CRIS — SELECTED PROJECTS SEARCH

Current Research Information System (CRIS)
USDA Funded Research – Selected Projects

Item No. 1 of 59
ACCESSION NO: 0402814 SUBFILE: CRIS
PROJ NO: 5325-41000-037-00D AGENCY: ARS 5325
PROJ TYPE: USDA INHOUSE PROJ STATUS: TERMINATED
START: 12 MAY 1999 TERM: 11 MAY 2004 FY: 2004

INVESTIGATOR: ROBERTSON G H; OFFEMAN R D; ORTS W J; WOOD D F

PERFORMING INSTITUTION:
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ENABLING TECHNOLOGIES FOR WHEAT STARCH AND PROTEIN SEPARATION, DRYING, AND UTILIZATION

OBJECTIVES: Devise large-scale technologies to enable the efficient and environmentally sound separation of wheat starch and protein from wheat flour, evaluate the quality of resulting products and co-products, and determine economic feasibility at different scales. Create and evaluate selective membrane barriers for the separation of ethanol-from-water and water-from-ethanol, and optimize appropriate films by applying chemical or enzymatic treatments.Develop models to improve separation strategies.

APPROACH: Invent improved separation concepts combining mechanical separation with fluid displacement methods based on the refrigerated ethanol method developed here, and evaluate scale-up potential.Apply the techniques to simulate pilot and commercial processes and produce large samples of gluten and starch. Use the fractions to quantify quality factors in conventional food uses (such as flour fortification) and in new uses (polymer formulations) that make use of the unique properties. Quality factors include drying rate of gluten; ease of fractionation, protein distribution, protein vitality, and functionality (vis-a-vis bread making). Analyze process potential for solubility-based subfractionation of the protein into gliadin and glutenin fractions.Test highly selective and permeable biomaterial films for their ability to separate ethanol from water and water from ethanol,ensuring that these films can withstand a large pressure drop encountered during industrial pervaporation.Optimize these films by chemical and enzymatic modifications. FY02 Prog. Inc.

PROGRESS: 1999/05 TO 2004/05
1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter? Over the past twenty years, exports of US wheat have been both unpredictable and declining. A potentially huge market for wheat is the biorefining and bioconversion of wheat into biobased products, including fuel-grade ethanol and fine chemicals. A key biorefining step is the fractionation of the wheat into starch and protein concentrates. Current separation technologies, which produce concentrates of starch and gluten, are costly and have difficulty competing with foreign imports. Contributing to the expense of the commercial methods are: (1) outdated, nonstandard mill technologies; (2) the inefficient use of energy; and (3) the generation of copious amounts of wastewater. This project researches new and relevant separation technologies and systems. One of these is the cold-ethanol process invented at WRRC, in which ethanol serves as a process fluid to largely replace the use of water and reduce energy use. The cold-ethanol method of separation has the potential to reduce energy and water usage, leading to optimal use of all co-product streams. Another important technology is the separation of ethanol and water, which is important to the cold ethanol method, as well as to the eventual production of fuel-grade ethanol from surplus starch. The overall project goal is to improve wheat

refining, increase wheat usage in bioproducts and biofuels (ethanol), and improve grain-to-ethanol processing efficiency. The total value of US wheat exports is 2/3 of its 1987 value, yet the total production has remained constant. The amount of wheat available for domestic non-food use is roughly 30 billion pounds and growing. Of this amount, the US gluten industry currently separates a little more than 2 billion lbs of wheat into starch and protein for food and non-food uses. This represents 58% of the total domestic gluten market; the rest of which is mostly imported from Europe due to their relative cost advantage. The potential of improving separation processes to improve starch and gluten separation would lead to increased domestic production of gluten, reduced imports, and improved attractiveness of all fractionated wheat components including starch for fuel-grade ethanol. Wheat starch as a fermentation feedstock could significantly expand grain-to-ethanol production in the US, help to reduce dependence on imported fuels and toxic oxygenates, and improve the nation's ability to produce ethanol in a wider range of diverse regional locations. Improved energy-efficiency through improved ethanol-water separations is crucial to the future of the wheat biorefinery. 2. List the milestones (indicators of progress) from your Project Plan. (FY 2002) a. Complete evaluation of lab scale separation using a screening/drying system to model full-scale operation in an analog of the conventional batter process. b. Complete evaluation of the influence of drying conditions (particularly temperature) on changes to gluten vitality. (FY 2003) a. Complete development of new project component for using biopolymer membranes to separate ethanol from water during fermentation to fuel- grade ethanol. b. Complete countercurrent extraction experiments on wheat separation. Evaluate opportunity for pentosan recovery. (FY 2004) a. Establish new project: biopolymer-based barriers to separate ethanol from water from ethanol fermentation fluids and from cold-ethanol separation of wheat gluten and starch. b. Establish collaborative research through MU or CRADA for additional evaluation and engineering assessment. c. Assess current project and develop project plan for next 5 years subject to peer review by OSQR. 3. Milestones: FY (2003) a. Completed countercurrent extraction experiments on wheat separation. Evaluated opportunity for pentosan recovery. Substantial progress. Reevaluation of this milestone suggested a more critical need for compositional studies including the assessment of soluble and insoluble protein removed during the washing of dough with cold ethanol. These studies were completed. Collaboration has been formed with WRRC scientist to assist in the pentosan assessment. FY (2004) a. Established new project: biopolymer-based barriers to separate ethanol from water from ethanol fermentation fluids and from cold-ethanol separation of wheat gluten and starch. Successfully met. New support position filled and analytical instrumentation installed and tested. Literature analysis of ethanol- selective membranes substantially completed and experimentation initiated. b. Established collaborative research using Memorandum of Understanding or CRADA for additional evaluation and engineering assessment. Successfully met. Three companies with interest in barrier separations, plant design, and grain refining have indicated the desire in writing to collaborate with us in the evaluation and development of this research. c. Assess current project and develop project plan for next 5 years subject to peer review by OSQR. Successfully met. New project plan formulated and in final evaluation received the highest scoring level by peer panel. B. Refer to the annual report for new CRIS project, pending OSQR process completion. 4. What were the most significant accomplishments this past year? A. Single Most Significant Accomplishments With unpredictable and declining exports of US wheat over the past 20 years, creation of new markets for US wheat hinges on efficiently separating or biorefining this grain into its constituents to optimize its value as a feedstock for bioproducts, fine chemicals and ethanol. Researchers at Western Regional Research Center (WRRC) developed and scaled-up cold-ethanol based methods for separating and refining wheat into its major constituents. During this year WRRC researchers developed and implemented technology that resulted in the ability to track each protein constituent and to identify how ethanol composition and temperature affected the removal, quality and end-use value of each protein. This new knowledge is being used to predict the full-scale capability of this process, define the separation equipment required, and to facilitate industrial adoption of improved wheat bio-refining. B. Other Significant Accomplishments Barrier-based separation of ethanol and water is critical in all ethanol fermentation processes, and thus has widespread implications. It is particularly pertinent in the present research because of the need to manage ethanol concentrations in the cold ethanol starch/gluten separation with the requirement to eliminate added processing water. Researchers at WRRC, Albany invented a "New Filtering Device Utilizing a Combination of Membrane Materials which Allows for Selective Isolation of Target Compounds from a Production Stream" which can be used for separating ethanol from fermentation broths. This invention, which has been approved by the Area-wide Patent Committee and will be filed shortly, is based on experimental evaluation of ethanol-selective extractants as components of a novel hybrid barrier system employing both selective barriers and solvent extraction. C. Significant Accomplishments/Activities that Support Special Target Populations None. D. Progress Report Barrier-based separation of ethanol and water: This effort is critical in all ethanol fermentation processess, and thus has widespread implications. A complete overview and analysis of the existing barrier literature is substantially complete. We initiated experimental evaluation of ethanol- selective extractants as components of a novel hybrid barrier system employing both selective barriers and solvent extraction. Wheat separation: Kilogram-scale wheat separation methods are now regularly employed to disperse and separate wheat fractions using enclosed vibratory screening. In addition to producing high quality vital gluten, this process has the added benefit of creating separate phases in the chilled ethanol starch "wash-off" that contain pentosans and soluble and insoluble protein. An improved understanding of the composition of the wash-off is important in the identification of co-product and process equipment and was sought during this period. Recovery of these components could have important implications to the overall attractiveness of the cold-ethanol method eg. the pentosans have specific food-ingredient and potential health benefits. Differences in both starch and protein functionality/composition between cold-ethanol-produced and water- produced vital products continue to be studied. Using the cold-ethanol process, gluten properties are generally better, and starch is more specifically fractionated into its morphological components, such as A- type starch, B-type starch. New processing capabilities have been acquired, installed, and tested by the team, including a solvent ready basket centrifuge, a solvent-ready decanter centrifuge, and capillary electrophoresis. Substantial progress was made in the development of thermoformed gluten polymers. A variety of crosslinking and formulation strategies were assessed for their impact on elastic properties of these pliable polymers. 5. Describe the major accomplishments over the life of the project, including their predicted or actual impact. This project developed the fundamental basis for optimalling separating wheat components in non-aqueous ethanol systems. It established significant new knowledge about the material and chemical properties of sensitive proteins and natural protein combinations following exposure to ethanol. This information is crucial to the development of the novel cold-ethanol method patented by WRRC. Specific accomplishments include: (1) discovery that the cold ethanol extraction method improves wheat gluten quality as determined in farinograph, mixograph, and baking evaluations. It maintains quality through severe drying conditions, (2) definition of the use of novel "dough-ball" and "dispersed-batter" methods for the production of wheat gluten concentrates, and (3) development of new fundamental knowledge about selective protein solubility and the composition of proteins in processing fluids applied to developed wet-dough. As a whole, these findings provide critical support for adoption of the cold-ethanol method as a processing technology that can produce improved wheat quality. This should lead to market advantages for domestically- produced wheat components. Further, the body of information developed within this project has recently stimulated interest by grain processing companies for further investigation. Advancement of this technology will require preliminary costing, further process definition and small pilot- scale evaluations. In addition, these findings help to improve the fundamental understanding of the chemical and functional properties of native and processed wheat fractions. The accomplishments were reported in 7 publications and one patent with additional manuscripts in preparation. Oral presentations and individual meetings with company representatives have been included in the ongoing effort to effect technology transfer. Substantially all scientific milestones have been, or will be addressed in these publications. This project was consistent with two components of the National Program 306 Action Plan (a) "New knowledge derived from improved understandings of the structure, properties, metabolism, and function of crop and animal components, particularly carbohydrates, proteins, and lipids, will generate development of a variety of new food, feed, and industrial products." and (b) "New technologies to convert commodities and processing byproducts into important value-added products such as fat substitutes, high-quality animal feeds, improved textiles, pharmaceutical ingredients, enzymes, and cosmetics will fill demonstrated needs." This project was consistent with two components of the National Program 307 Action Plan (a) "New technologies that integrate feedstock pretreatment, biological conversion and product recovery processes, as well as fundamental knowledge regarding fermentation, milling and membrane separations. The information gained will result in a reduction in capital and processing costs associated with biofuel production." (b) "Higher value coproducts generated from current low-value production byproducts. Envisioned coproducts include specialty oils, novel polysaccharides that will compete with imported gums, sugar alcohol food additives that are currently imported, enzymes, and inexpensive aquaculture feeds." 6. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end- user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products? The technology is currently available through patent and publications. Information has been conveyed to a number of interested companies through reference to web summaries, printed brochures and personal discussions at trade related meetings such as the Fuel Ethanol Workshop and the Corn Utilization and Technology Conference held this year. Particular targets have been grain-processing companies and design firms. Constraints: the separation technology affects many parameters of the overall process system and would require retirement of complex existing facilities and installation of new processing operations. Therefore, reliable data on product quality, drying properties, etc. and larger scale evaluations are essential to implementation. The technology may become available for small-pilot evaluation (outside WRRC) within the next 1-2 years as scale- up experiments proceed. 7. List your most important publications in the popular press and presentations to organizations and articles written about your work. "Creating and capturing total value of agricultural resources for biofuels and biobased products." 8pp. Posted at http://www.pw.usda.gov. Also distributed as brochure at trade meetings.

PUBLICATIONS (not previously reported): 1999/05 TO 2004/05
1. Robertson, G.H., Cao, T.K. Effect of processing on functional properties of wheat gluten prepared by cold-ethanol displacement of starch. Cereal Chemistry. 2003. v. 80. p. 212-217.
2. Robertson, G.H., Cao, T.K. Mixograph responses of gluten and gluten- fortified flour for gluten produced by cold-ethanol or water displacement of starch from wheat flour. Cereal Chemistry. 2002. v. 79. p. 737-740.
3. Robertson,G.H., Cao, T.K. Options and opportunities for Biorefining of Wheat. American Institute of Chemical Engineers. 2002. Abstract Page No. 293a.
4. Robertson, G.H., Cao, T.K. Altering gluten mixing and baking quality by method of separation and drying. American Association of Cereal Chemists. 2002. Abstract No. 419.

Item No. 2 of 59
ACCESSION NO: 0408878 SUBFILE: CRIS
PROJ NO: 5325-41000-045-00D AGENCY: ARS 5325
PROJ TYPE: USDA INHOUSE PROJ STATUS: TERMINATED
START: 12 MAY 2004 TERM: 06 JUN 2005 FY: 2005

INVESTIGATOR: ROBERTSON G H; OFFEMAN R D; ORTS W J; WOOD D F

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ENABLING TECHNOLOGIES FOR WHEAT STARCH AND PROTEIN SEPARATION, DRYING, AND UTILIZATION

OBJECTIVES: Devise large-scale technologies to enable the efficient and environmentally sound separation of wheat starch and protein from wheat flour, evaluate the quality of resulting products and co-products, and determine economic feasibility at different scales. Create and evaluate selective membrane barriers for the separation of ethanol-from-water and water-from-ethanol, and optimize appropriate films by applying chemical or enzymatic treatments.Develop models to improve separation strategies.

APPROACH: Invent improved separation concepts combining mechanical separation with fluid displacement methods based on the refrigerated ethanol method developed here, and evaluate scale-up potential.Apply the techniques to simulate pilot and commercial processes and produce large samples of gluten and starch. Use the fractions to quantify quality factors in conventional food uses (such as flour fortification) and in new uses (polymer formulations) that make use of the unique properties. Quality factors include drying rate of gluten; ease of fractionation, protein distribution, protein vitality, and functionality (vis-a-vis bread making). Analyze process potential for solubility-based subfractionation of the protein into gliadin and glutenin fractions.Test highly selective and permeable biomaterial films for their ability to separate ethanol from water and water from ethanol,ensuring that these films can withstand a large pressure drop encountered during industrial pervaporation.Optimize these films by chemical and enzymatic modifications. Formerly 5325-41000-037-00D (07/04).

