA and plasmid DNA, prepared from tetracycline-resistant *Z. mobilis* transconjugates, were used to conduct a Southern hybridization experiment using the Technology Management Services (TMS) tetracycline-resistance gene as a probe. Results indicated no hybridization with plasmid, but strong hybridization with chromosomal DNA. Further experiments will be needed to determine the nature of the integration and stability of the transconjugates. If verified, this will be the first observation of directed insertion into the *Zymomonas* genome. This genetic breakthrough could lead to more stable fermentation biocatalysts.

Technical and Scientific Progress for the Quarter

ABC Pretreatment Team

Work continued on evaluating cellulose hydrolysis options for the countercurrent pretreatment technology that uses the yellow poplar hardwood sawdust feedstock. The process, aimed primarily at hemicellulose hydrolysis and high pentose equivalent recovery, was extended to include a higher temperature stage (225°–245°C) to hydrolyze crystalline cellulose to hexose equivalents. We demonstrated a shrinking bed reactor concept, which will allow for high sugar yields while minimizing the volume of prehydrolyzate liquor generated. We also demonstrated yields of glucose equivalent from cellulose in excess of 85% in individual liquors from a three-stage process in early testing of this concept. Yields of xylose equivalents in this process exceed 95%. Most glucose equivalents are in the form of monomeric glucose; however, a significant fraction (about 60%) of the xylose equivalents are in oligomeric form. We are currently attempting to determine the yields in a true countercurrent process aimed at minimizing the volume of liquor generated. This will keep sugar concentration in a reasonable range for process economics.

A C-level milestone, "Complete Engineering and Economic Evaluation of Continuous Flow Dilute Acid Pretreatment against the NREL Base Case Process Assumptions and Make Recommendations," was submitted on January 15. It indicated that the high pretreatment yields and SSF ethanol yields from the pretreated solids result in an overall process economic cost that is about 20% lower than for batch or cocurrent pretreatment, even after accounting for the likely high capital costs of a countercurrent pretreatment system. We are now focusing on the fermentability of the prehydrolysis liquor generated from this process and the development of detoxification alternatives. Liquors from prehydrolysis and complete hydrolysis countercurrent scenarios appear to respond reasonably well to a combined overlimed/solvent extraction detoxification. However, this occurs only if the fine particulates, which presumably result from the high level of lignin solubilization in these pretreatments, is removed (currently by high-speed centrifugation).

We are beginning to evaluate the use of a variety of precipitating agents to enhance the removal of these compounds, either as a stand-alone detoxification method or in conjunction with a solvent extraction process that can remove acetic acid from the pretreatment liquor.

We are continuing to modify the 2-in. diameter percolation reactors to take advantage of the "shrinking bed" concept. A set of springs, which will compress the biomass particles as they are hydrolyzed, was fabricated. This results in a lower solids volume, which allows less overall liquor volume to be generated. It also maintains high linear flow rates, which will maximize the yields of sugar equivalents and minimize the formation of sugar degradation products. We are attempting to standardize the protocol for conducting the reaction to allow consistent countercurrent shrinking bed pretreatment liquors to be generated. These will be used in detoxification evaluations.

We are investigating a hot-water preextraction step to selectively remove extractives (and some lignin), with virtually no loss of biomass carbohydrates from the yellow poplar sawdust feedstock. We are now evaluating the preextraction liquors to determine whether this actually removes significant amounts of toxic compounds from the liquors that would eventually go to fermentation steps.

A series of preliminary hot-water pretreatment runs was recently completed. They were run in a single-stage percolation mode on yellow poplar sawdust. Temperatures ranged from 160° to 240°C, with no added acid. The solids and liquors are now being evaluated to determine the hydrolysis levels of the various carbohydrate fractions and the recovery of sugar equivalents. This information will be used to select multistage countercurrent pretreatment conditions for hot-water-only pretreatments. If successful, this approach will overcome several current difficulties.

A series of paddle reactor runs was conducted at Hazen Research to generate about 25 kg (dry wt basis) of pretreated yellow poplar sawdust. This material was sent to the University of Wisconsin to support work on a subcontract for cellulase production technologies.

We evaluated the responses from a direct mailing and an announcement published in the *Commerce Business Daily* to solicit interest in designing and developing high-solids countercurrent pretreatment reactors. (After an internal evaluation, we concluded that countercurrent pulping reactors and decanter centrifuges show the most promise.) We received 17 responses from companies with related process equipment experience in the pulp and paper industry, food processing equipment industry, and solids-liquid contacting and separation equipment suppliers. We are now developing a strategy for more formal discussions and negotiations with specific companies to structure an agreement to develop and test engineering-scale countercurrent pretreatment reactor technology.

A methodology to evaluate the alternate pretreatment methods being examined via subcontracts is being developed. A process flow diagram was constructed for each method. Representative pretreatment performance data, enzymatic hydrolysis and SSF conversion of pretreated solids, and prehydrolyzate fermentability were culled from data collected by the subcontracts. ASPEN-based models are being developed for each method, and an economic comparison will be conducted. These data will result in a formal C-milestone report next quarter.

ABC Cellulase Development Team

Analytical ultracentrifugation and time-of-flight laser mass spectroscopy have confirmed that the molecular weight of the native EI endoglucanase is near 70,000 daltons. Because the proteolytically derived catalytic domain and cellulose binding domain (CBD) comprise about 58,000 daltons, we now suspect the native enzyme may harbor multiple CBDs. C-terminal analysis is under way and should resolve this issue.

The first batch of site-directed mutagenized EI clones has been produced at NREL using the "*in vivo* Amber Suppression Mutagenesis System" from Promega. Although viewed primarily as proof of the transformation system, this experiment produced 12 mutant EI endoglucanase-producing strains of *Escherichia coli*, each strain substituting a different amino acid residue at the targeted site. Analyses of cell extracts are under way.