PROGRESS: 2004/05 TO 2005/06
1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter? Over the past twenty years, exports of US wheat have been both unpredictable and declining. A potentially huge market for wheat is the biorefining and bioconversion of wheat into biobased products, including fine chemicals and fuel-grade ethanol. A key biorefining step is the fractionation of the wheat into starch and protein concentrates. Current separation

technologies, which produce concentrates of starch and gluten, are costly and have difficulty competing with foreign imports. Contributing to the expense of the commercial methods are: (1) outdated, nonstandard mill technologies; (2) the inefficient use of energy; and (3) the generation of copious amounts of wastewater. This project researches new and relevant separation technologies and systems. One of these is the cold-ethanol process invented at WRRC, in which ethanol serves as a process fluid to largely replace the use of water and reduce energy use. The cold-ethanol method of separation has the potential to reduce energy and water usage, leading to optimal use of all co-product streams. Another important technology is the separation of ethanol and water, which is important to the cold ethanol method, as well as to the eventual production of fuel-grade ethanol from surplus starch. The overall project goal is to improve wheat refining, increase wheat usage in bioproducts and biofuels (ethanol), and improve grain-to-ethanol processing efficiency. The total value of US wheat exports is 2/3 of its 1987 value, yet the total production has remained constant. The amount of wheat available for domestic non-food use is roughly 30 billion pounds and growing. Of this amount, the US gluten industry currently separates a little more than 2 billion lbs of wheat into starch and protein for food and non-food uses. This represents 58% of the total domestic gluten market; the rest of which is mostly imported from Europe due to their relative cost advantage. The potential of improving separation processes to improve starch and gluten separation would lead to increased domestic production of gluten, reduced imports, and improved attractiveness of all fractionated wheat components including starch for fuel-grade ethanol. Wheat starch as a fermentation feedstock could significantly expand grain-to-ethanol production in the US, help to reduce dependence on imported fuels and toxic oxygenates, and improve the nation's ability to produce ethanol in a wider range of diverse regional locations. Improved energy-efficiency through improved ethanol-water separations is crucial to the future of the wheat biorefinery. This is a bridging project from 5325-41000-037-00D to 5325-41000-047-00D. 2. List the milestones (indicators of progress) from your Project Plan. This project is being terminated and replaced by 5325-41000-047-00D. Please see the report for 5325-41000-047-00D for milestones. 3a List the milestones that were scheduled to be addressed in FY 2005. For each milestone, indicate the status: fully met, substantially met, or not met. If not met, why. 1. This project is being terminated and replaced by 5325-41000-047-00D. Please see the report for 5325-41000-047-00D for milestones. Milestone Substantially Met 3b List the milestones that you expect to address over the next 3 years (FY 2006, 2007, and 2008). What do you expect to accomplish, year by year, over the next 3 years under each milestone? This project is being terminated and replaced by 5325-41000-047-00D. Please see the report for 5325-41000-047-00D for milestones. 4a What was the single most significant accomplishment this past year? Recovery of ethanol from water solutions is critical for fuel ethanol production and use of ethanol as a biorefining solvent in wheat fractionation. The energy balance for ethanol production using standard technology, distillation, is energy-intensive. Solvent extraction and membrane permeation are two low-energy alternatives. Researchers at WRRC, Albany have screened a large number of solvents for performance in extracting ethanol, including a variety of biobased solvents, and have developed structure/performance correlations useful for prediction. This has led to discovery of several solvents with significantly better extraction performance than those typically considered for this application. 4b List other significant accomplishments, if any. The use of wheat as a platform to produce new biobased products and biofuels can be advanced with the ability to produce stable and enriched wheat gluten protein subfractions with non-varying compositions and properties. These could be used as platform chemicals. WRRC scientists have conducted extraction studies to assess the separation of wheat gluten components using ethanol and reduced temperatures that lower the risk of protein denaturation; hence, both food and non-food markets may be met. The data obtained by capillary electrophoresis of flour extracts provide composition maps useful to the design of separation processes. 4d Progress report. Wheat Fractionation: Wheat protein and protein fraction solubility at low temperatures has not been previously reported except for very broad classes of protein. This information was acquired to develop strategies for fractionation of narrow classes of protein that can potentially be recombined without loss of functionality. The adoption of the technologies being developed here are partially dependent on valuation of the fractions produced. This is especially true for wheat gluten intended for bread flour fortification. We have elicited collaboration from a domestic producer, importer, and supplier of wheat gluten and have processed about 75 kilo of flour to produce both water-based and cold-ethanol based gluten from two commercial sources of flour (the collaborator and a local ingredient supplier). Samples will be provided to the collaborator and others expressing interest. Ethanol-Extraction Solvents: The reasons why isomers of the higher alcohol solvents show differences in ethanol extraction performance are unknown. Molecular simulation is being used to determine if intermolecular associations between solvent molecules, and between solvent, water, and ethanol molecules, can explain these observations. Preliminary indications are very positive, and will be a significant improvement in understanding the fundamentals of solvent performance. Membrane

Permeation: Membrane processes for recovery of ethanol from water solutions are attractive due to their low energy requirements, compared to distillation. Currently available ethanol-selective membranes cannot economically compete with distillation because of their low throughput, requiring large membrane area. Supported liquid membranes, and mixed matrix membranes incorporating fine particles into the polymer, can have enhanced properties compared to standard polymer membranes. We have built an apparatus for measuring the permeation performance of membranes, and have instituted preparation of new membranes for evaluation. A patent application, Spiral-Wound Liquid Membrane Module for Separation of Fluids and Gases" has been filed. 5. Describe the major accomplishments over the life of the project, including their predicted or actual impact. This is a bridging project. Prior reports for the parent project may be found in annual reports for project 5325-41000-037-00D and the continuation is found in 5325-41000-047-00D. The parent project developed the fundamental basis of separability of wheat components in non-aqueous ethanol systems and established significant new knowledge about the material and chemical properties of sensitive proteins and natural protein combinations following exposure to ethanol. This information is crucial to the development of the novel cold-ethanol method patented by WRRC. Specific accomplishments include: (1) discovery that the cold ethanol extraction method improves wheat gluten quality as determined in farinograph, mixograph, and baking evaluations, and maintains quality through severe drying conditions, and (2) definition of the use of novel "dough-ball" and "dispersed-batter" methods for the production of wheat gluten concentrates, and (3) development of new fundamental knowledge about selective protein solubility and the composition of proteins in processing fluids applied to developed wet-dough. As a whole, these findings provide critical support for adoption of the cold-ethanol method as a processing technology that can produce improved quality. This should lead to market advantages for domestically-produced wheat components. Further, the body of information developed by us has recently stimulated interest by both process development and grain processing companies for further investigation. Advancement of this technology will require preliminary costing, further process definition and small pilot-scale evaluations. In addition, these findings help to improve the fundamental understanding of the chemical and functional properties of native and processed wheat fractions. The accomplishments were reported in 10 peer reviewed publications and one patent with additional manuscripts in preparation. Oral presentations and individual meetings with company representatives have been included in the ongoing effort to effect technology transfer. Substantially all scientific milestones have been, or will be addressed in these publications. This project was consistent with two components of the National Program 306 Action Plan: (a) "New knowledge derived from improved understandings of the structure, properties, metabolism, and function of crop and animal components, particularly carbohydrates, proteins, and lipids, will generate development of a variety of new food, feed, and industrial products." and (b) "New technologies to convert commodities and processing byproducts into important value-added products such as fat substitutes, high-quality animal feeds, improved textiles, pharmaceutical ingredients, enzymes, and cosmetics will fill demonstrated needs." This project was consistent with two components of the National Program 307 Action Plan: (a) "New technologies that integrate feedstock pretreatment, biological conversion and product recovery processes, as well as fundamental knowledge regarding fermentation, milling and membrane separations. The information gained will result in a reduction in capital and processing costs associated with biofuel production." and (b) "Higher value coproducts generated from current low-value production byproducts. Envisioned coproducts include specialty oils, novel polysaccharides that will compete with imported gums, sugar alcohol food additives that are currently imported, enzymes, and inexpensive aquaculture feeds." 6. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end- user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products? The technology is currently available through patent and publications. Information has been conveyed to a number of interested companies through reference to web summaries, printed brochures and personal discussions at meetings such as the Fuel Ethanol Workshop (June 2005) and the 4th Starch Utilization Technology Conference (June 2005). Scientists on the staff have been invited to make oral reports for staff of a principal firm involved with ethanol plants. Particular targets have been grain processing companies and design firms. Constraints: the separation technology affects many parameters of the overall process system and would require retirement of complex existing facilities and installation of new processing operations. Therefore, reliable data on product quality, drying properties, etc. and larger scale evaluations are essential to implementation. The technology may become available for small-pilot evaluation (outside WRRC) within the next 2-3 years as scale-up experiments proceed.

PUBLICATIONS (not previously reported): 2004/05 TO 2005/06

1. Wong, D., Batt, S.B., Lee, C.C., Robertson, G.H. Rapid selection of alpha- amylase mutants in yeast libraries for raw starch activity. 2003. Society of Biomolecular Screening Annual Conference, Portland, Oregon.
2. Wong, D., Batt, S.B., Lee, C.C., Robertson, G.H. Engineering apha-amylase for enhanced activity by molecular evolution. American Society of Biochemistry and Molecular Biology Annual Meeting, June 12-16, 2004, Boston, Massachusetts. Abstract No. B38.
3. Wong, D., Batt, S.B., Lee, C.C., Robertson, G.H. 2004. Direct screening of libraries of yeast clones for alpha-amylase activity on raw starch. Journal of Biomolecular Screening. 10:459-468.
4. Robertson, G.H., Cao, T. 2004. Proteins extracted by water or aqueous ethanol during refining of developed wheat dough to vital wheat gluten and crude starch as determined by capillary-zone electrophoresis (cze). Cereal Chemistry. 81(5):673-680.

Item No. 3 of 59
ACCESSION NO: 0409351 SUBFILE: CRIS
PROJ NO: 5325-41000-046-00D AGENCY: ARS 5325
PROJ TYPE: USDA INHOUSE PROJ STATUS: NEW
START: 24 MAR 2005 TERM: 23 MAR 2010 FY: 2006

INVESTIGATOR: WONG D; LEE C C; SMITH M R; WAGSCHAL K C; WOOD D F

PERFORMING INSTITUTION:
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EVOLUTIONARY ENZYME DESIGN FOR IMPROVED BIOREFINING OF CROPS AND RESIDUES

OBJECTIVES: Objective 1: Develop novel starch-degrading enzymes and enzyme-based systems that can convert starch from corn, wheat, and barley to glucose more efficiently. The aim is to improve activity at low pH, and increase catalytic conversion to lower the chemical and energy cost of liquefaction and saccharification. Objective 2: Develop novel enzymes and enzyme-based systems for solubilization and saccharification of hemicellulose components of corn fibers, crop residues, straws and grasses to simple sugars. Special focus will be to engineer high activity xylanases with decreased end-product inhibition and that can be produced in microbes, which make active cellulases. Objective 3: Utilize and evaluate the evolved enzymes and enzyme systems for the hydrolysis of corn, wheat, and barley starch (Objective 1) and solubilization and saccharification of hemicellulose components of corn fibers, crop residues, straws, and grasses (Objective 2) in laboratory and pilot scale operations. The improved enzymes will be evaluated on industrial substrates. Large-scale process development will be conducted in collaboration with corn ethanol companies.

APPROACH: New biocatalysts are created through directed evolution, in which libraries of enzyme variants are created by the mutation of encoding genes. The libraries are screened for target properties, and the candidate molecules selected can further undergo cycles of mutation, amplification, and selection until the desired enzyme variant is obtained. To create novel starch-degrading enzymes toward improved performance, the following steps are involved: (1) isolate, clone and express amylase genes; (2) mutate the gene sequences to create libraries; (3) screen the libraries for desired functions; (4) Purify the evolved enzymes for biochemical studies; (5) define the structure-function relationship; and (6) construct novel hybrid enzymes. To develop enzymes for the solubilization of hemicelluloses, the following approach is used: (1) screen by molecular techniques for a full set of genes encoding xylanolytic enzymes that are individually or collectively highly active in xylan degradation; (2) clone and express the genes in suitable expression systems; (3) conduct directed evolution by computational and experimental enzyme mutation; (4) purify the evolved enzymes for biochemical studies; (5) test the enzymes by combinatorial approaches; and (6) produce the enzymes in microbial systems which also produce active cellulases. As the best-fit enzymes are evolved and selected, the enzyme will be purified to homogeneity for the determination of kinetic properties. A variety of microscopic techniques will be applied to elucidate the action of the individual enzymes developed in this research. Several formats of bench scale enzyme reactions will be evaluated. These include liquefaction and saccharification as a distinct process, liquefaction and saccharification integrated with fermentation, and differentiated liquefaction and saccharification at high solids concentrations. Replacing 5325-41000-041-00D (February 2005).

PROGRESS: 2005/10 TO 2006/09

Progress Report 1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter? This project is aligned with National Program 307 Bioenergy and Energy Alternatives and also contributes to National Program 306 Quality and Utilization of Agricultural Products. The conversion of crop components--corn or wheat starch, grain fiber, and crop residue--to a fermentation feedstock for the production of high value products such as ethanol always involves partial disassembly of natural polymers to achieve solubility and full disassembly to simple sugars like glucose or xylose. For grain starches this is accomplished by cooking followed by a two-step enzyme reaction. For fibers and residues this begins with a process like acid hydrolysis and is followed by enzymatic conversion of the cellulose to glucose. These disassembly steps are energy intensive and costly. This research seeks to reduce or eliminate the disassembly costs by developing new enzymes for these tasks using the emerging methodology of directed molecular evolution. The goal is to provide alternate polymer disassembly methods for fuel and chemical fermentation and sweetener manufacture. Currently the US grain ethanol industry produces nearly 3 billion gallons of ethanol per year. This is from corn grain (85-90%) or from wheat grain (10-15%). Grains or their starch granules are cooked and enzymatically converted with the use of 10-15% of the fuel value of the ethanol from the fermentation. This energy could be saved by the elimination of the cooking step. Grain fibers and straw residues also may be disassembled to monomer sugars for fermentation feedstock, but even more energy-intensive and enzyme-intensive operations keep the cost higher than that for grains. These low cost and plentiful sources of fermentable sugars have been of interest because of their potential for increasing the contribution of renewable fuels in the United States. Energy cost reduction was the highest priority action item for ethanol producers at the 17th International Fuel Ethanol Workshop (2001). Conversion to glucose is also an integral part of other fermentation processes such as those leading to isomerized glucose and sweeteners. 2. List by year the currently approved milestones (indicators of research progress) Year 1 (FY 2005). For amylases: (1) Isolation, cloning and expression of amylase genes; (2) Initiate directed evolution experiments to create libraries of mutants; (3) Initiate screening of libraries for desired activity; (4) Initiate purification of evolved enzymes for biochemical studies. For hemicellulases: (1) Initiate isolation by molecular techniques for a full set of genes encoding xylanolytic enzymes that are individually or collectively highly active in xylan degradation. Year 2 (FY 2006). For amylases: (1) Complete cloning and expression of individual amylases; (2) Continue directed evolution on individual amylases based on initial biochemical studies; (3) Initiate construction of hybrid enzymes; (4) Continue purification of evolved enzymes for biochemical studies; (5) Initiate kinetic evaluation of evolved enzymes. For hemicellulases: (1) Continue screening more environmental genomic DNA libraries; (2) Clone and express the genes in suitable expression systems; (3) Initial development of high-throughput assays for the discovery of hemicellulases; (4) Initiate directed evolution experiments. Year 3 (FY 2007). For amylases: (1) Complete directed evolution on individual amylases; (2) Complete purification of evolved enzymes; (3) Complete construction of hybrid enzymes; (4) Complete kinetic evaluations on individual enzymes; (5) Initiate directed evolution on hybrid enzymes; (6) Initiate scale-up purification and characterization using bioreactors. For hemicellulases: (1) Continue screening of microbial genomic libraries for novel enzymes; (2) Complete cloning and expressing the key enzymes in xylan degradation; (3) Continue directed evolution experiments. Year 4 (FY 2008). For amylases: (1) Complete directed evolution on hybrid enzymes; (2) Conduct scale-up production; (3) Test new enzymes by combinatorial approaches; (4) Initiate pilot scale fermentation. For hemicellulases: (1) Complete screening and isolation of enzymes from genomic libraries; (2) Complete biochemical studies of individual enzymes; (3) Produce the xylanolytic enzymes in microbial systems that co-express active cellulases; (4) Initiate scale-up purification and characterization of enzymes individually and combinatorially. Year 5 (FY 2009). For amylases: (1) Continue testing new enzymes; (2) Continue studies on pilot scale fermentation; (3) Continue process evaluation. For hemicellulases: (1) Conduct development of microbial systems co-expressing active cellulases and xylanolytic enzymes; (2) Perform scale-up enzyme purification and characterization; (3) Optimize conditions and enzyme combinations for high efficiency biomass degradation. 4a List the single most significant research accomplishment during FY 2006. Starch-degrading enzymes work synergistically at low temperatures: This project is aligned with National Program 307 - Bioenergy and Energy Alternatives:Component 1: Ethanol and NP306 - Quality and Utilization of Agricultural Products: Component II.(d)New and Improved Processes and Feedstocks: A key energy intensive and costly step in the bioconversion of starch is high temperature cooking of the starch granules so that they can be fermented rapidly. BCE scientists cloned and characterized raw starch-degrading enzymes that act synergistically at low temperatures and eliminate the need for cooking. This research leads to markedly improved economy and efficiency in biofuel production from starches thus with the potential to increase markets for cereal crops and reduce dependence on foreign oil. 4b List other significant research accomplishment(s), if any. High-throughput assays validated for enzyme screening: This project is aligned with National Program 307 - Bioenergy and Energy Alternatives:Component 1: Ethanol and NP306 - Quality and Utilization of Agricultural Products: Component II.(d)New and Improved Processes and Feedstocks. A significant problem for making fuels and chemicals from biomass is the lack of suitable, highly efficient enzymes for breaking the biomass down so that it can be chemically or biologically convered. Furthermore, finding new enzymes for converting biomass into fuels and chemicals is hampered by the lack of appropriate high-throughput screening techniques . BCE scientists developed assays for the detection and characterization of enzymes using natural substrates and especially a high-throughput assay for screening enzymes for starch and biomass degradation and utilization. The screening technique will accelerate the discovery and isolation of improved enzymes that are key to the production of biofuels and chemicals. 5. Describe the major accomplishments to date and their predicted or actual impact. This project is aligned with National Program 307 - Bioenergy and Energy Alternatives:Component 1: Ethanol and NP306 - Quality and Utilization of Agricultural Products: Component II.(d)New and Improved Processes and Feedstocks. Scientific firsts for this project include: first expression of active barley- amylase in yeast, first expression of barley alpha-amylase in E. coli, first application of directed molecular evolution to plant source enzyme, first attempt to utilize directed molecular evolution on enzymes as large as those of the amylase family. Specific major project accomplishments include the following. (1) Isolated both low and high pI alpha-amylase genes from barley cDNA library. (2) Constructed the barley gene into suitable vectors to achieve expression in yeasts and E. coli. (3) Selected and identified peptide ligands that bind to barley low pI alpha-amylase. (4) Purified alpha-amylase and identified a putative raw starch binding domain. (5) Expressed the functional raw starch-binding domain in E. coli and yeast. (6) Developed a novel assay for monitoring enzymatic digestion of solid substrates. (7) Developed immunodetection methods to quantify recombinant amylase in individual clones, which enable the fine analysis of enzyme activities. (8) Isolated a glucoamylase gene, and cloned it into Saccharomyces for expression. (9) Construction and screening of libraries and tens of thousands of mutants leading to several enzyme clones with increase in total activity. (10) Isolated more than 20 novel genes of xylanolytic enzymes by direct screening of genome collections of microbial populations in unique ecological environments. (11) Developed novel solid-phase assays for functional selection of genes in metagenomic libraries. (12) Developed activity assays for alpha-xyloxidase and other xylanolytic enzymes using natural substrates that can be adapted to high-throughput formats. (13) Cloned and expressed several xylanolytic enzymes (endo-xylanase, alpa- xylosidase, arabinofuranosidase, ferulic acid esterase, acetylxylan esterase, and endoglucanase), some of which are bifunctional enzymes, in E. coli or yeast systems. 6. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end- user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products? We have established collaboration with a corn ethanol producer to test our improved enzymes. This work has collaborations with (1) faculties in the Animal Science Department and in the Department of Molecular and Cellular Biology at the University of California, Davis, and (2) faculties at the University of Saskatchewan. This work also has co- investigations with faculties of the University of Kentucky on State of Kentucky and NRI grants. Our work also results in Material Transfer Agreements with faculty members in Italy, and other scientists in the United States. A CRADA has been started this year working on key xylanolytic enzymes. A second CRADA is also in place, working on starch hydrolysis. 7. List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below). (1) "Break it Down Now" by Jessica Williams summarizing oral presentation at June 2005 meeting. January 2006 issue (http://www.ethanol-producer.com/article.jsp? article id=319&q=robertson&category id=37); and (2) Long summary of JAgFoodChem. "Native Starch Digestion..." Biofuels Journal. bySusan Reidy. January-March issue. 2006. Hemicellulose: (1) "USDA Researchers Modifying Yeast for Cellulosic Ethanol Production" Green Car Congress © 2006 BioAge Group, LLC (http://www.greencarcongress. com/2005/11/usda researcher.html); (2) A Mushrooming Approach for Biofuels, http://www. renewableenergyaccess.com/rea/news/story?id=40154; (3) Better ethanol production through GM technologyhttp://archives. foodsafetynetwork.ca/agnet/2005/11-2005/agnet.nov.30.html.

PUBLICATIONS (not previously reported): 2005/10 TO 2006/09

1. Wong, D., Batt, S.B., Lee, C.C., Wagschal, K.C., Robertson, G.H. 2005. Characterization of active lentinula edodes glucoamylase expressed and secreted by saccharomyces cerevisiae. Protein Journal. vol 24, pp 455-463.

2. Wong, D. Feruloyl esterase - a key enzyme in biomass degradation. 2005. Applied Biochemistry and Biotechnology, 133:87-112.
3. Wagschal, K.C., Franqui Espiet, D., Lee, C.C., Robertson, G.H., Wong, D. 2005. Enzyme-coupled assay for beta-xylosidase hydrolysis of natural substrates. Applied and Environmental Microbiology. 71:5318-5323.
4. Robertson, G.H., Wong, D., Lee, C.C., Wagschal, K.C., Smith, M.R., Orts, W. J. 2006. Native or raw starch digestion: A Key step in energy efficient biorefining of grain. Journal of Agricultural and Food Chemistry, 54:353- 65.
5. Smith, M.R., Zahnley, J.C. 2005. Production of amylase by arthrobacter psychrolactophilus. Journal of Industrial Microbiology and Biotechnology. 32:277-283:56
6. Smith, M.R., Zahnley, J.C. 2005. Characteristics of the amylase of arthrobacter psychrolactophilus. Journal of Industrial Microbiology and Biotechnology, 32:439-448.
7. Lee, C.C., Smith, M.R., Accinelli, R., Williams, T.G., Wagschal, K.C., Wong, D., Robertson, G.H. 2006. Isolation and characterization of a psychrophilic xylanase enzyme from flavobacterium sp. Current Microbiology, 52(2):112-116.
8. Wong, D., Robertson, G.H., Lee, C.C., Wagschal, K.C. 2006. Synergistic action of barley alpha-amylase and lentinula edodes glucoamylase on raw starch hydrolysis. Approved 10/27/2005. American Society of Biochemistry & Molecular Biology, San Francisco, CA. April 1-4, 2006, Program 73.16, vol A27.
9. Lee, C.C., Accinelli, R., Wagschal, K.C., Robertson, G.H., Wong, D. 2006. Cloning and characterization of cold-active xylanase enzymes. 28th Symposium on Biotechnology for Fuels and Chemicals. Paper No. 1A-41.
10. Robertson, G.H., Wong, D. 2005. Raw starch digestion or non-cooking breakdown of starch prior to fermentation. Fuel Ethanol Workshop and Trade Show, June 28, 2005, Kansas City, MO. Paper No.8:1.

Item No. 4 of 59
ACCESSION NO: 0067383 SUBFILE: CRIS
PROJ NO: CA-D*-MCB-3146-H AGENCY: CSREES CALB
PROJ TYPE: HATCH PROJ STATUS: REVISED
START: 01 OCT 2006 TERM: 30 SEP 2011 FY: 2006

INVESTIGATOR: Doi, R. H.

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STRUCTURE, FUNCTION, AND REGULATION OF CLOSTRIDIUM CELLUVORANS CELLULASE

NON-TECHNICAL SUMMARY: We will investigate the structure and function of the Clostridium cellulovorans cellulosome - an extracellular enzyme complex that can degrade plant cell walls. The efficient degradation of plant biomass can lead to alternate sources of fuel energy such as ethanol. The rate limiting step is the degradation of plant cell wall material by enzymes to sugars. Since the cellulosome contains a large number of cellulolytic enzymes and the composition can vary, we will determine which combination of enzymes can be most active in the degradation of plant cell walls. Furthermore we will try to convert non-cellulose degraders to cellulose degraders. This could lead to utilization of agricultural waste as inexpensive substrates for the synthesis of value-added products.

OBJECTIVES: Studies on synergism between Clostridium cellulovorans cellulosomal cellulases and hemicellulases to improve the function of cellulosomes. Production of more efficient C. cellulovorans cellulosomes by in vitro and in vivo methods. Designer cellulosomes will be constructed by genetic engineering and by growth of the organism on specific substrates. Conversion of non-cellulose utilizers to cellulose degrading organisms. We will convert Bacillus subtilis into a cellulose user. This could serve as a model system to convert industrially important organisms into cellulose utilizers and result in use of less expensive substrates for the production of valuable products.

APPROACH: We have cloned and sequenced five family 9 and two family 5 glycosyl hydrolase genes. We will add his tags to these genes and express and produce these enzymes in E. coli and purify each by nickel columns. We will test for synergy within and between family members to see which combinations may have the highest activity. We will then test the activity by combining with mini-scaffolding proteins (mini-CbpA) to form mini-cellulosomes. We will also produce mini-cellulosomes containing cellulases and hemi-cellulases and test their activity on artificial and natural lignocellulose substrates. These tests should demonstrate which combinations are most active on pure cellulose substrates and on natural substrates such as corn fiber. Preliminary experiments have shown that growth of C. cellulovorans on different carbon sources yield cellulosomes with different enzymatic compositions. We will obtain cellulosomes grown on cellulose, xylan, and pectin and combine the cellulosomes and test the activity of the combination on natural substrates. These tests should determine whether a combination of natural cellulosomes would be more effective in degrading natural substrates. We are currently attempting to express C. cellulovorans cellulosomal genes in Bacillus subtilis. We will transform mini-CbpA gene and cellulase genes, engB and engE and hemicellulase gene, xynB, into B. subtilis. We hope that B. subtilis will express these genes and make mini-cellulosomes. This will be a first step in demonstrating that B. subtilis can be converted into a cellulose utilizer. This should serve as a model system for the conversion of non-cellulose utilizers into cellulose utilizers. This may have an important impact in reducing the substrate costs for organisms used in industry.

PROGRESS: 2006/01 TO 2006/12
A large amount of agricultural waste such as rice straw, corn stalks, and wheat straw is currently produced in this country. If these cellulosic materials could be degraded to sugars, and the sugars fermented to ethanol, it would solve many problems in terms of the environment and energy, Our research is continuing on the enzymatic degradation of lignocellulosic materials by investigating the structure, function, regulation, and assembly of the cellulosome from Clostridium cellulovorans. The cellulosome is an extracellular enzyme complex that is capable of degrading cellulose and hemicellulose, the major components of plant cell walls. Our recent studies have shown that the cellulosome is not a homogeneous enzyme complex, but consists of many subpopulations of cellulosomes that differ in terms of enzymatic composition. The subunit composition varies depending on the growth substrate. Thus the organism is capable of degrading a variety of plant cell walls that it may encounter. In another study we have found that an apparent redundant population of family 9 cellulosomal endoglucanases actually allows the cellulosome to attack substrates with differing structures. In addition the products that were produced by the family 9 endoglucanases also were varied indicating that the family 9 enzymes attacked substrates in a differing manner and increased the versatility of the cellulosome. Further studies on the interaction of cellulosome with non-cellulosomal enzymes indicate that there is synergistic activity between the cellulosome and non-cellulosomal enzymes. The non-cellulosomal hemicellulases apparently attack the plant cell wall and expose the cellulosic components and facilitates the activity of the cellulosome. These studies will allow us to construct more efficient designer cellulosomes with greater capacity for degrading lignocelluloses.

IMPACT: 2006/01 TO 2006/12
These studies will lead to better methods for disposing and utilizing cellulosic agricultural and forest wastes. The enzymatic digestion of plant cell wall materials to sugars and the conversion of the sugars to ethanol will provide biofuel. These methods will help to keep the environment cleaner and will lead to use of plant biomass for biofuels that could partially reduce our dependence on foreign petroleum fuels.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
1. Arai, T., Kosugi, A., Chan, H., Koukiekolo, R., Yukawa, H., Inui, M. and Doi, R.H. 2006. Properties of cellulosomal family 9 cellulases from Clostridium cellulovorans. Appl. Microbiol Biotechnol. 71: 654-660.
2. Kosugi, A., Arai, T. and Doi, R.H. 2006. Degradation of cellulosome-produced cellooligosaccharides by an extracellular non-cellulosomal ?-glucan glucohydrolase, BglA, from Clostridium cellulovorans. Biochem. Biophys. Res. Commun. 349:20-23.

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Item No. 5 of 59

ACCESSION NO: 0211297 SUBFILE: CRIS
PROJ NO: CA-D*-VIT-7693-CG AGENCY: CSREES CALB
PROJ TYPE: NRI COMPETITIVE GRANT PROJ STATUS: NEW
CONTRACT/GRANT/AGREEMENT NO: 2007-35504-18332 PROPOSAL NO: 2007-02140
START: 01 SEP 2007 TERM: 31 AUG 2010 GRANT YR: 2007
GRANT AMT: $455,000

INVESTIGATOR: Block, D. E.

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A SYSTEMATIC STUDY OF THE EFFECT OF CELL LIPID BILAYER COMPOSITION ON RESISTANCE TO ALCOHOL-INDUCED CHANGES

NON-TECHNICAL SUMMARY: While production of biofuels such as ethanol and butanol through fermentation presents an attractive alternative to traditional petroleum-based liquid fuels, low alcohol tolerance of production microorganisms has proven to be a limiting factor in the economic feasibility of these processes. The purpose of this study is to improve alcohol tolerance in production microorganisms by altering the composition of the cell membrane lipid bilayer in a systematic manner.

OBJECTIVES: We are proposing to extend our studies of the effects of cell membrane composition on the interdigitation response to alcohol, leading to a better understanding of the components of alcohol tolerance. This will allow us to rationally select yeast and bacterial strains for higher alcohol production. We will pursue the following four related objectives to achieve our technical goals: (1) Correlation of membrane characteristics with ability to complete fermentations. Phospholipid headgroup composition, fatty acid chain length and saturation level, and ergosterol level will be measured for a series of fifteen common wine yeast strains from our existing fermentation database and for several Clostridium strains of various butanol tolerance levels. Multivariate statistics will be used to identify the most critical composition factors for alcohol tolerance. (2) Identification of the role of membrane components most associated with resistance to alcohol induced changes, e.g. interdigitation, using purified lipid/sterol bilayers. Purified lipids and sterols will be used to construct and evaluate model cell membranes in the presence of ethanol and higher alcohols. Atomic Force Microscopy (AFM) and fluorescence will be used to look for interdigitation and other microstructural changes as a function of composition. As a new technology, fluorimeter experiments will be developed to identify interdigitation in cell membranes to feed into aim 4, leading to a new high throughput method for alcohol tolerant strain selection. (3) Identification of membrane components most associated with resistance to alcohol induced changes using a multiscale simulation approach. Systematic multiscale molecular simulations will be performed to analyze the effects of membrane composition on resistance to interdigitation and other structural changes in the presence of ethanol and higher alcohols. Multiscale simulations ensure that all relevant length scales are treated correctly. (4) For Objective 1, we propose to first analyze the lipid composition of all 15 commercial yeast strains in our database using established chromatographic/GC methods. This analysis will also be performed on several C. acetobutylicum strains with increased butanol tolerance. Once the data on lipid composition is collected for all of the strains, datamining methods will be used to examine the relationship between the concentrations of individual components of the cell membranes and the kinetic and alcohol tolerance parameters measured for all strains.

APPROACH: For Objective 1, we propose to first analyze the lipid composition of all 15 commercial yeast strains in our database using established chromatographic/GC methods. This analysis will also be performed on several C. acetobutylicum strains with increased butanol tolerance. Once the data on lipid composition is collected for all of the strains, datamining methods will be used to examine the relationship between the concentrations of individual components of the cell membranes and the kinetic and alcohol tolerance parameters measured for all strains. For Objective 2, we propose to study the impact of ethanol, propanol, and butanol on ternary and quaternary lipid model membrane systems containing ergosterol. The major lipid class in yeast membrane is phosphatidylcholine (PC), thus we will mainly focus on ternary and quaternary mixtures containing PC headgroups and acyl chain variations (chain length, degree of (un)saturation) and ergosterol. We will determine the partitioning of ergosterol by the behavior of the DPPC domains (i.e. do they behave like a fluid or a solid) and napthopyrene partitioning. In this stage, AFM and FM of SLBs and FM of GUVs will be used. Subseqently, alcohol will be added, and we will look for interdigitation using AFM, changes in microstructure (using both AFM and FM), and changes in napthopyrene partitioning. In addition, we plan to study the impact of inclusion of key lipid head groups which are associated with ethanol tolerance, namely negatively charged phosphatidylinositol (PI) and phosphatidylserine (PS). In Objective 3, atomistically detailed simulations to elucidate the effects of alcohols on the bilayer structure will be extended from our preliminary work. For this kind of modeling we rely on a highly accurate lipid model. Structural changes in the bilayer resulting from alcohol will be characterized, e.g. by order parameter, area per headgroup, degree of interdigitation and bilayer thickness, and compared to experiments. We will focus on the comparison of low molecular weight alcohols (up to butanol) to understand the underlying mechanisms of the experimentally observed Traube rule. We are additionally going to measure the influence of alcohol on the structure, including the binding sites of the alcohols, and on dynamic quantities, like diffusion and lipid rotation. In Objective 4, we will construct proof-of-concept yeast and Clostridium strains with improved alcohol tolerance in order to clearly assess the feasibility for application in a biorefinery setting. For yeast, we plan to genetically modify strains of Saccharomyces to overproduce or underproduce specific membrane components. We will chose existing commercial yeast for manipulation, one with relatively low ethanol tolerance and one with relatively high tolerance. For each of these strains, genetic modifications will be made, that, based on the information from the above objectives, should increase ethanol tolerance and other modifications that should decrease ethanol tolerance. For Clostridium, we will take a similar approach with similar targets. Genetically altered strains will be assessed for alcohol tolerance and production kinetics.