ABC Metabolic Engineering Team

Research has been initiated to develop *Z. mobilis* strains with combined xylose- and arabinose-fermenting capabilities. The initial approach will focus on the cotransformation of *Z. mobilis* strains with individual plasmids carrying either xylose or arabinose-fermenting genes. *Z. mobilis* strains cured of the xylose- and arabinose-fermenting plasmids will be used as hosts for cotransformation, because uncharacterized host mutations may be required for successful fermentations.

A series of pH-controlled fermentation with the arabinose-fermenting *Z. mobilis* showed the strain grew faster and achieved a higher final cell density as the pH was increased from 4.5 to 6.0. Residual arabinose was considerably lower at pHs > 5.5 than previously observed in shake flasks without pH control. Despite lower residual arabinose concentration, process yields were no higher because of by-product formation at higher pHs.

Ethanol Process Development (EPD)

Summary of Technical Achievements or Results

The Ethanol Process Development Team is reporting on the bioethanol (sawdust) process development project, on a mini-pilot system for process development activities, and on general pilot plant improvements to support the Amoco Corporation/ NREL CRADA, the Gridley project, and sawdust process development.

The sawdust process development project accomplished the following:

- Developed two processes for conditioning hydrolysates for effective fermentative conversion of sugars to ethanol
- Developed a recombinant xylose-fermenting *Zymomonas* strain adapted to perform better in unconditioned prehydrolysate
- Selected a new standard condition for dilute-acid pretreatment that uses the Sunds pilot-scale reactor.

These achievements have moved the team steadily toward completing the two technical milestones scheduled to be completed during the second half of FY 1996. Substantial progress was made toward completing the milestone to define performance requirements for an integrated bioethanol process. The team successfully developed a recombinant xylose-fermenting *Zymomonas* that can grow and produce ethanol in 65% (v/v) overlimed yellow poplar hemicellulose hydrolysate.

The team has developed two hydrolysate conditioning processes that produce liquors which, at a level of 85% (v/v), can be fermented by *Zymomonas* at the same rate and yields as are achieved in pure sugar solutions. Eighty-five percent (v/v) is the maximum concentration of hydrolyzate anticipated in the process because other streams dilute the hydrolyzate by approximately 15%).

The team established an improved dilute-acid pretreatment standard based on operating the Sunds pilot-scale reactor at 200°C. The team is therefore confident that the process qualifier milestone will be met (and possibly exceeded) on schedule.

In support of the second technical milestone on cellulase enzyme hydrolysis, past cellulase production studies were analyzed. Results were used to develop a set of key experiments and sensitivity analyses needed to accomplish the enzymatic hydrolysis milestone.

Technical progress on commissioning and improving the mini-pilot-scale fermentation system continued this quarter. Activities are proceeding on schedule for planned process demonstration work to be conducted in this equipment late in FY 1996.

The clean-in-place (CIP) system was installed this quarter, which completes installation of equipment associated with the Phase 3a process development unit (PDU) improvement activities. Ongoing activities now focus on improving the PDU's overall operability and data-gathering capabilities to better support the CRADA and sawdust process development teams.

Technical and Scientific Progress for the Quarter

EPD Sawdust Process Development Project

The bioethanol process development project has weathered the changes wrought by reduced funding and restructuring at NREL. Many team members left, and the AOP was rewritten with milestones moved further into the future. Research productivity has remained high, however, and the team has made substantial technical progress. It continues to work toward the two FY 1996 technical objectives described in the October 1995 C-level milestone report entitled, "Sawdust SSCF Technology Demonstration Plan Milestone Report." These objectives are summarized below (detailed versions of the plans are available):

Objective 1: Achieve process performance qualifiers for moving into process demonstration work (P-milestone due August 30, 1996).

The pretreatment, detoxification, cellulase production, and fermentation-integrated process must meet or exceed the following minimal performance qualifiers to be moved into the pilot/integration scale demonstration.

1.1 **Ethanol Yield**. Conversion of cellulose and hemicellulose to ethanol must be at least 50% of the initial biomass or 60% of the sugars into simultaneous saccharification and cofermentation (SSCF)

1.2 **Hydrolysate Concentration**. Hydrolysate concentration into SSCF must be at least 50% of the initial hydrolysate concentration (based on at least 20% total solids into pretreatment)

1.3 **Fermentation Time**. Total SSCF time must be $\leq$ 7 d.

Objective 2: Evaluate enzymatic hydrolysis and plan for future action (C-Milestone due June 30, 1996).

The following tasks will be performed to achieve this milestone:

2.1 Evaluate the cost of NREL cellulase production and use in context of a sawdust-to-ethanol conversion process, using specific activity, volumetric productivity, sugar consumption, other *T. reesei* fermentation parameters, and SSCF cellulase dosage information and process configuration considerations

2.2 Hypothesize and evaluate all potential *T. reesei* system improvements, as well as ethanol process and process configuration improvements, that could be made to the enzymatic hydrolysis process. Determine the resources needed to implement each improvement as well as the likely impact on process cost. Estimate the projected cost of enzymatic hydrolysis using the best possible improvements.

Considerable progress has been made toward meeting the process performance qualifier for producing ethanol from hardwood sawdust. The team is confident that these qualifiers can be met, and perhaps exceeded. The groundwork laid in previous quarters, for which milestones were established with the entire team's input and buy-in, is apparently paying significant dividends.

Technical progress toward these objectives is reported in the sections, Pretreatment, *Zymomonas*-Based SSCF, Cellulase Production, and Hydrolysate Conditioning. Pilot plant improvements are summarized in the Pilot Plant Improvement section.

EPD Pretreatment

A key pretreatment objective is to have small- and large-scale equipment that will treat the biomass material equivalently. This would allow testing of pretreatment conditions that use the small-scale equipment and would provide a reactor that can reproduce the small-scale results at the pilot scale for large sample preparation. Because the favored conditions for dilute-acid cocurrent pretreatment are high temperature and short time (less than 5 min), equipment engineering is important. This quarter, experimental runs that use the new (bench- or minipilot scale) batch digestion system and the Sunds (pilot-scale) reactor were completed to compare the performance of the bench- and pilot-scale pretreatment devices. The batch digestion system was brought to experimental readiness and initial experiments were conducted. The Sunds reactor was also modified for better control, and hydrolysates and solids were made for the ethanol project's use.