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Item No. 6 of 59
ACCESSION NO: 0185940 SUBFILE: CRIS
PROJ NO: CALK-2000-03113 AGENCY: CSREES CALK
PROJ TYPE: SMALL BUSINESS GRANT PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 00-33610-9455 PROPOSAL NO: 2000-03113
START: 01 SEP 2000 TERM: 31 AUG 2003 FY: 2003 GRANT YR: 2000
GRANT AMT: $265,000

INVESTIGATOR: Kelly, J.

PERFORMING INSTITUTION:
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LOW COST FUELS PRODUCTION FROM AGRICULTURAL WASTES USING CHEMICAL REGENERATION

NON-TECHNICAL SUMMARY: Bioconversion of ligenocellulosic wastes to fuels and chemcials requires pretreatment ot enhance conversion and reduce overall costs. Texas A&M University (TAMU) has tested an effective and low cost lime pretreatment process that can ben3efit from lime regeneration. Altex has identified the Low Cost Lime Regenerator (LCLR), which is more compact, less costly and more efficient than conventional regeneration systems. Based on equipment supplier cost inputs, the LCLR, lime regeneration concepts in a 2 TPD test system.

OBJECTIVES: Demonstrate, at a scale of 2 TPD, the capability of the Low Cost Lime Regeneration (LCLR) system to regenerate lime for use in the Texas A&M University (TAMU) bioconversion system.

Determine regeneration quality and costs, bioconversion pretreatment effectiveness and system captial and operating costs. Conclude on commercial potential of the system.

APPROACH: TAMU will process the biomass in their proprietary bioconversion process, using their pilot-scale reactor with lime to pretreat the biomass. This will generate precipitated calcium carbonate (PCC) that will have to be regenerated. The PCC will then be formed into pellets, using special low cost binders, and then processed in Altex's pilot-scale special kiln into a reactive lime product. During kiln processing, exhaust emissions and fuel use will be monitored to determine pollution potential and energy use. Equipment manufacturer input on estimated capital costs, and operating cost estimates derived from project results will then be used to determine system cost benefits.

PROGRESS: 2000/09 TO 2003/08
The regeneration of lime mud derived from a biomass pretreatment process was demonstrated using the Low Cost Lime Regenerator (LCLR) system by Altex, and lime mud received from Texas A&M University (TAMU). For reference, identical tests were also performed on lime mud from a paper plant, furnished by the Weyerhaeuser Corporation. The LCLR process consists of two critical steps. Spent lime mud is first pelletized using a commercial clay extruder, then calcined in a vertical shaft kiln. Extrusion tests have shown that lime mud from the biomass pretreatment process extrudes as well as paper plant lime mud and forms good quality pellets. In the green state, lime mud pellets have excellent handling characteristics and are readily conveyed using conventional equipment. Shaft kiln processing requires good pellet strength. Paper plant lime mud pellets have the necessaty strength because sodium contaminants in the lime mud act as an effective high temperature binder. However, the sodium content of lime mud from biomass pretreatment is much less. To increase the sodium content to a level comparable to that of paper plant lime mud, a lignosulfonate was added to the TAMU lime mud prior to pelletizing. LCLR pellet strength test results showed that, with the binder addition, TAMU lime mud pellets were just as strong as the paper plant lime mud pellets, and can therefore survive shaft kiln processing. Also, ASTM reactivity test results showed that a high quality lime product was produced, with reactivity times of less than three minutes. The ultimate test of the LCLR product was its performance in the TAMU biomass pretreatment process. A sample of LCLR regenerated lime mud pellets was returned to TAMU where samples of switchgrass and bagasse were pretreated with the LCLR lime regenerated at Altex and compared with identical samples pretreated with a commercial lime. A comparison of the resultant sugar yields showed that the LCLR lime is as effective as commercial lime. These results demonstrate the capability of the LCLR process to regenerate lime mud from a biomass pretreatment process. Relative to conventional rotary kilns the LCLR system has the following important benefits. Uniform robust pellets are produced that, because of uniformity, can be optimally processed in a shaft kiln. Pellet forming equipment is simple, inexpensive, and durable. The organic energy content in the mud is fully recovered, reducing fuel costs by 50 percent, and these organics are fully oxidized, eliminating hydrocarbon pollutants from the exhaust. Shaft kilns are simple with few moving parts, can be heavily insulated for high efficiency, are low in cost, and unlike rotary kilns, are filled with the process solids, making them more compact. Fuel burning occurs within the bed, and in the presence of solids, for uniform and efficient combustion with low emissions (less than 40 ppm CO and 40 ppm NOx, versus 200 ppm NOx for rotary kilns). Also, due to pellet forming and shaft kiln processing, dust and related control problems are reduced. The greatest benefit of the LCLR is a 55 percent reduction in cost, relative to those for a rotary kiln.

IMPACT: 2000/09 TO 2003/08
Wherever the LCLR system is implemented, it will have many positive benefits on the production of biomass waste derived fuels. Lime pretreatment will be less costly. Lime regeneration with the LCLR system reduces the cost of producing alcohol to $0.55/gal. This is considerably less costly than ethanol from corn ($1.40/gal) and MBTE ($0.90/gal). The current fuel market for ethanol derived from corn is 1 billion gallons per year. Assuming that the market for lignocellulose derived fuels is the same size, then application of the process would result in cost savings to the industry of approximately $800 million per year. These are substantial economic benefits that will help drive the production of alcohol from waste biomass. Also, the process would consume 10 million tons/year of wastes, leaving behind a residue that is only 28 percent of the volume of the original waste. Therefore, applying the process will extend landfill use by a factor of 3.5. In addition, a lime pretreatment method avoids the hundreds of thousands of tons of waste produced per year by acid pretreatment processes, or by processes that do not regenerate the lime. These waste reductions will decrease disposal costs, which can exceed $15/ton, and the associated negative

environmental impacts. Lastly, NOx and CO2 greenhouse gas emissions would be reduced by over 100,000 lbs/yr and 400,000 tons/yr, respectively, using the LCLR process rather than a conventional lime kiln.

PUBLICATIONS (not previously reported): 2000/09 TO 2003/08
1. United States Patent No. 5,824,244 Kelly, et al. October 20, 1998
2. United States Patent No. 5,865,898 Holtzapple, et al. February 2, 1999

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Item No. 7 of 59
ACCESSION NO: 0200576 SUBFILE: CRIS
PROJ NO: CALK-2004-02681 AGENCY: CSREES CALK
PROJ TYPE: SMALL BUSINESS GRANT PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2004-33610-15001 PROPOSAL NO: 2004-02681
START: 01 SEP 2004 TERM: 31 AUG 2007 GRANT YR: 2004
GRANT AMT: $296,000

INVESTIGATOR: Clough, R. C.

PERFORMING INSTITUTION:
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CELLULASES FOR BIOMASS CONVERSION FROM THE TRANSGENIC MAIZE PRODUCTION SYSTEM

NON-TECHNICAL SUMMARY: The production of ethanol from lignocellulosic biomass can utilize large volumes of agricultural residues that are untapped today. Ethanol fuels burn cleanly, thus the use of ethanol for partial replacement of petroleum fuels will have a positive impact on the environment. The production of ethanol fuels from lignocellulosic biomass is ready to be developed into a viable industry. Our results will show that the maize production system can meet the sensitive cost targets for cellulase enzyme production for the biomass to ethanol industry. In addition, they will show that maize has the flexibility to produce any new cellulase enzymes that are engineered to have improved performance on the vast array of lignocellulosic substrates that can be utilized in this industry.

OBJECTIVES: This Small Business Innovation Research Phase II project is designed to demonstrate the feasibility of cost-effective conversion of lignocellulosic biomass to sugars. The project has two major objectives--to demonstrate the activity of the transgenic corn-derived enzymes (from Phase I) on cellulosic and pretreated lignocellulosic material, and to demonstrate the breadth and power of the recombinant production system by expressing an array of cellulases in maize seed.

APPROACH: We will produce grain from maize lines identified in Phase I experiments that express high levels of cellulase. Enzyme will be extracted from this grain, concentrated, and used in application tests. In addition, other cellulase genes will be expressed in the recombinant maize system. A breeding program will be initiated to select for high expressing lines.

PROGRESS: 2004/09 TO 2007/08
The principal objective in Phase II was to develop convincing data that a maize-produced cellulase could be a viable alternative to supply the large volume and low cost requirements for the conversion of lignocellulose biomass into ethanol. This centered on 1) developing a production model for supplying enzymes including economic parameters that predict the expression requirements of cellulase in grain, 2) demonstrating that maize lines that are capable of expressing cellulase at these concentrations, and 3)

demonstrating that the maize-produced cellulase will work on complex insoluble cellulose similar to what would be encountered in cellulose biomass. A model for using maize-produced cellulase for ethanol conversion was developed to illustrate the advantages and requirements that this may have over other systems. This model included the assumptions for expression levels required to be competitive for the biomass conversion of ethanol. This model estimated that concentrations in the range of 1g cellulase/kg grain would be required to meet the minimum economic incentives assuming that the maize-produced enzyme was the sole source of enzymes for ethanol conversion. Using transgenic material containing cellulase, activity of the enzymes was routinely examined using a soluble substrate. In addition, chemical and biological assays were conducted to evaluate activity on insoluble cellulase. These assays confirmed activity of maize-produced cellulase on insoluble cellulose and future work is planned to correlate this on a quantitative basis with the soluble assay results used for routine screening. Five generations of cellulase-expressing plants were grown and selected based on expression levels in the seed. This included using elite germplasm and crossing multiple alleles into the same plant. The expression levels were increased up to five-fold in bulk inbred seed. Using the production schemes with hybrid lines, we would expect to raise expression an additional two-to-four fold. These results demonstrated the potential for these seeds to reach the expression target assumed in the production model. Alternative constructs were made in an attempt to enhance expression. These included using alternative promoters, targeting sites and alternative genes. However, as of this date, they did not provide an increase in the expression in T1 seed over what had been shown earlier. Based on our results we are now in discussion with three different companies to commercialize a maize-produced cellulase. We expect to enter Phase III of this project with at least one of these companies under a contractual agreement signed by the end of this year.

IMPACT: 2004/09 TO 2007/08
The enzymes for cellulose conversion into ethanol are required at large volumes and low cost. The findings from this study indicate that it may be feasible to supply the necessary enzymes from maize. Having the enzymes produced from a crop that is also used to produce ethanol offers the possibility for a fully integrated system. This will not only have the potential to reduce cost but will also put less stress on the environment since there is no need to produce separate crops for cellulose and separate production systems for the enzymes.

PUBLICATIONS (not previously reported): 2004/09 TO 2007/08
1. John Howard and Elizabeth Hood. 2007. Methods for growing nonfood products in transgenic plants. Crop Sci.47:1255 to 1262.
2. Elizabeth E. Hood, Robert Love, Jeff Bray, Richard Clough, Kamesh Pappu, Carol Drees, Kendall R. Hood, Sangwoong Yoon, Atta Ahmed, and John A. Howard. 2007. Subcellular targeting is a key condition for high-level accumulation of cellulase protein in transgenic maize seed. Plant Biotechnology (in press).

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Item No. 8 of 59
ACCESSION NO: 0202388 SUBFILE: CRIS
PROJ NO: IDA01291 AGENCY: CSREES IDA
PROJ TYPE: HATCH PROJ STATUS: NEW
START: 01 JUL 2004 TERM: 30 JUN 2009 FY: 2006

INVESTIGATOR: Shrestha, D. S.

PERFORMING INSTITUTION:
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FUEL FROM OIL CROPS: PROTECTION, FEASIBILITY AND TECHNOLOGY

NON-TECHNICAL SUMMARY: Environmental and energy advantages of using vegetable oil for biodiesel production is unknown. B Biodiesel quality is an issue that must be address to increase its utilization and acceptance. This research will justify the use of Pacific Northwest oil crops for biodiesel production. Farmers will be benefited by knowing how much area to devote for oil production for their profit optimization. This research is also directed to develop a quick, reliable and economical sensor which can be used to quickly assess biodiesel quality.

OBJECTIVES: 1. Study the feasibility of Pacific Northwest (PNW) oil crops and its derivatives to predict the future of biodiesel production from these feedstocks. 2. Improve biodiesel compatibility with diesel engines.

APPROACH: Experiment 1: Life cycle analysis for energy and environment assessment of oil crops and biodiesel production In this study energy and environmental analysis for locally grown major oil crops in Pacific Northwest region will be carried out. Various forms of energy going into the production versus total energy coming out of a crop will be compared in terms of amount of energy and price. Research will be conducted in cooperation with farmers and farmer associations. A research proposal will be written and submitted to various donor organizations. Direct and indirect sources of energy inputs in various oil crops production will be studied. Direct energy is required to perform various tasks related to crop production processes such as land preparation, irrigation, intercultural operation, harvesting and transportation of agricultural inputs and processing. Indirect energy, on the other hand, consists of the energy used in the manufacture, packaging and transport of fertilizers, pesticides and farm machineries. Energy output from the crop includes energy contained in biodiesel, byproduct glycerol, oil cake and biomass. Energy as well as price of inputs to outputs will also be considered. Opportunity cost of oil crops will be calculated to estimate net gain in economical sense by using oil to produce biodiesel. Experiment 2: Biodiesel characterization for suitability in running a diesel engine Biodiesel from different feed stocks will be exposed under electromagnetic radiation sources of different frequencies. Depending on size and concentration of molecule compositions in the biodiesel, some wave bands will be absorbed more than others. This will produce a modified transmitted and refracted radiation spectrum. We expect that depending on biodiesel composition, the measured spectrum characteristics will vary as it is exposed to the electromagnetic radiation of various wave lengths ranging from microwaves to infrared. The reflectance and refractance of the oil will be measured using radiation spectrum sensor. The oil composition will also be determined as per ASTM specified standard methods to correlate with the measured spectrum. A multivariate statistical analysis will be carried out to correlate between spectrum absorbance of biodiesel to its inherent constituents. After the transmitted and refracted spectrum measurement, the engine performance will be tested. Brake horse power, emission characteristics and other important engine performance parameters will be measured to correlate fuel quality with measured spectrum. In addition to pure biodiesel, biodiesel with different fuel additives especially to reduce NOx will be studied. Spectrums will be measured with different fuel additives and successive engine testing will be performed. As far as condition permits, both two and four stroke engines will be tested in this experiment to study the usefulness of biodiesel in running diesel engines.

PROGRESS: 2006/01 TO 2006/12
Biodiesel field sensor development This research is to develop affordable field sensors that could be used 1) to detect percent biodiesel in biodiesel-diesel blend and 2) to measure free and total glycerol in pure biodiesel. Use of ultra violet light showed a good promise in detecting blend level regardless of diesel and biodiesel type use. Two conference papers were published and one paper has been submitted for journal publication from this research. An application has been made to patent the technology. A prototype of the field sensor is being developed and industrial partners are being sought to fund further research in this area. Glycerol sensing method used a chemical reaction that produces heat and measure of the temperature rise which is then correlated to glycerol content. This research is taking the ground but more research is needed to improve the initial research results. One graduate student is doing the research to perfect the technique. If successful, this technology will sense the alcohol content along with total and free glycerin. Biodiesel Utilization Issues This research is focused to understand the cold flow behavior of biodiesel. I have investigated the effect of different fuel additives to improve cold flow and based on the results, published a conference proceeding and a tech-note. It was concluded that the commercial cold flow fuel additives available in the market was ineffective in lowering the gelling point of biodiesel. Life cycle analysis of biodiesel It has been argued by some researchers that the production of biodiesel requires more fossil

energy than the energy contained in the fuel itself. If that is the case, biodiesel is a non renewable source of energy. This is an important issue to ensure that the bioenergy is renewable source of energy to make it sustainable. One technical presentation, one technical review and one conference paper have been published from this research. A research grant was received from USDA to update the life cycle energy balance study report. One journal paper is being submitted form the review of past researches with partially updated data. Biodiesel Education The purpose of this project is to educate the public about biodiesel, its benefits and limitations. I have given several presentations in conferences, workshops and seminar. I have also given several unofficial presentations to the tour group at UI biodiesel research lab and answered numerous questions to the public about biodiesel. Precision agriculture Besides assigned research area for year 2006, I have extended my research area to include precision agriculture. A proposal was submitted to USDA's Animal and Plant Health Inspection Service (APHIS) to develop an automated soil sampler which will be used to conduct a national soil survey. Consequently I received a grant from USDA to develop an automated soil sampler to collect soil samples from a potato harvester. The prototype is under development and two undergraduate students are working on this project.

IMPACT: 2006/01 TO 2006/12
Biodiesel field sensor development Need of research on biodiesel quality was rated as number 1 among all research needs for biodiesel by the experts in the field. One of the problems that the producers, the distributors and the users of the biodiesel are concerned about is its quality assurance. I envision that these inexpensive field quality sensors will enhance the acceptance of biodiesel and more quality fuels in the market means fewer fuel related problem. This would definitely increase the demand for biodiesel. Biodiesel Utilization Issues Unfavorable cold flow property and higher oxides of nitrogen (NO_x) emissions are two most important issues with biodiesel. NO_x is a serious air pollutant and green house gas which limits its use in urban areas. Success of this research will have a big effect on acceptability of biodiesel without any doubt. Life cycle analysis of biodiesel This is an important issue to ensure that the bioenergy is renewable source of energy to make it sustainable. Biodiesel Education Awareness about biodiesel both for consumers, producers are crucial in success of the biodiesel. Biodiesel education program educates people about benefits of biodiesel to make them readily accept biodiesel as alternate fuel and producers to run a successful biodiesel industry that produces quality fuel. Precision agriculture Precision agriculture has great benefits of reducing application of agro chemicals while improving yield. With ever increasing demand on higher productivity the importance of precision agriculture can not be over emphasized.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
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2. A. Zawadzki, D. S. Shrestha, B. He, 2006. Biodiesel blend level detection using ultraviolet absorption spectra. Applied Engineering in Agriculture
3. Pradhan, A. and D. Shrestha. 2006. An update on life cycle study of soybean oil biodiesel production. ASAE Paper No. 066142 St. Joseph, Mich.: ASAE.
4. Zawadzki, A. and D. Shrestha. 2006. Ultraviolet absorption spectra for biodiesel quality sensing. ASAE Paper No. 063043 St. Joseph, Mich.: ASAE.

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Item No. 9 of 59
ACCESSION NO: 0405277 SUBFILE: CRIS
PROJ NO: 3620-41000-101-00D AGENCY: ARS 3620
PROJ TYPE: USDA INHOUSE PROJ STATUS: TERMINATED
START: 16 FEB 2002 TERM: 08 AUG 2004 FY: 2004

INVESTIGATOR: ERHAN S Z; LIU Z

PERFORMING INSTITUTION:

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CHEMICAL SYSTEMS FOR THE CONVERSION OF VEGETABLE OILS TO INDUSTRIAL PRODUCTS

OBJECTIVES: Convert vegetable oil (emphasizing soybean oil) for industrial materials by polymerizing, by improving the oxidative stability and by derivatizing unsaturated acids for increased reactivity. Utilize these compounds in sheetfed, heatset, flexographic and gravure printing ink formulations, in interior/exterior architectural paint formulations and as a base stock in hydraulic fluids and lubricants.

APPROACH: Modify chemical and physical properties of soybean oil or its fatty acids to enhance their use as additives, or major components of lubricants, plastics, inks, surface coatings and other industrial materials. Use isomerization and addition reactions to form liquid products having improved thermal and oxidative stability and increased reactivity. Develop 100% soy oil-based sheetfed, heatset, flexographic and gravure printing inks. Improve the quality of existing architectural (interior/exterior) oil based paints by using soybean oil and solving the yellowing problem. Continue to develop soy oil based base stock for hydraulic fluids and lubricants. Test chemical, physical and rheological properties of the products and compare with existing commercial (petroleum based) products. Develop (1) reliable methodology for characterizing the products, (2) costeffective technologies and (3) preliminary marketing data.

PROGRESS: 2002/02 TO 2004/08
1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter? The major use of vegetable oils (such as soybean oil) is directed to food products, salad and cooking oils, shortenings, and margarines in particular. Less than 3% is utilized in non-food applications, since a number of former vegetable oil markets are lost to petroleum products. In the years following World War II, petroleum derived products rapidly displaced vegetable oil-based materials. Certain performance and technical properties can be easily achieved using seed oil derivatives. Such products based on agro raw materials are at par with available petroleum based products in the market. The importance of natural products for industrial applications becomes very clear with increasing social emphasis on the issues of environment, waste disposal, and depleting non-renewable resources. Seed oils and products based on seed oil derivatives suffer from some technical drawbacks such as oxidation, cold flow behavior and extended stability. For applications such as composites, lubricants, plastics, fuel, and chemical intermediates; soybean oil and other vegetable oils are too viscous and too reactive toward atmospheric oxygen to establish significant markets. Limitations in molecular weight and crystallization properties restrict the uses of vegetable oils in solvents, plastics, fuels, and industrial fluids. In addition to above problems, this research project will address low volatile organic chemicals (VOC), high biodegradability in ink formulations, and low or no VOC improved oxidative stability/low temperature properties in biodegradable lubricants. U.S. Environmental Protection Agency (EPA), Clean Air Act Amendment, and U.S. Department of Labor, Occupational Safety & Health administration, (OSHA) regulations exert ever growing pressure on the graphic arts (inks, paints, coatings), lubricants and hydraulic oils, detergents, emulsifiers, and the polymer industries on limiting the use of organic solvents, creating less toxic waste, using a renewable resource, and producing more biodegradable products. Parties affected by these regulations and parties interested in enhanced marketability of soybean (American Soybean Association, United Soybean Board) and other oilseed crops will use the results and/or products of this research project to expand the market for these commodities. This project falls within component "New Processes, New Uses, and Value- Added Foods and Biobased Products" of National Program 306. This project establishes new bio-based fluid technology and their feasibility for industrial applications. This project will have direct impact on the utilization of local grown seed oils (mainly soybean oil) for non-food applications and therefore improve the local agro-economy. The research project will identify new oil-based industrial products and optimize conditions for laboratory scale up followed by field tests in close collaboration with industrial partners. 2. List the milestones (indicators of progress) from your Project Plan. Milestones and expected outcomes: Modifications, formulations and laboratory testings of the products will be completed by the end of the third year and scale-up, field testing and publications/patents will be completed at the end of the fifth year. Progress will be documented by publications in peer-reviewed journals, presentations at technical meetings, in-house annual progress reports and if applicable, patents. All of the above milestones have been accomplished and two vacant Research Chemist positions are filled. 3. Milestones: A. List the milestones (from the list in Question #2) that were scheduled to be addressed in

FY 2004. How many milestones did you fully or substantially meet in FY 2004 and indicate which ones were not fully or substantially met, briefly explain why not, and your plans to do so. A new project plan was certified by the Office of Scientific Quality Review as having completed NP 306, Quality and Utilization of Agricultural Products Panel Review on 7/15/04. In addition to completing the milestones from the expiring plan, preliminary experiments for the new project plan were conducted. B. List the milestones (from the list in Question #2) that you expect to address over the next 3 years (FY 2005, 2006, & 2007). What do you expect to accomplish, year by year, over the next 3 years under each milestone? This is the final Report of Progress (AD-421) for this project, which has been replaced with project 3620-41000-117-00D. The new project plan was certified by the Office of Scientific Quality Review as having completed NP 306, Quality and Utilization of Agricultural Products Panel Review on 7/15/04. The milestones of the new project plan are listed below: First year 1. Seed oil-based metalworking fluid for aluminum hot rolling will be formulated. 2. Complete initial preparation of grease samples with Li metal, fatty acids and check for consistency and smooth paste-like texture conforming to NLGI specifications. 3. Complete preparation of synthetic lubricant basestocks using Epoxidized vegetable oils, long chain/branched alcohols and catalyst; and further reaction on above product using acid anhydrides. 4. Complete the reaction optimization of vegetable oils with different sulfur compounds (which may or may not contain an amine functionality) using a catalyst (perchloric acid) to obtain product in higher yields and characterization using nuclear magnetic resonance (NMR), Fourier transformed infrared (FTIR) and pressurized differential scanning calorimetry (PDSC). 5. Complete intial preparation of composite samples with non-coated flax fibers using compression molding method and study their mechanical and water absorption properties. 6. Complete the sample preparation. Thermo and mechanical properties will be studied. 7. Hydrogels prepared from ring-opening polymerization and hydrolysis of obtained polymers will be studied for their temperature sensitive and pH sensitive properties. 8. Epoxidized soybean oil (ESO) will be cured will polyamine compounds to form sheets for mechanical, thermophysical properties tests. 9. Ring opening polymerization will be carried out using Lewis catalysts. Second year 1. Seed oil-based metalworking fluid for aluminum hot rolling will be tested for various performance and tribochemical properties in-house. 2. Characterization of grease samples for their penetration, oxidation and thermal stability using PDSC, micro-oxidation, oven test, rotary bomb oxidation test (RBOT). 3. Complete ring-opening reaction of epoxidized seed oil followed by esterification of resulting hydroxyl group to give lubricant base fluid. Characterize the chemically modified vegetable oil fluids for their oxidative stability and pour point and check to see if the process significantly improved the low temperature fluidity and thermal stability of the oil. 4. Complete the reactions of vegetable oils with different amines using same catalyst to synthesize bio-based anti-wear, anti-oxidants additives and characterization using NMR, FTIR and PDSC. 5. To prepare composites samples reinforced with coated flax fibers and to characterize their binding properties using transmission electron microscope (TEM). Three point bend and tensile tests will be performed on samples fabricated under task 1 (first year) using noncoated fibers and coated fibers. Compare their mechanical and water absorption properties. Evaluate polyamine curing agents. 6. Optimum clay loading, curing conditions and study the structures of clay dispersion in the host polymers. 7. Study drug loading and release rate. Effect of dissolution media such as temperature, pH on the release rate will be investigated. 8. Complete material selection and formulation. Glass transition temperatures (Tgs) will be determined using differential scanning calorimetry (DSC). 9. Initiator evaluation for ring opening polymerization will be performed. Optimization of polymerization conditions necessary to obtained polymers in high yield will also be investigated. Third year 1. Tribochemical evaluation and physical property determination of metal working fluids will continue based on feed back from Cooperative Research and Development Agreement (CRADA) partner. 2. Develop methods to study wear-friction properties of grease samples. Try to establish structure-property relationship using above data. 3. Complete the formulation and blending of chemically modified vegetable oil fluids with other seed oils and suitable PPD, anti-oxidant and anti- wear additives. Evaluate the final products for pour point, viscosity, viscosity index, oxidative stability using PDSC, friction and wear characteristics using Falex to study the effect of structural variation in the oil molecules. 4. Complete the reactions of vegetable oils with different alcohol-amines using same catalyst to synthesize bio-based anti-wear, anti-oxidants additives and characterization using NMR, FTIR and PDSC. 5. Optimization of curing agents for epoxidized soybean oil and curing conditions such as pressure, temperature, and reaction time; study of fiber loading influence on the mechanical properties of composites; and analysis of water swelling data of composites. 6. Work will continue on the characterization of composites using x-ray and TEM. 7. Study drug release kinetics followed Fickian law or nonFickian law. 8. Damping and falling weight properties of soybased materials will be evaluated. 9. Hydrolysis of obtained soybean oil based polymers using sodium hydroxide to remove glycerin small molecule, purification of the samples for gel permeation chromatography (GPC) analysis.

Surface tension and contact angle measurements are performed. The critical micelle concentration (CMC) will be determined. 4. What were the most significant accomplishments this past year? A. Single most significant accomplishment during FY 2004: A soy oil based elevator hydraulic fluid technology was developed based on a request from National Park Service (Statue of Liberty, Ellis Island, NY). The bio-based fluid was developed at the NCAUR facility of USDA and independently evaluated by industrial partner (OTIS elevator). Subsequently the fluid was scaled up and retrofitted in the Statue of Liberty elevator and has been successfully operating for nearly two years. A product license has been applied for by a leading lubrication company for commercial production of the fluid. Based on this technology development activity, the NCAUR/USDA team has been awarded the FLC-2004 award for "Excellence in Technology Transfer". B. Other significant accomplishment(s), if any: 1. A US patent 6,583,302, "Chemically modified vegetable oil-based industrial fluid" was granted. 2. A U.S. Patent 6,528,571 "Extrusion Freeform Fabrication of Soybean Oil- based Composites by Direct Deposition" was granted. 3. A CRADA with Advanced Ceramic Research, Tucson, AZ, has been signed. C. Significant activities that support special target populations: None. 5. Describe the major accomplishments over the life of the project, including their predicted or actual impact. Technologies developed by the involved scientists and currently undergoing commercial development include lithographic news ink, sheet- fed and heat-set inks consisting of pigments and 100% vegetable oil (soy oil) based vehicles. U.S. Patent (5,122,188) has been awarded for news ink technology, and the potential domestic market is more than 500 million pounds per year for this technology. Ink prepared by this technology and commercial news inks consisting of vehicles prepared with petroleum resin base and either mineral oil or vegetable oil solvents were evaluated for their potential biodegradation. Gravimetric (using mixed cultures of soil microorganisms) and Modified Sturm Test (using activated sludge) methods were used. With both methods, USDA's ink degraded faster and more completely than either hybrid soy oil based or petroleum based inks. Also, comparison of de-inking properties and analysis of Volatile Organic Compound (VOC) content (using method 24, 24A, and 30) showed the superiority of vegetable oil based inks over petroleum resin based inks. For sheet-fed and heat-set ink, the market is more than 100 million pounds and 400 million pounds per year, respectively. Sheet-fed and heat- set web offset ink vehicles were prepared in the absence of petroleum products. Inks with these vehicles were formulated and tested for the physical properties, and the results were comparable to conventional mineral oil based vehicles. U.S. Patent (5,713,990) has been awarded for sheet-fed and heat-set ink technologies. Total non-food use of soybean oil is only 300 million pounds per year. The above ink technologies alone present an opportunity to triple the market. A company is in the process of completing the licensing application. 6. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end- user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products? Ink and hydraulic elevator fluid technologies have been transferred to a company. Two Cooperative Research and Development Agreements signed. One Cooperative Research and Development Agreement in progress. Three U.S. Patents allowed. Two U.S. Patent applications. One U.S. Patent application in process Two Material Transfer Agreements. These products will be available to the public in a year. 7. List your most important publications in the popular press and presentations to organizations and articles written about your work. Erhan, S.Z., Adhvaryu, A., Liu, Z. 2003. Chemically Modified Vegetable Oil-based Industrial Fluid. U.S. Patent 6,583,302 Erhan, S.Z., Adhvaryu, A., Sharma, B.K. 2004. Poly (hydroxy thioether) Vegetable Oil Derivatives Useful as Lubricant Additives. U.S. Patent Application S/N 10/887,127.

PUBLICATIONS (not previously reported): 2002/02 TO 2004/08

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2. Sharma, B.K., Adhvaryu, A., Liu, Z., Erhan, S.Z. 2004. Tribological and oxidation properties of chemically modified vegetable oils as lubricants. Annual Meeting and Expo of the American Oil Chemists' Society. p. 78.
3. Shogren, R.L., Petrovic, Z., Liu, Z., Erhan, S.Z. 2004. Biodegradation behavior of some vegetable oil-based polymers. Polymers and the Environment. 12(3):492351. p. 173-178.
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5. Erhan, S.Z., Liu, Z. 2004. Soybean oil based composites. International Soybean Processing and Utilization Conference. p. 738-744.

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Item No. 10 of 59
ACCESSION NO: 0408515 SUBFILE: CRIS
PROJ NO: 3620-41000-110-00D AGENCY: ARS 3620
PROJ TYPE: USDA INHOUSE PROJ STATUS: NEW
START: 03 MAY 2004 TERM: 02 MAY 2009 FY: 2006

INVESTIGATOR: SKORY C D

PERFORMING INSTITUTION:
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PATHWAY ENGINEERING OF FUNGI FOR IMPROVED BIOPROCESS APPLICATIONS

OBJECTIVES: The overall objective of this project is the genetic modification of filamentous fungi for improved synthesis of value added products. The primary emphasis will focus on improving the efficiency of lactic acid production by Rhizopus oryzae through metabolic engineering. Additionally, the techniques developed in this research will be used to assess the potential of using this industrially robust organism for directed protein expression.