Batch Digestion System

Shakedown experimentation with the batch digestion system (BDS), which consists of a 4-L steam-explosion digester, a 3-L stirred-tank reactor (ZipperClave), and a 100-L Atmospheric-Pressure Acid Impregnator (APAI), was completed. Because

of its small size and processing flexibility, the BDS is ideal for pretreatment testing and optimization before scaling up to the pilot plant.

Testing of the APAI and the digester using yellow poplar sawdust has begun. Based on preliminary work, the steam-explosion digester will be used for comparison work. Preliminary test runs of the ZipperClave indicate that the equipment and operating procedure must be modified to reduce the time required to bring biomass to the required temperature. The steam-explosion digester will be used. When time and resources permit, we will resume modification of the ZipperClave, which is planned to be used for slurry pretreatment (< 20 wt % total solids), whereas the steam-explosion digester is designed to handle high solids (> 20 wt % total solids).

The APAI is important for reduced pretreatment time. It uniformly disperses the acid catalyst before the biomass is subjected to reaction temperatures. At high temperatures and short times the result is a more uniform pretreatment that requires less acid and produces fewer sugar breakdown products. Preliminary experimental operating parameters call for the APAI dilute acid to circulate at 50°C through the basket of sawdust for 3 h. The acid concentration in the bath initially drops because of dilution from water in the green poplar sawdust and begins to level off after 2 h. Circulation is limited to 3 h to minimize the dissolution of hemicellulose. In one experiment, after 4 h of circulation of 0.3% sulfuric acid at 50°C through a basket containing 10.5 kg (dry wt) of the green sawdust, 1% of the original glucan, 4% of the xylan, 46% of the galactan, 16% of the arabinan, and 11% of the mannan were hydrolyzed and leached into the acid solution. The high values for galactan, arabinan, and mannan are most likely due to variance in the analysis as the concentration of these sugars are near the lower detection limit of the instrument. Investigation of temperature and time combinations that ensure thorough acid impregnation into the sawdust particles and minimize carbohydrate removal is planned.

Three steam-explosion digester runs and a Sunds pretreatment reactor experiment at similar conditions were completed. Preliminary HPLC results indicate that pretreated poplar sawdust from the digester characteristics very similar to the Sunds. We are evaluating the digestibility and fermentability of pretreated material, and will include in the next semiannual report results that show the pretreatment equipment at the bench and pilot scales produces the same results.

Sunds Pretreatment Reactor

The Sunds pretreatment reactor experiment was used to test new control procedures and generate hydrolysate and solids for the rest of the ethanol project's use. The operation of the modified Sunds level control system was tested and refined, and a new procedure that more precisely controls acid addition was established. This procedure uses pH measurement of the pretreated material as it exits the flash tank, rather than the flow measurement of the acid input, as the acid addition control parameter. The standard pretreated material was processed at pH 2, 200°C, and 4-min residence time. Using a basket centrifuge, the liquor was separated from the pretreated fiber right after the flash tank, and then stored in 5-gal pails at 4°C. The liquor is being used in detoxification, cellulase production, and *Zymomonas* adaptation experimental activities.

EPD Zymomonas-Based SSCF

Strain Development via Adaptation

Efforts continued this quarter to use evolutionary adaptation for developing improved *Zymomonas* strains that can tolerate higher concentrations of inhibitory yellow poplar hemicellulose hydrolyzates. This quarter, tests that compared the performance of an apparently adapted strain with that of the unadapted parent strain. A key experiment compared the growth and SSCF performance of the stock process strain, 39676 pZB4L, with that of the putative adapted daughter strain. Results of this experiment, which was performed using overlimed Sunds-generated hydrolyzate, showed that growth of the

adapted strain in seed production using 30% (v/v) hydrolyzate was far superior to that achieved using the unadapted strain. In overnight seed culture, the adapted strain outgrew the unadapted parent strain by more than twofold. However, results regarding the superiority of the adapted strain in SSCF were inconclusive. The adapted strain utilized sugars much more rapidly than the parent strain during the initial stages of SSCF, but apparently stalled after about 48 h (as inferred by glucose accumulation); both strains achieved similar 7-d final ethanol yields. Despite inconclusive results in SSCF, this experiment clearly demonstrated that the adapted strain exhibits a much higher tolerance to hydrolyzate than did the stock strain.

A second focus of adaptation research has been to assess the stability of the adapted strain to long-term storage. The objective here is to confirm that the traits of the adapted strain are stable enough that the culture can be maintained over time. This work is ongoing, and more complete results will appear in the next semiannual report. However, preliminary results suggest that long-term storage of the adapted culture will not be a problem; adapted cultures stored either in cryovials at -70°C or on agar plates at 4°C were able to grow in 40% (v/v) overlimed hydrolyzate. Tolerance to hydrolyzates is maintained after the culture is revived, which strongly suggests that the adaptation has a genetic basis.

Additional experiments to assess the long-term stability of adapted strains are under way. Studies are planned to characterize the performance of adapted strains as a function of SSCF processing conditions. Finally, we will continue to pursue our promising adaptation strategy in an effort to develop even hardier strains.

SSCF Process Development

Several significant accomplishments were made this quarter that greatly advance integration of inoculum seed production and SSCF processing steps. Early in the quarter, a two-level, four-factor fractional factorial experiment was completed to characterize SSCF performance as a function of cellulase (enzyme) loading, inoculum (cell) loading, pH, and yellow poplar hemicellulose hydrolyzate concentration. Statistical analysis of the results indicated that, within the experimental design space, cell loading did not affect batch SSCF performance, whereas the other three factors did. The major implication is that we should be able to inoculate the SSCF process with significantly reduced cell loadings.