APPROACH: Utilize metabolic engineering technologies to develop fungal strains with improved production capability for lactic acid. The general approach of this work is to sequentially increase enzymatic activities responsible for the production of lactic acid, while reducing those involved in the production of unwanted byproducts. The effects of these changes will be examined through traditional methods (i.e., fermentations, enzymatic activities, and northern data), as well as some of the newer genomic and metabolic flux analyses that will be incorporated into our work. Additionally, we will continue to develop methods for improved genetic recombination and RNA interference in Rhizopus. We will also design and employ strategies for the production and secretion of recombinant proteins of industrial interest using the fungus Rhizopus. Initial efforts of this work will include development and study of promoter/terminator constructs in Rhizopus. We will determine the importance of copy number compared to transcription promoter strength. Lastly, much of the work is to be focused on the necessary requirements for efficient folding and secretion. BSL-1 (2) Recertified February 15, 2007.

PROGRESS: 2005/10 TO 2006/09
Progress Report 1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter? Fossil fuels are by far the most exploited natural resource used today. The whole infrastructure of modern society is built upon oil, which has a limited reserve. Petroleum serves as the foundation for not only most of the USA's energy needs, but also for many products, such as solvents, organic acids, and numerous polymers and plastics. In 2004, the USA consumed about 5.7 billion barrels of petroleum with approximately 2-5% being used for

the production of plastics and solvents (Department of Energy (DOE) Petroleum Supply Annual 2004). Although many benefits to society are derived from the use of petroleum, potential problems, in the form of litter, landfill depletion, environmental pollution, global warming, and increased atmospheric carbon dioxide are associated with its use. Means to help alleviate these problems include the development of viable technologies for the production of industrial chemicals using renewable feedstocks (e.g., starch or cellulosic biomass) and the development of more efficient fermentation processes. Finding new uses for agricultural crops and improving the efficiency of how we utilize them is imperative for competing in today's global market. The broad objective of the CRIS Work Unit (CWU) is to develop improved technologies and novel systems for increasing yields and reducing costs of converting agricultural commodities/residues to value-added products (e.g., biofuels and organic acids) through enzyme and fermentation technology. The CWU concentrates primarily on studying the genetic control mechanisms for production of several metabolites and enzymes by the filamentous fungus Rhizopus oryzae. This fungus is used for large- scale manufacture of lactic acid. However, problems exist with fermentations because of synthesis of several unwanted byproducts that must be separated from the final product and decrease the overall yield of lactic acid. This fungus is also recognized as a robust organism that is routinely used for industrial enzyme production (e.g., glucoamylase, lipase, peptidase). Currently, its use is limited by the enzymes that the organism naturally produces. We have developed technologies that can now be used to genetically manipulate this fungus so that a host of useful proteins can be produced. This research contributes to the objectives of National Programs 306, Quality and Utilization of Agricultural Products. Corn and its processing byproducts (e.g., corn stover and corn fiber) will initially serve as the primary agricultural crop for this research, although technology is applicable to alternative crops. Customers of this work include: producers/consumers of lactic acid or its end-products (e.g., polylactic acid (PLA) plastic); industries requiring enzymes (e.g., biofuels, biomass conversion, food and feed, specialty chemicals); corn processors; new markets for agricultural commodities; agricultural community; scientific community with interests in fungal metabolic engineering; and general public. Cooperative Research and Development Agreements (CRADA) with appropriate companies are currently used to transfer products of this research. Much of the research focuses on the production of lactic acid because of the potential market growth of the biodegradable plastic, polylactic acid, and solvent ethyl lactate. Domestic production of lactic acid is greater than 50,000 tons/yr and will likely increase exponentially if the growth for PLA and ethyl lactate develops as expected. However, fermentation efficiency must be improved to ensure the economic feasibility of the anticipated market expansion and to ensure that the U. S. maintains its leadership role. Additionally, considerable effort is being directed towards new technologies for improved enzyme production, which currently are estimated to have annual sales in excess of $1 billion. The ability to genetically improve the production of these enzymes, as well as other novel recombinant enzymes (e.g., cellulase, xylanase), is expected to have significant implications. Furthermore, the genetic knowledge gained from this work can easily be adapted to aid research with numerous other fungi that have industrial value. 2. List by year the currently approved milestones (indicators of research progress) Year 1 (FY 2004) Test feasibility of using Rhizopus oryzae for expression of desired proteins. Continue to modify expression of various genes involved in the synthesis of lactic acid and various unwanted byproducts. Year 2 (FY 2005) Begin study of protein secretion pathways for Rhizopus. Continue study of synthesis pathways for unwanted products formed during lactic acid production. Develop improved methods of integration and gene-knockout. Year 3 (FY 2006) Design improved methods of protein secretion. Apply technology of gene integration and gene-knockout to ameliorate production of unwanted by-products. Initiate development of biochemical and analytic tools for monitoring the metabolic state of Rhizopus during fermentation. Initiate work to investigate inhibitors of lactic acid production in Rhizopus. Year 4 (FY 2007) Utilize new analytical techniques to study modified strains and identify new targets for further improvements in lactic acid production and protein synthesis. Begin testing of pilot and large scale fermentations of modified strains. Identify targets to overcome inhibition of lactic acid production. Year 5 (FY 2008) Test improved protein production systems using several enzymes with industrial interest. Study methods to improve product stability during lactic acid production. Utilize technology of gene modification to improve lactic acid production in the presence of inhibitors. Continue pilot and large scale testing of modified strains. 4a List the single most significant research accomplishment during FY 2006. INHIBITION OF GENE EXPRESSION USING RNA INHIBITION - performed under NP 306, Quality and Utilization of Agricultural Products. Lactic acid is produced by fermentation technology using renewable agricultural products and has significant potential for market growth as a result of new interest in the biodegradable plastic, PLA. Rhizopus is a filamentous fungus that is currently used for industrial scale production of lactic acid, but also accumulates several unwanted by-products that decrease the potential yield and necessitate further purification. We have identified methods to decrease expression of genes and should be able to apply this technology to eliminate by-products forming through other metabolic pathways. The results of this work are currently being exploited for the development of recombinant strains that will be tested by a major lactic acid producer. This accomplishment directly addresses National Program 306 - Quality and Utilization of Agricultural Products (100%), in particular Component 2, New Processes, New Uses, and Value-Added Foods and Biobased Products. 4b List other significant research accomplishment(s), if any. IMPROVED METHODS OF PROTEIN PRODUCTION BY RHIZOPUS - performed under NP 306, Quality and Utilization of Agricultural Products. Rhizopus is recognized as a robust organism that is routinely used for industrial enzyme production (e.g., glucoamylase, lipase, peptidase), but its use is limited by the enzymes that the organism naturally produces. We continued our development of expression vectors to allow secretion of important proteins with Rhizopus. Furthermore, these vectors contain binding sites that will be of potential benefit for purification of the final protein. The application of this technology is expected to have direct benefits to numerous industries involved in production of enzymes, including those involved in biomass conversion. 5. Describe the major accomplishments to date and their predicted or actual impact. A major thrust of this project has been to use molecular biology techniques for the development of microbial strains with improved lactic acid production. We have isolated several genes from Rhizopus that are associated with lactic acid formation and more recently have been collaborating in the sequencing of Rhizopus genome. Numerous methods have been developed to introduce genetically modified genes back into the fungal host in order to decrease production of unwanted byproducts and increase accumulation of lactic acid. Each modification of a particular enzymatic conversion step resulted in varying degrees of improvement for fermentations using this fungus. This work has been so successful that it led to a two Cooperative Research and Development Agreements (CRADA) with a major industrial partner over the last seven years. We have also begun to utilize some of these molecular techniques to investigate the ability to express proteins of interest in Rhizopus. This work has been very promising and also resulted in the formation of a CRADA with a midwestern biotechnology company to express important industrial enzymes. 6. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end- user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products? We have had a CRADA for the last seven years with a major industrial partner so that they may assist in our efforts to increase lactic production in the fungus Rhizopus. Strains modified for improved synthesis of lactic acid were routinely transferred as part of this CRADA. The numerous techniques developed by our laboratory served as a foundation to allow the formation of another CRADA with a major midwestern biotechnology company to develop improved methods of enzyme synthesis using R. oryzae. Several of our expression systems have been tested with various genes from the CRADA partner. Ultimately, the farmer and consumer will benefit from all of this research, since it relies on renewable agricultural materials for the final product. Other scientists also benefit from this work, since we often assist other laboratories by providing strains, DNA, and knowledge from our research.

PUBLICATIONS (not previously reported): 2005/10 TO 2006/09

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Item No. 11 of 59
ACCESSION NO: 0408950 SUBFILE: CRIS
PROJ NO: 3620-41000-121-00D AGENCY: ARS 3620
PROJ TYPE: USDA INHOUSE PROJ STATUS: NEW
START: 19 SEP 2004 TERM: 18 SEP 2009 FY: 2006

INVESTIGATOR: BISCHOFF K M; HUGHES S R; LIU S

PERFORMING INSTITUTION:
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MICROBIAL CATALYSTS TO PRODUCE FUEL ETHANOL AND VALUE ADDED PRODUCTS

OBJECTIVES: The broad goal of the proposed research is to develop new microorganisms and biocatalysts that can be employed in the fermentative conversion of renewable agricultural materials to fuels and other value-added products. The research entails engineering existing fermentative microorganisms to possess desirable traits for industrial fermentation of lignocellulosic material or searching for new microorganisms that possess these traits.

APPROACH: Novel microorganisms, genes, and enzymes will be sought that can be employed in the fermentative conversion of agricultural commodities into biofuels and bioproducts. Specific approaches include: (1) application of high throughput screening procedures to develop, by use of directed evolution and gene shuffling, microbial strains and enzymes with superior ability to convert agricultural materials to biofuels and bioproducts, (2) apply metabolic engineering and genetic manipulation methods to existing and newly discovered/developed microbial strains to improve on ability to perform in an industrial fermentation environment and to optimize production of desired products, and (3) determine the potential for use of microorganisms from extreme environments (e.g., extreme thermophiles, halophiles, acido/alkalophiles) as biotechnological agents. BSL-1; Recertified February 9, 2006.

PROGRESS: 2005/10 TO 2006/09
Progress Report 1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter? The effective bioconversion of agricultural materials to fuels and other valuable products has been encumbered by the relatively small number of organisms suitable for use under harsh (industrial) environments and by the gaps in our understanding of the biosynthetic pathways that produce these products. New microorganisms and enzymes are needed that utilize lignocellulosic biomass to produce the desired products in high yield under conditions of extreme temperature, pH, osmotic pressure, and concentrations of substrate or end-products. These new biocatalysts may be developed through the application of metabolic and molecular screens to identify novel organisms and genes for exploitation. Once identified, the candidate organisms, genes, or enzymes can be evaluated, new expression systems developed, and bioconversion strategies optimized for production of biofuels and other microbial products. The development of new biocatalysts that will function in industrial environments will benefit not only commercial partners but farmers as well by providing new markets for agricultural based feedstocks. Expansion of the production of fuel ethanol, in particular, would reduce the nation's dependence on foreign oil and improve the environment by developing alternate energy sources from renewable resources. The broad goal of this research project is to develop new microorganisms and biocatalysts that can be employed in the fermentative conversion of renewable agricultural materials to fuels and other value-added products. The research entails engineering existing fermentative microorganisms to possess desirable traits for industrial fermentation of lignocellulosic material, and searching for new microorganisms that possess these traits. The specific objectives of the project are as follows: 1) create efficient xylose-fermenting Saccharomyces cerevisiae strains; 2) engineer lactic acid bacteria to make ethanol, and 3) determine the potential for microorganisms from extreme environments to serve as biotechnological agents in the fermentation industry. This research is expected to increase the efficiency of conversion of biomass to liquid fuel, and to discover new uses for agricultural by-products. Therefore, portions of the research fall under National Program 307 (Bioenergy and Energy Alternatives) and under National Program 306 (Quality and Utilization of Agricultural Products). Specifically, the research contributes to Component I (Ethanol) of the NP 307 Action Plan and to Problem Area 2b (New Uses for Agricultural By- products) of the NP 306 Action Plan. 2. List by year the currently approved milestones (indicators of research progress) Year 1 (FY 2005) 1.1. Construct functional proteomic robotic workcell. 1.2. Cloning pyruvate decarboxylase gene from Sarcina ventriculi and Zymobacter palmae. Year 2 (FY 2006) 2.1. Construct library of shuffled XI genes. 2.2. Transformation of pdc shuttle vector constructs into lactic acid bacteria. 2.3. Confirmation of transformants and examination of enzymatic activities. 2.4. Screening G. stearothermophilus strains for growth on xylan substrates. Year 3 (FY 2007) 3.1. In vitro transcription of XI library. 3.2. Construct Pichia library. 3.3. Characterization of xylan utilization enzymes and cloning of respective genes. Year 4 (FY 2008) 4.1. Mass transformation of S.

cerevisiae with Pichia library and high throughput screening. 4.2. Transform S. cerevisiae strains with shuffled XI clones. 4.3. Evaluate recombinant XI yeast strains and Pichia transformed strains for anaerobic growth on xylose and EtOH production. 4.4. Construct stable strains by chromosomal integration of plasmid- encoded genes from strains producing ethanol. 4.5. Inactivation of undesired genes like als, ldh, ack, and pdh if needed. 4.6. Application of new biocatalysts against natural xylan substrates (corn fiber). Year 5 (FY 2009) 5.1. Integration of optimized strains to large scale fermentations. 5.2. Analyze and evaluation of strains for ethanol and other value- added products production. 5.3. Genome shuffling and screening for best combinations of genes required for ethanol. 5.4. Integration of new biocatalysts in large scale fermentations. 4a List the single most significant research accomplishment during FY 2006. Construction of a Proteomic Workcell. The Ethanol component of the NP307 Action Plan seeks to reduce the cost of producing ethanol from biomass through technological advances in enzymology and microbiology, and in chemical, biochemical and process engineering. One approach to identifying new biocatalysts is to use an automated high-throughput strategy to screen tens of thousands of candidates for improved variants. In collaboration with Hudson Control Group, Inc., Springfield, NJ, scientists in the Bioproducts and Biocatalysis Research Unit at the USDA-ARS-National Center for Agricultural Utilization Research, Peoria, Illinois have developed a plasmid-based proteomic workcell that integrates all of the molecular, microbiological, and biochemical techniques used for the high-throughput strategy into a single robotic platform. Mechanical construction of the workcell is complete, and operational protocols have been tested using a multiplexed mutagenesis strategy of the CelF endoglucanase enzyme from the anaerobic fungus Orpinomyces PC-2. This workcell will ultimately be used for identifying important xylose-utilization genes and for improving strains of yeast that ferment xylose to ethanol. 4b List other significant research accomplishment(s), if any. Co-expression of ethanol production genes in lactic acid bacteria. The Ethanol component of the NP307 Action Plan seeks to reduce the cost of producing ethanol from biomass through technological advances in enzymology and microbiology, and in chemical, biochemical, and process engineering. Lactic acid bacteria are well suited to industrial fermentation environments and have the potential to be developed into biocatalysts for the production of ethanol from agricultural feedstocks. Scientists in the Bioproducts and Biocatalysis Research Unit at the USDA- ARS-National Center for Agricultural Utilization Research, Peoria, Illinois have genetically modified a strain of Lactobacillus brevis to express genes required for ethanol production. Genes for pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) were successfully cloned and expressed in L. brevis, but ethanol production in recombinant strains did not increase. Significant optimization and engineering of metabolic pathways in lactic acid bacteria remains to be explored for the development of new biocatalysts to convert agricultural materials to biofuels. Purification and characterization of thermophilic cellulase. The Ethanol component of the NP307 Action Plan seeks to reduce the cost of producing ethanol from biomass through technological advances in enzymology and microbiology and in chemical, biochemical, and process engineering. Thermophilic bacteria are a potential source of robust enzymes for use in the fermentation industry. Scientists in the Bioproducts and Biocatalysis Research Unit examined a collection of thermophilic bacteria for enzymes that degrade cellulose and hemicellulose. An endoglucanase enzyme from a thermophilic strain of Bacillus licheniformis was purified, identified, and characterized. The broad pH range and thermophilic properties of this enzyme may prove suitable for application in the conversion of biomass to glucose for production of fuel ethanol or other valuable fermentation products. Antimicrobial susceptibility of bacterial contaminants from fuel ethanol plants. The Ethanol component of the NP307 Action Plan emphasizes the need for technologies to reduce the cost of producing ethanol from cornstarch. Bacterial contamination of commercial fermentation cultures is a common and costly problem to the fuel ethanol industry. Scientists in the Bioproducts and Biocatalysis Research Unit have examined bacterial species isolated from a wet-mill and from a dry-grind ethanol plant for susceptibility to penicillin and virginiamycin, two antimicrobial agents commonly used to control contamination. Isolates from the dry-grind plant were less susceptible to this virginiamycin than isolates from the wet-mill plant, but most isolates had minimum inhibitory concentrations lower than the maximal application rate used for virginiamycin. This information is important to ethanol plant managers and will help guide intervention strategies that control bacterial contamination. 5. Describe the major accomplishments to date and their predicted or actual impact. The plasmid-based proteomic workcell developed by scientists in the Bioproducts and Biocatalysis Research Unit represents an advancement in the field of laboratory automation. The robotic integration of microbiological, molecular, and biochemical techniques will facilitate the development of improved biocatalysts for converting biomass to fuel and valuable products. The workcell will also find broad application in other fields of agricultural and pharmaceutical biotechnology. 6. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end- user (industry, farmer, other scientists)?

What are the constraints, if known, to the adoption and durability of the technology products? Information derived from this research has been disseminated to producers, industry representatives, and other scientists through the presentation of data at national and international scientific meetings. Two provisional patents covering the workcell and a third covering its associated biology have been written and will be placed in conjunction with the completion of the workcell. Presently, our collaborative partner has constructed a second integrated robotic platform that has been sold for use in the pharmaceutical industry.

PUBLICATIONS (not previously reported): 2005/10 TO 2006/09

1. Hughes, S.R., Riedmuller, S., Mertens, J.A., Li, X., Bischoff, K.M., Cotta, M.A., Farrelly, P. 2005. Development of a liquid handler component for a plasmid-based functional proteomic robotic workcell. Journal of the Association for Laboratory Automation. 10(5):287-300.
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5. Hughes, S.R., Riedmuller, S., Mertens, J.A., Li, X., Qureshi, N., Bischoff, K.M., Jordan, D.B., Cotta, M.A., Farrelly, P. 2005. Plasmid-based functional proteomic robotic workcell process for high-throughput screening of multiplexed libraries of mutagenized clones [abstract]. Optimization high-throughput Cultures for Bioprocessing 2005. p. 3.
6. Hughes, S.R., Riedmuller, S., Li, X., Qureshi, N., Liu, S., Bischoff, K.M., Cotta, M.A., Farrelly, P. 2006. Mass transformation of plasmid libraries of cdna or mutagenized clone sets into yeast or bacteria using a functional proteomic robotic workcell [abstract]. PepTalk 2006. p. 10.
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9. Bischoff, K.M., Skinner-Nemec, K., Leathers, T.D., Hughes, S.R. 2005. Antimicrobial susceptibility of bacterial contaminants from a wet-mill ethanol plant [abstract]. Society for Industrial Microbiology. Poster P42.
10. Bischoff, K.M., Li, X., Rooney, A.P., Liu, S., Hughes, S.R. 2005. Characterization of carboxymethylcellulase activity from Geobacillus stearothermophilus [abstract]. Society for Industrial Microbiology. Paper #P38.
11. Bischoff, K.M., Skinner, K.A., Leathers, T.D. 2006. Antimicrobial susceptibility of lactobacillus species isolated from fuel-ethanol [abstract]. American Society for Microbiology. Poster No. O-038.
12. Liu, S. 2006. Genetically engineered lactic acid bacteria for the production of fuels and chemicals [abstract]. 10th International Symposium on the Genetics of Industrial Microorganisms. Paper #015.

Item No. 12 of 59
ACCESSION NO: 0210649 SUBFILE: CRIS
PROJ NO: ILLU-000-531 AGENCY: CSREES ILLU
PROJ TYPE: NRI COMPETITIVE GRANT PROJ STATUS: NEW
CONTRACT/GRANT/AGREEMENT NO: 2007-55618-18160 PROPOSAL NO: 2007-02945
START: 01 SEP 2007 TERM: 31 AUG 2010 GRANT YR: 2007
GRANT AMT: $399,001

INVESTIGATOR: Agarwal-Tronetti, R.; Khanna, M.; Sonka, S.; Long, S.; Jain, A.

PERFORMING INSTITUTION:
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ENVIRONMENTAL, ECONOMIC AND BUSINESS OPPORTUNITIES IN THE BIOFUELS INDUSTRY FOR SMALL/MEDIUM FARMS

NON-TECHNICAL SUMMARY: To be viable substitutes for fossil fuels, alternative energy sources such as biofuels should be economically competitive and environmentally sustainable. This project intends to 1) provide a detailed assessment of land use and environmental implications of biofuels from multiple feedstocks; 2) explore the industry evolution of the emerging cellulosic-based biofuels sector; and 3) examine how the liability of smallness may be mitigated through inter-firm cooperation.

OBJECTIVES: We propose to conduct an integrated, interdisciplinary research project that will contribute to the CSREES strategic goal of enhancing economic opportunities for agricultural producers. The research results and associated outreach efforts will examine how present and future development of the biofuels industry in the US can be exploited to the advantage of small and medium-sized farmers. There are agronomic and logistic characteristics of potential feedstock sources which may be favorable for small and medium-sized farmers. The logistical challenges of producing, assembling and transporting feedstocks suggest that there may be a need for new organizational entities to provide these functions. The collective efforts of small and medium-sized farmers are one means by which this gap could be satisfied. Therefore, it is critically important that research be focused on the strategic issues and constraints associated with the future development of cellulosic feedstock supply chains at the same time that the technological R&D efforts are underway. Discovery and documentation of targeted market channel innovations by which small and medium-sized producers can effectively support future cellulosic feedstock channels needs to occur before the marketplace need exists. Similarly, small and medium-sized producers need to be informed and educated relative to the opportunities and actions needed for them to capitalize on this potential. To address these issues, we propose a three-pronged, inter-disciplinary and integrated approach: 1) Provide a detailed assessment of the on-farm production opportunities and environmental consequences associated with production of a dedicated energy crop. The proposed research will focus on the Midwestern region, which currently is the major source of food and feedstock. The assessment of Land Use and Environmental Implications of Biofuels from Multiple Feedstocks will detail opportunities for dedicated energy crop production on currently underutilized land and in competition for corn and soybean acres. 2) Explore the industry evolution dynamics likely to characterize the emerging cellulosic-based biofuels sector. This investigation of the Liability of Smallness and Inter-firm Cooperation: A Contingent Perspective of Biofuels Technology Evolution will discover key insights from the relatively rapid emergence of the grains-based biofuels sector and interpret them through the lens of the industry evolution and strategic management to establish templates for alternative evolutionary paths in which small and medium sized farms may benefit in the cellulosic-based biofuels sector. 3) Deliver active learning based tools and information which will better enable Small and Medium-Sized Farmers Achieve Success as Providers of Cellulosic-Based Feedstocks. Intensive, active learning sessions will be conducted with targeted producers and leaders of associated producer organizations. In addition to informing these participants, these outreach and educational sessions themselves will contribute to knowledge creation efforts that can be extended to broader audiences of small and medium-sized producers.

APPROACH: This project is composed of three interrelated components: 1) The first component examines the optimal allocation of existing cropland for mix of feedstock production in Illinois. We will use detailed GIS data, at a 2x2 km level, for Illinois on soil quality, climate and land use to determine the costs and revenues from producing row crops, stover and perennial grasses, with simulation models MISCANMOD. To investigate the implications of utilizing CRP land for biofuel production, we will identify the land parcels (at the 2x2 km level) that are currently enrolled in the CRP and include those with the cropland parcels considered above, so as to determine the optimal allocation of land for biofuel production and the mix of feedstock that will be produced at various prices of corn, gasoline and ethanol and at various levels of incentive payments for using CRP land for bioenergy crops. To determine optimal mix of feedstock and spatial allocation of land use, we will estimate the greenhouse gas mitigation potential of various feedstocks with models including ISAM-2 and life cycle analysis. 2) The second component explores the industry evolution dynamics of the emerging biofuels sector (both grain and cellulosic-based), with four objectives. First, we will investigate the effect of biofuels technology evolution on the strategic decisions and performance of small and medium-sized firms in terms of their survival, likelihood of successfully participating in supply chains and the entry decision of small/medium sized farms to the biofuels industry, with a multiple levels (national, regional and state level) and methods (quantitative and qualitative) approach. Second, we will analyze quantifiable differences in survival and growth rates based on firm size with all firms engaged in the biofuels industry in the United States from 1990-2006, using the Generalized Estimation Equation (GEE) and the Cox proportional hazard survival methodology. Third, to analyze the effects of key industry, environmental, and regional factors which differentially advantage small firms that adopt the new technology, we will integrate findings from the first component into our survey data from a stratified sample (based on locational characteristics) of small and medium- sized. Finally, we will use survey method and limited dependent regression techniques such as probit and logit analysis to study the impact of strategic relationships on potential entry into grain-based and cellulosic technology regimes. 3) An outreach component that will deliver active learning-based tools and information to facilitate small and medium-sized farms as providers of cellulosic-based feedstocks. The outreach efforts will be accomplished in three stages; materials development and testing, delivery of producer workshops, and implementation of web-based tools/information. These workshops will employ state of the art computer-based tools that illustrate the challenges and opportunities associated with cellulosic-based feedstocks for biofuels, and provide simulation-based learning modules emphasizing the dynamics that dictate success within supply chain alliances.

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Item No. 13 of 59
ACCESSION NO: 0205057 SUBFILE: CRIS
PROJ NO: IND045076H AGENCY: CSREES IND
PROJ TYPE: HATCH PROJ STATUS: NEW
START: 01 OCT 2005 TERM: 30 SEP 2010 FY: 2006

INVESTIGATOR: Tyner, W.

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ECONOMIC AND POLICY ANALYSIS OF BIOMASS ENERGY ALTERNATIVES

NON-TECHNICAL SUMMARY: Higher oil prices have led to increased interest in economic and policy analyses with respect to renewable energy. This project will provide economic and policy analyses for specific renewable energy technologies and will estimate national impacts of certain renewable energy policy alternatives.

OBJECTIVES: 1)To evaluate the economics of biomass energy alternatives under a wide range of technical and economic assumptions relative to current and future technical change and current and future alternative energy pricing scenarios. 2)To examine the consequences of energy policy alternatives with respect to development of alternative fuels, impacts of the policy alternatives on the agricultural sector, and impacts on the environment. 3)To design and evaluate new energy policy options such as alternative subsidy or tax policies.

APPROACH: This research will draw upon a standard collection of tools used for benefit-cost analysis, policy analysis, and environmental impact analysis. Some of the tools to be employed are as follows: Descriptive-engineering (or process) modeling - Descriptive engineering models are used to capture the physical and economic flows in some engineering process such as ethanol production from corn. The models contain multiple triggers that can be set on or off to test sensitivity to different assumptions on processing technology. They then can be used to test the impacts of changes in technology on the economics of the underlying processes. Also, they can be used to test the impacts of different policy alternatives on the economic viability of the technology. Monte Carlo simulation models - Monte Carlo simulation can be used with descriptive engineering models or other policy models to trace the inherent uncertainty in input or conversion values through the system to the impact on output variables. In other words, this approach yields output variable distributions that reflect the uncertainty in the different input values. Policy simulation models - these models are constructed for each unique policy problem to be addressed. They normally are spreadsheet models containing all the needed data and parameters for the policy simulation. Integer mathematical programming - this technique will be used to examine optimal location of biomass facilities. Cellulosic biomass is costly to transport, and we envision evaluating alternative transport mechanisms and possible location of regional slurry sites to reduce costs. Life-cycle costing - this technique, which borrows extensively from the methods used in benefit-cost analysis, will be used for evaluating life-cycle costs of technological alternatives. Standard regression techniques - regression will be used to capture historical trends and variability in prices of inputs and outputs such as corn, ethanol, and gasoline. The variability and trend estimates can then be fed into the Monte-Carlo simulation models described above. Environmental impact analysis - mostly techniques from benefit-cost will be used for quantifying and valuing the environmental impacts of various technical and policy alternatives. Depending on the environmental question at hand, we might used dose response methods, contingent valuation, or alternative cost calculation methods. Some of the anticipated specific application areas are as follows: Estimating the impacts of using an ethanol subsidy that varies with the price of corn and the price of gasoline (or ethanol) instead of being fixed at 51 cents per gallon as at present. Estimate the economic impacts of new technologies for treating and processing distillers dried grains and solubles (DDGS) to increase conversion of some of this material to ethanol. Integer programming and life-cycle costing will be used to determine the optimal configuration for collecting, transporting, and processing corn residue (or other cellulosic biomass) into ethanol.

PROGRESS: 2006/10 TO 2007/09
OUTPUTS: The outputs of this project for 2006/07 include the following: journal articles, conference proceedings, extension publications, conference presentations, media interviews and reporting, and graduate student research. The publications are listed in the section below. Tyner made more than 20 conference presentations during the year and did more than 200 media interviews. He was quoted in The Economist, New York Times, Wall Street Journal, DTN, Bloomberg, Reuters, and many other sources. He did multiple TV and radio interviews and two half hour radio programs. The outputs described here focused on the objectives of this project, being analysis of alternative biofuels energy policies. His work was used by members of Congress, and he did briefing sessions for Congressional staffers on biofuels policy alternatives. PARTICIPANTS: Post doc: Farzad Taheripour. Current energy graduate students: Dileep Birur, David Perkis, Jayson Beckman, Bhwana Bista, Sarah Brechbill, Craig Rismiller, Delphine Simon. Professional development: Did a session on energy economics and policy for Indiana K-12 economics teachers. Farm Foundation has provided travel support. Purdue has generously funded the post-doc. We have obtained funding from DOE and NSF to continue this work. TARGET AUDIENCES: There are six target audiences: academics, government officials, members of Congress and state legislature, industry, media, and the general public. Tyner has made use of the media to reach many of these audiences with hundreds of media interviews. He works directly with Congressional staff and with state legislators. he did a briefing for the state budget committee on the Indiana legislature. He has done multiple briefings for staffers on Capital Hill. He has reached academic, government, and industry audiences through conference presentations. The journal publications also reach academic audiences.

IMPACT: 2006/10 TO 2007/09
Because of the research and extension activities under this project, multiple audiences now have a much better understanding of biofuels policy alternatives available to our nation. Senator Richard Lugar included one of the alternatives developed by Tyner in legislation proposed in the Senate. At a recent conference, Tyner was introduced as follows: "Whether I talk to folks in industry, academia, media, or politics, they all say that the go-to person in the country for biofuels policy is Dr. Wallace Tyner." The person doing the introduction was someone Tyner had never met. This is just one indication that the work conducted under this project has had significant impact. We have been able to characterize and describe the impacts of a wide range of biofuels policy alternatives including staying with current policy, a subsidy that varies with the price of crude oil, a two-part subsidy designed to target energy security and reduced greenhouse gas emissions, a national renewable fuels standard, cellulose specific subsidies, and a RFS combined with a variable subsidy.

PUBLICATIONS (not previously reported): 2006/10 TO 2007/09
1. Quear, Justin, and Tyner, Wallace E. Development of A Variable Ethanol Subsidy and Comparison with the Fixed Subsidy. Staff paper 06-16, Department of Agricultural Economics, Purdue University, December 2006.
2. Hurt, Chris, Tyner, Wally, and Doering, Otto. Economics of Ethanol. Purdue Extension Publication ID-339, December 2006. http://www.ces.purdue.edu/bioenergy.
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Item No. 14 of 59
ACCESSION NO: 0194612 SUBFILE: CRIS
PROJ NO: KS1007 AGENCY: CSREES KAN
PROJ TYPE: HATCH PROJ STATUS: NEW MULTISTATE PROJ NO: S-1007
START: 01 OCT 2002 TERM: 30 SEP 2007 FY: 2006

INVESTIGATOR: Sun, X. S.; Wang, D.