The ability to inoculate at much lower cell loadings and avoid cell concentration before SSCF processing would greatly reduce seed production costs and facilitate overall process integration. Therefore, studies were conducted to confirm that batch SSCF is effective even when inoculated at a much lower level than previously used. Results showed conclusively that SSCFs can indeed be inoculated using 10% (v/v) whole inoculum culture broth; this approach has now been adopted as the standard SSCF inoculation procedure. In related experiments, we also showed that the seed culture can be effectively grown in the presence of 30% (v/v) overlimed hydrolyzate. Seed cultures so prepared (using the adapted strain) are effective for SSCF processing when inoculated at a level of 10% (v/v) whole broth. For comparison, seed had previously been prepared using pure sugars (i.e., not in the presence of hydrolyzate), then concentrated by centrifugation prior to inoculating SSCFs. The demonstrated ability to use 10% (v/v) whole broth inoculum (and perhaps less) suggests that a large fermentation train will not be required for *Zymomonas* seed production for a large-scale SSCF process.

Having made substantial advances in seed production and integration that and SSCF processing steps, experimental work now focuses on developing a better understanding of the factors that limit overall SSCF ethanol yields. Preliminary results suggest that process yields are constrained more by the extent of enzymatic hydrolysis than by fermentation performance. Detailed results of this work will be communicated in the next report.

EPD Cellulase Production

Further work that uses the MTC-a-13 was conducted to determine how it performs in the sawdust

SSCF environment. The focus is on utilizing cellulose and xylose from pretreated sawdust to produce enzyme, then testing that enzyme on pretreated sawdust in an SSCF process that utilizes the xylose-fermenting recombinant *Z. mobilis*. Preliminary tests indicate that the MTC-a-13 cellulase whole broth (prepared using lactose feed) does not inhibit fermentation by recombinant *Zymomonas*. This result is important, as the concern was that the *T. reesei* fungal strain might produce antibiotics that would be carried into SSCF. The most favored process is to put the cellulase-containing broth directly into the SSCF vessel with no post-cellulase production processing.

During the next reporting period we expect to complete our initial evaluation of enzymatic hydrolysis using a *T. reesei* cellulase production system. To do this, cellulase produced on xylose and cellulose will be directly utilized in SSCF to provide critical loading data. We will then perform a complete process analysis to estimate our current enzymatic hydrolysis process cost and the likely projected cost of utilizing enzymatic hydrolysis for bioethanol. This will represent an improvement over previous projected costs because the performance of cellulase preparations in SSCF will have been evaluated experimentally.

Hydrolysate Conditioning Detoxification

Acid hydrolysates from hardwood sawdust pretreatment contain several compounds such as acetic acid (formed from the hydrolysis of the acetyl groups in hemicellulose), phenolics (produced from lignin degradation), and furfurals (derived from the degradation of pentose and hexose sugars), that are toxic to the fermentative organism. Because of their great impact on the fermentation performance of most organisms, they must be hydrolysate conditioned or removed to achieve reasonable ethanol process yields.

The evaluation of the first class of methods for conditioning hardwood hydrolysates to improve fermentability performance (started last quarter) is complete. Overliming, chemical oxidation using hydrogen peroxide, activated carbon adsorption, and combinations thereof, were tested. Conditioning with zeolites is also complete. All these methods successfully reduced lignans and other color components, but they did not reduce acetic acid concentration, which seems to be the most significant toxin for most ethanologenic organisms, including *Z. mobilis* and yeast. As a result, the fermentability of the treated hydrolysates by the recombinant *Z. mobilis* improved only marginally.

This quarter, two additional processes were evaluated for conditioning sawdust hydrolysates before fermentation. The first is based on liquid-liquid extraction and the second on ion-exchange adsorption. Both remove acetic acid very efficiently. They also remove other acidic and nonpolar inhibitors, such as fatty and resin acids, lignans, and other phenolic components, as well as the sugar degradation products hydroxymethyl furfural and furfural. The fermentability of the solvent extracted hydrolysate with the recombinant *Z. mobilis* strain was as good as the pure sugars control up to a 60% (v/v) hydrolysate level. The performance of a hydrolysate-adapted *Z. mobilis* strain on the same material was even better.

These results represent a major breakthrough in hydrolysate conditioning for the sawdust-to-ethanol process. The performance objectives that were set in the first milestone (fermentability at 50%–60% hydrolysate level) were met and could be substantially exceeded by the developed technologies. In parallel to the experimental work, a process model for the liquid-liquid extraction process was set up to help evaluate the economic impact of the hydrolysate conditioning step on the overall process. As the effectiveness of the solvent extraction and ion-adsorption processes has been demonstrated in the laboratory, the economic viability of both will be evaluated to determine the best option.

EPD Pilot Plant Improvement

The following Phase 3a improvement acitivities were completed during the quarter:

- A skid-mounted CIP system, which reduces the labor required to clean fermentation and related tanks

- Exhaust vent condensers for the 1450-L and 9000-L fermenters, which allow better retention of ethanol and other volatile components in the fermentors

- A flexible conveyor to service the Amoco pretreatment device

- A set of modified discharge valves for the Sunds reactor

- Feedstock supply system modifications, including dust control for the Gridley project

- Many minor projects to improve operability or meet the Amoco/NREL CRADA needs.

Additionally, engineering to allow for piping and conduit attached to the PDU roof to be relocated to floor supported piping racks is complete. Transfer of the piping to the piping racks is nearly complete. This ensures the structural integrity of the building.

Future pilot plant improvements will continue to meet the Amoco/NREL CRADA and Gridley project needs. Planning and initial procurement activities to support the implementation of detoxification and countercurrent pretreatment in the pilot plant or mini-pilot plant will occur during the next semiannual period.

Chemical Analysis and Testing Team

Summary of Technical Acheivements or Results

Method development efforts by the Chemical Analysis and Testing (CAT) Team for this quarter focused on new or enhanced procedures to support the PDU and other in-house research efforts. The group also continued to provide a certified analytical service to support the in-house, PDU, CRADA, new business, and subcontract research efforts by analyzing complex samples. This quarter 1,653 samples that support 14 ethanol project work packages were analyzed and summary reports issued. The team's effort, which included sample analysis, training, method devleopment, instrument maintenance and repair, laboratory QC program enhancement, and streamlining data entry and documentation procedures, focused primarily on support to the PDU.

Technical and Scientific Progress for the Quarter

Method Development

The established protocol for determining total sugars in liquid process samples (LAP-014) was streamlined to allow the recoveries of all five monmeric sugars from a single chromatographic run to be calculated. The current procedure was updated into a draft protocol to reflect this change. It will be statistically assessed, then reissued as an official Laboratory Analytical Procedure (LAP).

The standard method for analyzing organic acids and inhibitors in liquid samples (LAP-015) was enhanced by incroporating the quantification of xylitol and ethanol. A draft protocol that reflects these changes was issued. The procedure will be reissued as an official LAP once the formal QA evalution is complete.

Three new analytical protocols were developed to support the PDU:

- **Enzyme sensitivity (reactivity) assay**. Developed at the request of Amoco Corporation, this indicates the accessibility of cellulose in pretreated biomass to enzymatic attack, and is currently being used to assess the effectiveness of various pretreatment conditions.

- **Enzymatic starch assay**. This was developed to measure the amount of starch present in a sample, independent of the cellulose content. The sample is digested to make the starch more accessible, then amyloglucosase is added to break down the starch into glucose, which can be easily quantified. The new approach is quicker and more reliable than the current Amoco-based protocol.

- **Enhancement of the high-performance anion exchange chromatography-pulsed amperometric detection (HPAEC-PAD) method for monosaccharides to include cellobiose**. This was used to analyze a wide range of PDU samples. An improved sensitivity for all sugars and elimination of baseline (coelution) problems that plague the conventional HPLC approach were noted. Because better mass closures were obtained when HPAEC-PAD values were used in the calculations, all mass balance PDU samples are currently being analyzed by this technique.

A study was conducted to discover the source of glycerol (added to most filters to prevent brittleness through drying) that was contaminating project samples. The Gelman #4192 filters commonly used in the laboratories contained an average of 1.3 g/L glycerol. The ethanol project researchers and the manufacturer were notified about this potentially serious problem.

Sample Analysis

The CAT Team continues to provide a certified analytical service to support the in-house, PDU, CRADA, subcontract research efforts, and new

business ventures by analyzing complex samples. The following table summarizes the chemical analysis support provided to the ethanol project during the first quarter.

Some routine solid and liquid sample analyses are performed at Hauser Chemical Research under the subcontracts "Compositional Analysis of Lignocellulosic Samples" and "Compositonal Analysis of the Liquid Fraction of Biomass Process Samples." Both subcontracts are scheduled for renewal in April 1996, when they will be combined to minimize administration and monitoring efforts. The statement of work (SOW), written as part of the subcontract renewal packet, reflected this change, as well as enhancements to analytical protocols. These efforts have streamlined the work required by the ethanol project and by Hauser.

First Quarter 1996					
	Number of Samples				
Work Package	Solid	Liquid	GC	Other	Total
BF620301 CAT Method Development	32	22		8	62
BF621242 (WU630101) BRI CRADA	1	2		2	5
BF621320 Enzyme Engineering		8		8	16
BF622011 Fermentation Technology	3	4			7
BF622021 Sawdust Pretreatment	7	4			11
BF622023 Sawdust Detoxification		89		39	128
BF622261 Coors CRADA	12	11		23	46
BF623001 Amoco CRADA Benchscale Research	16	276	70	21	383
BF623002 Amoco CRADA, PDU Task #2	37	347	80	41	505
BF623003 Amoco CRADA, PDU Task #3	22	114	39	30	205
BF623004 Amoco CRADA, PDU Task #4	8	133		14	155
BF623002 Amoco CRADA, PDU Analytical Support				58	58
BF623103 New Energy CRADA	11	12		23	46
BF628208 Partnership Development	13			13	26
Totals for first Quarter 1996	162	1022	189	280	1653

Quality Assurance

The CAT Team continued to implement the Ethanol Project Quality Assurance Plan (QAP) project-side. R. Ruiz and D. Templeton were awarded an "NREL Outstanding Team Award" in recogniziton of their work in this critical area.

The PDU analytical support laboratory successfully implemented a laboratory QC program that meets the unique needs of the PDU. During this quarter the efforts of all CAT Team members were directed toward improving the data documentation process without compromising data quality, primarily by enhancing the calculation and data entry spread-sheets streamlining the QC checkoff procedures.

Partnership Development

Summary of Technical Achievements or Results

Wood Industries Company Report

The Wood Industries Company (WICO) final report, *Feasibility of Reducing Costs of Liquid Fuels and Electricity from Dedicated Biomass Feedstocks and Waste-to-Resource Management in California* for subcontract AAC-5-13326-1,1 was received and approved this quarter. The WICO study indicates two significant feasibility issues:

1. Conversion technology and dedicated feedstock supply system (DFSS) development are the two highest priority areas for R&D to achieve the lowest price per gallon of ethanol. However, feedstock sourcing, supply and processing, and integrated waste-to-resource management also offer great potential for lowering ethanol costs.

2. Early biomass-to-ethanol plants must rely on low-cost or disposal fee-based waste feedstocks. However, to meet the nation's sustainable alternate energy needs, DFSS must be developed. Commercial developers must be cautious with baseline feedstock price structuring to facilitate the gap closure necessary to bring in DFSS; i.e., to pay for more expensive DFSS feedstocks necessary to ensure constant supply, an adequate tipping fee structure needs to be developed for waste-derived feedstocks.

Coors/NREL CRADA

Phase 1 of the Coors/NREL CRADA continued this quarter. The project is on schedule to meet the June 30, 1996, C-milestone, "Coors CRADA Phase 2 Go/No-Go Decision."

Gridley Project

A letter subcontract was awarded to Stone & Webster Engineering Corporation (SWEC) on February 1, 1996, for a feasibility study on rice straw-to-ethanol production in Gridley, California. A project kickoff meeting was held February 12, 1996, in Davis, California. SWEC, along with seven project partners, will determine whether rice straw can be economically converted to ethanol, power, and chemical coproducts. Bench- and pilot-scale studies will be conducted at NREL to demonstrate conversion technology and obtain critical process data. The scheduled completion date is July 1997.

Communications

Major accomplishments in communications for this quarter are:

- Displayed the new "Ag industry" exhibit at the American Farm Conference in Reno, Nevada, during January. Approximately 6,000 people attended the conference. There were 500 to 600 booth visitors.

- Displayed the "ethanol business partnerships" exhibit at the Third Enterprise Forum in San Francisco during February

- Displayed "Ag industry" exhibit at the NREL Staff awards expo during February

- Installed visitors' wall display at the Alternative Fuels User Facility (AFUF) in February

- Supplied articles on AFUF and PDU and environmental impacts of bioethanol to *Biologue* magazine. Because of short notice and non-AOP funding designation, this was an update and a condensation of an *NREL in Review* article.

Technical Evaluation and Planning

Summary of Technical Achievements or Results

DynCorp examined the DOE "Assessment of Costs and Benefits of Flexible and Alternative Fuel Use in the U.S. Transportation Sector" draft report dated September 22, 1995, and derived DOE/PO projections of ethanol content for E85, fuel extender, and oxygenate use in light-duty vehicles. A reference case for the year 2000 was gleaned from various report tables. The 2010 benchmark and unconstrained cases were derived given ethanol contents contained in the report to arrive at the projected numbers. A detailed explanation of these derivations and the necessary assumptions was supplied to NREL with a final graphic showing these projections.

P. Lusk completed a review report of the Regional Biomass Energy Program (RBEP) Performance Metrics. This document focuses on RBEP core business areas, goals, objectives, performance metrics, and performance metrics analysis. The RBEP performance metric goal for the year 2000 is to deploy a range of bioenergy technologies that would reduce annual fossil fuel consumption by more than 65 trillion Btu of conventional fossil fuels. For the years beyond 2000, the goal is more than 350 trillion Btu.

TMS submitted a report, *Review of Candidate Evaluation Factors*, dated March 15, 1996 as prepared for the Program on Partnership for a New Generation of Vehicles. This program's goals is to support the development of vehicles comparable in cost to today's cars with as much as three times greater fuel economy.

Technical and Scientific Progress for the Quarter

DynCorp completed the following:

- Delivered a draft list of U.S. ethanol plant producers that shows company names, production capacities, mailing addresses, and telephone numbers.

- Delivered a draft report that summarizes all information gathered from several sources on the GRAS (Generally Regarded As Safe) status of genetically engineered bacteria, specifically *E. coli* and *Z. mobilis* in the United States. The report focuses on the status of certified uses of these genetically engineered bacteria, especially their potential for producing ethanol from biomass. The report also addresses the concern raised by regulatory agencies to eliminate all pathways to man and other animals, caused by the pathogenic and toxic potential of some strains of *E. coli* and *Z. mobilis*. After we receive comments, we will revise and finalize the report.

- Began to collect and assess information on biodiesel emissions from heavy-duty engines. This includes gathering information on biologically derived waste oils and grease, especially in wastewater and sludge streams.

- Updated the master database of addresses for the upcoming BioEnergy '96 conference (based on input from Tennessee Valley Authority [TVA]), and provided TVA with a diskette that contain the edited list of 15,000+ names and addresses.

- Began to review a Draft Report on Federal Tax Credits Options for the Rapid Expansion of the U.S. Ethanol Industry in the Period 1992–2010, as encouraged by significant success in the DOE R&D program on new biofuels technology. Several issues are addressed:

  - What is the impact on the federal Treasury of a large expansion in cellulosic ethanol production?

- Will new producers of ethanol who use DOE-developed, low-cost technologies reap a windfall from the current tax credits?

- What is the likelihood of pressure to extend the tax credits past 2000 and what impact will that have on the federal Treasury and total ethanol production?

- Will rapid development of new markets and new regulations for ethanol under the Clean Air Act Amendments interact with the tax credits in unexpected ways?

- Are there minor policy corrections that would rectify any unintended outcomes?

- Continued to review and comment on the final scoping document for the DOE/USDA Biodiesel Life Cycle Study. This document, which is sent to the members of the Biodiesel Stakeholder Group, was consolidated with previous input and comments on the draft document and revised considerably. Because detailed project parameter decisions are yet to be made, new input and comments are requested from biodiesel stakeholders to shape this project.

- Completed and delivered the Quarterly Summary report for all tasks, covering the period October 1, 1995 through December 31, 1995. It includes support for the DOE Biofuels Program in data collection and analysis, technical reviews, program planning and coordination support, and briefings and presentations.

- Assessed the RBEP's "Strategic Plan: Building a Sustainable Economic and Environmental Future," to "determine the extent to which biofuels and transportation sector issues were addressed compared to biopower." The two sectors, fuels and power, were quite evenly represented. When an item appeared to favor electric power, e.g., technology transfer seminars for landfill gas conversion, it may have been due to the more developed nature of the individual technology.

- Continued to develop a work plan designed to enhance modeling capabilities at headquarters. The intent is to meet the BSD's needs at HQ level, for rapid turnaround results in support of budget, Q&A, and OTT management and policy activities. A draft work plan was submitted and is currently being reviewed. The following activities were performed for this reporting period:

  - Met internally and with TMS to scope task levels of effort for conducting the major phases of the effort, i.e., providing a catalog of models being used by DOE/EIA/policy/OTT/EERE and related organizations such as USDA, Argonne National Laboratory, Oak Ridge National Laboratory, and NREL; analyzing the models for desired features; and developing or adapting a PC-based spreadsheet model for use by BSD HQ.

  - Developed and delivered to BSD a draft model review methodology template to be used during the catalog and analysis phases of the effort. The template is a checklist of parameters related to the features, characteristics, and structures of the models to be analyzed. It will ensure consistency in the amount and type of information gathered, as well as conformity of the analysis.

The IEA Bioenergy Agreement Task XIII Biomass Utilization received minutes from the Biomass Combustion Activity meeting, November 8, 1995, Nijmegan, The Netherlands. This meeting was held immediately following the International Symposium on Co-Combustion of Biofuels, November 7, also in The Netherlands. It addressed progress in combustion studies of wood and wood-derived fuels; characterization of biofuels; furnace design and combustion control to reduce emission; and co-combustion of biomass with other feedstocks.

J. Easterly pursued the suggestion to potentially have DynCorp maintain and provide a master visual aids file for use by all five RBEP managers when-

ever they need materials for programmatic discussion and presentations. If approved, this will become a task in 1996 in support of the RBEP.

TMS submitted its annual technical progress report for support services to NREL. The report, dated January 21, 1996, covers all tasks for 1995:

1. Outreach and Program Stakeholder Support

2. Technical/Economic Issue Analysis

3. Support to Analytical Working Group

Noteworthy under Technical/Economic Issue Analysis is the final report *MTBE from Biomass Methanol—A Viable Option?*

C. Wallace completed IEA Bioenergy Task XIII Biomass Utilization Progress Update for the period November 1995 to May 1996 in preparation for the next Executive Committee meeting in May. Administrative matters and research progress in biomass combustion, thermal gasification, pyrolysis for liquid products, biotechnology for conversion of lignocellulosics, technoeconomic analysis, and integrated bioenergy systems are discussed.

Biofuels Scientific Publications, Presentations, and Other Activities

Scientific Publications

Advanced Biochemical Conversions

The following book chapters are in press: "Cellulases: Structure, Function, and Application", by M. Himmel, W. Adney, J. Baker, R. Nieves, and S. Thomas, and "Hemicellulases: Diversity and Application," by J. Brigham, W. Adney, and M. Himmel, in *Handbook on Bioethanol: Production and Utilization* (C.E. Wyman, ed.), Taylor & Francis: Washington, DC, 1996.

The following manuscripts have been submitted for consideration:

"Expression of *Microbispora bispora* Bgl B Beta-Glucosidase in *Streptomyces lividans*," by X. Xiong, W. Adney, T. Vinzant, Y.-C. Chou, M. Himmel, and S. Thomas, *Appl. Environ. Microbiol.*, 1996.

"Crystal Structure of Family 5 Endocellulase EI from *Acidothermus cellulolyticus* in Complex with Cellotetraose," by J. Sakon, W. Adney, M. Himmel, S. Thomas, and P. Karplus, *Biochemistry*, 1996.

"The Roles of Key Residues in the 4/7 Superfamily of Glycosyl Hydrolases Revealed by Cellulase: Substrate Complex," by J. Sakon, S. Thomas, M. Himmel, and P. Karplus, Nature *Structural Biology*, 1996.

Technical Evaluation and Planning

The proceedings of the seminar "IEA Bioenergy Agreement Progress and Achievements 1992–1994," Oslo, Norway, March 23–24, 1995 was just published as a special volume of *Biomass & Bioenergy*, Vol. 9, Nos. 1–5, 1995. The volume includes an overview of progress in each task plus papers by the activity leaders covering details of the individual activities. C. Wallace presented the overview for biomass utilization.

The IEA Bioenergy Annual Report 1995 has been published by NUTEK, Stockholm, Sweden. This report includes achievements and assessments of completed tasks for 1992 to 1994, progress in 1995 in ongoing tasks (including biomass production, harvesting and supply, biomass utilization, energy recovery from municipal waste, and greenhouse gas balances of bioenergy systems), and participants and budgets for 1995–1997. A few copies are available from C. Wallace.

P. Lusk wrote a paper, "Deploying Anaerobic Digesters: Current Status and Future Possibilities," for *CADDET Renewable Energy Newsletter*, March 1996. The paper addresses some of the main conclusions drawn from a special session on developments in anaerobic digestion as part of the Second Biomass Conference of the Americas, August 1995.

Technical Presentations/Posters

Advanced Biochemical Conversions

The following papers were accepted for the 18th Symposium on Biotechnology for Fuels and Chemicals held in Gatlinburg, Tennessee: "Cellulase Superfolds: Diversity of Structure and Convergence of Function" and "A Membrane-Reactor Saccharification Assay to Evaluate the Performance of Cellulases and Substrate Pretreatments under Simulated SSF Conditions."

Metabolic Engineering Team

Abstract for a poster on "Metabolic Engineering of an Arabinose-Fermenting *Zymomonas mobilis*" was accepted for presentation at the 18th Symposium on Biotechnology for Fuels and Chemicals in May, 1996, in Gatlinburg, Tennessee.

S. Picataggio and M. Finkelstein submitted a paper on "Advances in Biocatalyst Development for Conversion of Corn Fiber to Ethanol," which will be presented by M. Finkelstein at the Corn Utilization Conference in St. Louis, June 4–7, 1996.

Patents or ROIs Filed

Advanced Biochemical Conversions

Cellulase Development Team

NREL/DOE CIP to 94-08: "The Cellulose Binding Peptide of a Thermal Stable Endoglucanase from *A. cellulolyticus*" is in preparation at NREL.

Metabolic Engineering Team

A letter from B. Brantley to T. Anderson indicated that MRI decided not to pursue national or PCT filing on "Recombinant *Zymomonas* for Pentose Fermentation" (NREL IR#95-26) and "Pentose Fermentation by Recombinant *Zymomonas*" (NREL IR#95-27) because of coverage by a previous NREL patent.

Project Operation/Total Quality Management and ES&H

Advanced Biochemical Conversions

A 2-month no-cost extension entitled, "Provide X-Ray Structures of Cellulases," was initiated for the subcontract to Cornell University. The SOW was drafted for FY 1996 funding for continuing this subcontract, which should be placed soon.

Metabolic Engineering Team

S. Picataggio and M. Zhang provided K. Dimitrov, a graduate student at Baylor College of Medicine, an overview of current biomass-to-ethanol technology as he considers this area of research as a possible career choice.

S. Picataggio spoke to S. Hart, who is interested in possible use of *Zymomonas* for producing alcoholic beverages. Mr. Hart will write to N. Hinman to propose an economic evaluation of a hypothetical process based on his input data and our conversion data under some type of CRADA.

S. Picataggio sent information on NREL's *Z. mobilis* to C. Karper, Knouse Foods Technical Center (Biglerville, Pennsylvania), in response to his interest in converting apple pumice wastes to ethanol.

S. Picataggio provided information and references to R. Goldberg, National Institute of Standards and Technology, on the importance of xylose fermentation for economical biomass conversion to ethanol.

ABC2000 and EPD members participated in round-table discussions on *Zymomonas* technology with P. Rogers of the University of New South Wales (Sydney, Australia), a world-renowned expert on *Zymonomas*. M. Finkelstein and M. Zhang provided Rogers a brief overview on Biofuels and *Zymomonas* strain development efforts at NREL.

M. Zhang provided M. Finkelstein and N. Hinman a draft of experiment plan for confidential test evaluation of xylose- and arabinose-fermenting *Z. mobilis* under the observance of CRA representatives. This will support a research grant application in response to a solicitation from the Illinois Corn Marketing Board to support the development of a corn fiber-to-ethanol conversion process based on the use of xylose- and arabinose-fermenting *Z. mobilis* strains.

Chemical Analysis and Testing Team

The CAT Team provided training for PDU staff members on data entry into the PDU database and on the use of CAT Team calculation and QA/QC spreadsheets.

Technical Evaluation and Planning

P. Lusk, P. Wheeler (U.K.), and C. Rivard (NREL) edited the document *Deploying Anaerobic Digesters: Current Status and Future Possibilities*, January 1996. NREL/TP-427-20558. It is drawn from a special session of the Second Biomass Conferences of the Americas and is available from NTIS.

P. Lusk informed RBEP managers on the project that will develop a number of RBEP-related case studies suitable for publication as technical bro-

chures by the IEA Centre for the Analysis and Dissemination of Demonstrated Energy Technologies (CADDET) or by the Federal Energy Management Program (FEMP). The goal is to provide greater visibility to the accomplishments of the RBEP, and to create greater awareness of recent deployment initiatives.

P. Lusk had discussions with the Biomass Energy Alliance (BEA) on anaerobic digestion and the section 29 energy tax credit. The BEA recently participated in the 50th annual meeting of the National Association of Conservation Districts. In addition to energy crops, the convention attendees came looking for new farming methods, including a good deal of interest in anaerobic digestion. In the Congress, the BEA was working with a previously proposed extension to Section 29 that includes a change to the "user-producer rule," which restricts producers of biomass gas from burning it and selling the resulting electricity on-grid.

On January 16, P. Lusk and the RBEP/DOE manager met with the principal investigators of a Swedish project concerning the competitiveness of bioenergy. The purpose of the meeting was to describe interesting activities, such as green pricing programs, projects, and organizations active in the field. The principal investigators are from the Development Projects Centre of the Swedish University of Agricultural Sciences. They were accompanied by the Secretary of the Energy and Housing Section of the Swedish Association of Local Authorities. They discovered the RBEP as a result of contacting the DOE Energy Efficiency and Renewable Energy Clearinghouse.

C. Wallace completed revisions in the IEA Bioenergy Agreement Annual Report for 1995. This report will have a slightly different format than in previous years, with more descriptive material for government officials and less technical content. The IEA Secretary drafted a brief introduction to the document covering goals and objectives of the agreement.

NREL received *Biomass Resources for Gasification Power Plants*, a report prepared for the IEA Bioenergy Biomass Gasification Activity by researchers in the United Kingdom. Topics include:

- The global biomass resource under headings of agricultural and forestry residues, biomass from existing forests, and biomass plantations for energy
- Estimates of the long-term markets for electricity from biomass
- A description of the changing market structures that will accompany market development
- Indications of priority long-term markets on the basis of fuel availability, power shortages, infrastructure, and policy framework.

The report is available from C. Wallace

General Presentations/Outreach

Advanced Biochemical Conversions

Pretreatment Team

A meeting was held with representatives from Alfa Laval Sharples on February 29 to discuss concepts related to the use of decanter centrifuges for large-scale pretreatment processes.

A presentation on general pretreatment technology was given to Hercules Inc. on March 19.

Cellulase Development Team

M. Himmel attended the Symposium on "Enzyme Biotechnology" held in Lake Buena Vista, FL.

M. Himmel gave a presentation entitled "Cellulase Technology at NREL" to a group from the Corn Growers Association, and a presentation entitled "Cellulase and Industrial Enzymes R&D at NREL" to a group from Hercules.

S. Thomas attended a symposium on "The Extracellular Matrix of Plants: Molecular, Cellular and Developmental Biology" held in Durango, CO.

S. Thomas was contacted by B. Hooker from Pacific Northwest Laboratory to discuss potential collaboration and support for work on potato expression of enzymes (cellulases).

NREL continues to generate strong interest regarding cellulase development activities. New companies to initiate contact with NREL include Unilever (The Netherlands), and Recombinant Biocatalysis (La Jolla, California).

Metabolic Engineering Team

S. Picataggio provided an overview of *Zymomonas* cofermentation capabilities to representatives from Arkenol, Inc., on January 12.

S. Picataggio presented V. Sarisky-Reed an overview of metabolic engineering plans and milestones. In response to reduced resources, we recommended modifying the *Zymomonas* work package and postponing the *Lactobacillus* work package until FY 1997.

Chemical Analysis and Testing Team

F.P. Eddy, a member of the Colorado Roundtable, attended the Seventh American Forest Congress in Washington, DC, where she met with participants to develop a common vision for America's forests and define a set of principles and next steps to create policies that will govern how our forests will be managed in the future.

Technical Evaluation and Planning

The IEA Integrated Bioenergy Systems Activity held its working group meeting, Rotorua, New Zealand, March 10–12 to continue the technoeconomic modeling of various bioenergy systems. The Bioenergy Assessment Model is being further developed to include a greater range of feedstocks and energy product outputs. Environmental parameters such as carbon dioxide balance and solid waste (ash) balance are also being incorporated.