PERFORMING INSTITUTION:
GRAIN SCIENCE AND INDUSTRY
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THE SCIENCE AND ENGINEERING FOR A BIOBASED INDUSTRY AND ECONOMY

NON-TECHNICAL SUMMARY: Fossil resource crisis has become global concern, which is driving to seek alternatives. This research will develop technologies of utilizing agriculture materials for industry uses, mainly for fuels and bioplastics, to meet national needs.

OBJECTIVES: I. To expand the scientific knowledge leading to significant economic improvements in biofuel production processes (Objective 2); II. To develop, evaluate, and optimize integrated processes to convert biomass resources into biomaterials with commercial applications (Objective 3).

APPROACH: For objective I (objective 2), co products or by products from grain processing line will be used as substrate for ethanol fermentation. Suitable microorganisms will be selected and evaluated for hydrolysis and ethanol production. Pretreatment methods will be developed and evaluated to improve the yield of soluble sugars with reduced loadings of cellulase enzymes, thereby reducing projected costs. Efficiency of enzymatic hydrolysis of cellulose and semicellulose is critical. Pretreatments such as grinding and chopping, thermal/pressure treatments with acid or base, biological treatment with fungi, and chemical fractionation.will be used to increase the hydrolysis of cellulose and semicellulose. These pretreatments are able to reduce molecular bounding strength and crystallinity of the cellulose and semicellulose at molecular level and increase specific surface area, which will improve sugar conversion rate. The combined actions of enzymes and bacteria will be conducted to increase and optimize the ethanol fermentation and production. System optimization including the physical and chemical pretreatment conditions, hydrolysis conditions, and fermentation conditions for high yield and low coast will be studied. For Objective II (Objective 3), one of the objectives in this part is to characterize and evaluate rheological and thermal behaviors of starch and protein polymers as affected by their structure. Structure variations of starche and protein will be obtained using chemical or enzymatic treatments and various sources. Rheological properties and behaviors will be studied using both static and dynamic rheometers. Thermal behaviors and phase transition properties, such as glass transition, melting, and crystallization, will be characterized using differential scanning calorimetry (DSC). Another objective in this part is to develop bioplastics from starch based materials. Coupling reagents will be used to improve mechanical properties and performance. Existing facilities for processing petroleum based plastics will be evaluated for the starch based plastic manufacturing. Formulas will be optimized to meet industry specification for various products for disposable applications.

PROGRESS: 2006/01 TO 2006/12
I. Sorghum fiber conversion for ethanol: Sorghum is a major cereal crop in the United States. However, sorghum has been underutilized as a renewable feedstock for value-added products and industrial applications. The objective of this research is to develop pretreatment method to increase bioconversion rate of sorghum for ethanol production. During the past year progress has been made in developing and optimizing pretreatment methods to improve enzymatic hydrolysis of sorghum fiber and to increase ethanol yield by increasing starch loading in the fermentation broth. The major chemical composition of sorghum fiber includes starch, cellulose and hemicellulose. Sorghum fiber is considered a low-value byproduct. More than 5.6 lb of fiber can be obtained from each bushel of sorghum, which could be converted to approximately 3.19 lb of fermentable sugars. These fermentable sugars can theoretically yield about 0.27 gal of ethanol. Methods for pretreatment and enzymatic hydrolysis of sorghum fiber involved hot water treatment, acid hydrolysis, starch degradation, and enzymatic hydrolysis with cellulase and hemicellulose enzymes. Using a combination of starch degradation, optimum hot water treatment, and optimum enzymatic hydrolysis resulted in maximum total sugar yield up to 75%. Using decortication as pretreatment method, the ethanol yields increased from 9 to 17% depending on percentage of decortication. II. Biodegradable Agriculture Mulch Film: In 2006, we successfully developed first generation of mulch film with about 0.5 to 1 mm thickness. The film was tested in field for durability, and results were encouraging. Because of this, we received $268,000 USDA/SBRI phase II funding for developing biodegradable agriculture mulch films in collaboration with AgRenew, Inc. Phase II project started Nov 1 2006, and everything is in progress. We continue working on PLA/starch based formula for large scale mulch film production in collaboration with a large plastic company in KS. We aim to reduce the thickness of the film to 0.1 mm by improving stretching properties in 2007. III. Soy Protein Adhesives for Foundry Industry: As identified in 2005, viscosity and curing time were two main drawback of the soy protein adhesive for foundry industry. We solved the viscosity problem and identified microwave curing technology that took only about 2 min for curing. The adhesion strength also met the performance requirements. In 2006, we focused on shelf life, temperature stability of this technology. Shelf like of this adhesive was about 1-2 months and temperature stability met the required application environment. However, most current foundry industries still use gas curing. The hurdle for commercializing this technology is the capital investment by replacing the gas curing with microwave technology. Therefore, in 2006, we focused on gas curing. No significant result has been achieved so far, but we received $120,000 from industry starting Feb 1 2007. We aim to solve gas curing problem in 2007.

IMPACT: 2006/01 TO 2006/12
I. Sorghum fiber for ethanol: A dramatic increase in ethanol production using the current grain-starch-based technology may have resource limitations because grain production for ethanol will compete for the limited agricultural land needed for food and feed production. It is obvious that other feedstocks, such as agricultural residues, wood, municipal solid wastes, and wastes from pulp and paper industry, are potential resources for low-cost ethanol production. The future of ethanol production must lie on conversion of cellulosic biomass, not starch. II. Biodegradable agriculture mulch film: About $300 million annual sales for agriculture mulch film. Current materials used for mulch film are those derived from petroleum based plastics, which causing soil pollution. Besides, the cost of current mulch film depends on petroleum resources. If this technology is successful and commercialized, the much film is biodegradable and will improve soil quality, and reduce dependence on petroleum resources. III. Soy protein adhesive for foundry: current adhesive for foundry are all environmental hazardous materials, such as formaldehyde, isocyanate based, poly vinyl acetate. Environment pollution is the major reason to seek environmental friendly resins for foundry.

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Item No. 15 of 59
ACCESSION NO: 0203106 SUBFILE: CRIS
PROJ NO: KS324 AGENCY: CSREES KAN
PROJ TYPE: HATCH PROJ STATUS: NEW
START: 01 OCT 2005 TERM: 30 SEP 2010 FY: 2006

INVESTIGATOR: Walawender, W. P.; Fan, L. T.

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VALUE-ADDED MANUFACTURE OF CARBON ADSORBENTS FOR NATURAL GAS AND HYDROGEN STORAGE FROM BIOMASS

NON-TECHNICAL SUMMARY: Kansas leads the nation in producing wheat and sorghum; however, their market values are often exceedingly low because of over-production. The manufacture of carbon adsorbents for the onboard storage of natural gas and hydrogen storage from such grains will profoundly add value to them. "Economic Development through Value-added Products" is one of the five core areas singled out in the recently issued Five-Year Work Plan of the K-State Research and Extension for 2005-2009. Naturally, the proposed research will contribute towards the goals of this core area. Moreover, the consumption of natural gas (NG) and hydrogen (H2) that emit far less pollutants than the consumption of fossil fuels will immensely contribute to the quality or sustainability of environments. This proposed research aims at establishing the technical and economic feasibility of manufacturing high-quality carbon adsorbents from biomass, mainly grains including wheat and sorghum, which are suitable for the onboard storage of natural gas and hydrogen.

OBJECTIVES: (1) To investigate the effects of modes and conditions of the operations for preparing the fine particles from grain-based activated carbons on the properties of the resultant fine particles (yr 1). (2) To investigate the effects of modes and conditions of the operation for preparing the carbon pellets for natural gas storage from the fine particles on the properties of the resultant carbon pellets (yrs 1 and 2). (3) To investigate the effects of modes and conditions of the operation for preparing the carbon pellets containing a transition metal for hydrogen storage on the properties of the resultant pellets (yrs 3 and 4). (4) To estimate the costs and assess the sustainability of various options for manufacturing the carbon pellets based on the resultant data and information (yrs 4 and 5).

APPROACH: (i) To achieve objective 1, the fine carbon particles from activated-carbon substrates will be prepared by various methods of milling and grinding, such as attrition, impact, and compression, and some combinations thereof. To characterize the properties of the resultant fine carbon particles, their particle-size distributions will be determined by sieving and microscopy; their shapes, by microscopy and image analysis; and their bulk densities, by directly weighing in a vessel with a known volume. The surface areas, pore-volumes, and pore-size distributions of the fine particles will be characterized with a gas sorption analyzer through the adsorption of N2, Ar, and/or CO2 to minimize erroneous results which might arise from specific interactions of any of these gases with carbonaceous materials. Their surface areas will be determined by the BET (Brunauer, Emmett, and Teller) equation; their total pore volumes, estimated from the liquid volume of the adsorbate (N2); their micropore volumes, by the DR (Dubinin-Radushkevich) method; and their pore-distributions, by the density functional theory (DFT). (ii) To achieve objective 2, the carbon pellets for natural gas storage will be molded first by binding the fine carbon particles with the addition of a synthetic binder, such as furfuryl alcohol polymers, followed by the compaction of the bound particles into the pellets by single-step or multi-step procedures of varying modes, intensities, and durations with or without the aid of mechanical vibration. The properties of the resultant carbon pellets will be characterized similar to the fine carbon particles. In addition, the adsorption capacities of the pellets for natural gas will be experimentally measured at room temperature and moderately high pressure by adopting the procedure and facilities established in the current terminating project. For convenience, the measurements will be performed first with methane, the major component of natural gas, prior to the measurements with natural gas. (iii) To achieve objective 3, the carbon pellets for hydrogen storage will be impregnated with a transition metal by adding the chloride solution of this metal prior to or after the molding of the fine carbon particles. The transition metals to be explored include Pd, Pt, and Ru with the emphasis on the last: Catalytic activity of Ru is higher than that of either Pd or Pt. Subsequently, a dehydration step and an inert-gas purge step will be performed to remove impurities, such as moisture and chloride. Finally, wet reduction will be carried out in the presence of hydrogen to activate the metal. The properties of the resultant carbon pellets will be characterized similar to the fine carbon particles. Moreover, the adsorption capacities for hydrogen will be experimentally measured similar to natural gas. (iv) To achieve objective 4, the costs and sustainability of various manufacturing options for the onboard storage systems for natural gas and hydrogen will be assessed by resorting to the methodology of total cost assessment (TCA) and the evaluation of the thermal efficiencies of these manufacturing options based on energy as well as exergy (available energy).

PROGRESS: 2006/01 TO 2006/12

Carbon adsorbents prepared by our group from biomass have large surface areas, high microporosity, and narrow pore-size distributions (PSD's), thereby indicating that they can be immensely effective in adsorbing and storing gases, such as natural gas and hydrogen. These characteristics of carbon adsorbents from biomass are similar to those of carbon molecular sieves (CMSs). A stochastic model for the early stage of the formation of CMSs has been derived as a pure-birth process with a constant intensity of transition. The model has been validated with available experimental data; its mean value is in excellent accord with these data. Moreover, substantial effort has been made to relocate our laboratory and upgrade our experimental facilities.

IMPACT: 2006/01 TO 2006/12
Kansas leads the nation in producing wheat and sorghum; however, their market values are often exceedingly low because of over-production. The manufacture of carbon adsorbents for the onboard storage of natural gas and hydrogen storage from such grains will profoundly add value to them. Our investigation has shown that carbon adsorbents prepared by our group from biomass have very high adsorption capacities, thereby indicating that they can be immensely effective in adsorbing and storing gases, such as natural gas and hydrogen. The consumption of natural gas and hydrogen that emit far less pollutants than the consumption of fossil fuels will immensely contribute to the quality of the environment.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
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Item No. 16 of 59
ACCESSION NO: 0200418 SUBFILE: CRIS
PROJ NO: KYSTROBEL AGENCY: CSREES KY.
PROJ TYPE: NRI COMPETITIVE GRANT PROJ STATUS: EXTENDED
CONTRACT/GRANT/AGREEMENT NO: 2004-35504-14678 PROPOSAL NO: 2004-01092
START: 01 SEP 2004 TERM: 31 AUG 2007 FY: 2006 GRANT YR: 2004
GRANT AMT: $330,000

INVESTIGATOR: Strobel, H. J.; Lynn, B.

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PROTEOMIC ANALYSIS OF ETHANOL SENSITIVITY AND TOLERANCE IN THERMOPHILIC AND ANAEROBIC BACTERIA

NON-TECHNICAL SUMMARY: The continued use of non-renewable resources for the production of fuels and other chemicals has negative impacts on the environment, economy, and national security. There is renewed interest in the development of technologies for producing chemicals, materials, and energy using biologically-based processes. The production of bio-ethanol offers considerable promise for decreasing fuel oil consumption and increasing domestic markets for agricultural and forestry commodities. Most bio-ethanol is currently produced using yeast fermentation of corn but the use of anaerobic and thermophilic bacteria (microbes that grow in the absence of oxygen and at high temperatires) offers the promise of fermenting other types of relatively inexpensive biomass. However, one of the technological barriers to efficient use bacteria is a relatively low ethanol tolerance. This sensitivity currently limits the commercial utility of many bacteria. This project will use state of the art approaches to study protein

expression by anaerobic thermophilic bacteria. These proteomic studies will help determine the physiological reasons why the bacteria are either sesitive or tolerant to ethanol.

OBJECTIVES: There is renewed interest in the development of technologies for producing chemicals, materials, and energy using biologically-based processes. Much of this has been prompted by general recognition that continued use of petroleum and fossil fuels will have negative impacts on the environment, economy, and national security. The production of bio-ethanol offers considerable promise for decreasing fuel oil consumption and increasing the domestic markets for agricultural and forestry commodities. Bioconversion of fibrous organic material by thermophilic anaerobic bacteria circumvents some of the problems associated with yeast fermentations that are currently used to produce most bio-ethanol, but one of the technological barriers to efficient biomass conversion by bacteria is the relatively low ethanol tolerance that nearly all prokaryotes have when compared to yeast. Other microbial processes of commercial importance also are negatively impacted by fermentation end products, and this sensitivity constrains the commercial utility of many microorganisms. There is still relatively little detailed information on the mechanisms causing physiological cellular alterations that occur in response to ethanol. Until recently, it was tedious and time-consuming to examine the many possible changes in protein expression that a cell can exhibit in response to an environmental stress such as ethanol exposure. However, state-of-the-art proteomic approaches are now available to efficiently identify protein expression changes. Our basic hypothesis is that exposure of thermophilic anaerobic bacteria to ethanol causes specific alterations in protein expression patterns, and the overall project goal is to identify and define these changes using proteomic approaches. Clostridium thermocellum and Thermoanaerobacter ethanolicus are thermophilic anaerobes that produce ethanol, degrade polymeric carbohydrates, and have been proposed for use in biomass conversion systems. These organisms will be used to model the specific effects that ethanol has on the proteomes of thermophilic anaerobic bacteria. Our experiments will provide basic information needed to overcome present process limitations and to optimize microbial conversion of biomass to alcohol, solvents and other useful products. The specific objectives are to: Objective 1: Characterize alterations in the proteomic profile of C. thermocellum and T. ethanolicus in response to ethanol challenge. Objective 2: Determine the proteomic profile of ethanol resistant strains. Objective 3: Examine if proteomic changes elicited by ethanol are similar to those caused by environmental stresses including temperature, pH, and organic solvents. Objective 4: Evaluate alternative approaches to identify and quantify changes in proteomes of thermophilic bacteria.

APPROACH: The underlying hypothesis that will drive and direct this project is that exposure of thermophilic anaerobic bacteria to ethanol causes specific alterations in protein expression patterns. The overall goal is to identify and define these changes using proteomic approaches. Specifically, Clostridium thermocellum and Thermoanaerobacter ethanolicus strains will be used to model the effects that ethanol has on the proteomes of thermophilic anaerobes. The general approach is based on the well-established observations that (i) thermophlilic anaerobic bacteria are relatively ethanol sensitive, (ii) alterations in membrane composition, enzyme activity, and metabolic pathways occur in response to ethanol challenge, and (iii) organisms adapted to ethanol often have altered cellular characteristics when compared to sensitive parent strains. In Objective 1, the proteomic profiles of ethanol sensitive wild-type strains will be evaluated in the absence and presence of ethanol (i. e., ethanol challenge). During this phase of the project, protocols will be developed and refined to optimize protein separation and identification from the thermophilic bacterial samples. The results of these experiments will be used for subsequent objectives and will provide the baseline proteome for both organisms. The protein profiles of wild-type and ethanol-challenged cells will then be compared to ethanol-adapted cultures in Objective 2. Although there may be similarities between ethanol-challenged and ethanol-adapted cultures, we predict that qualitative and quantitative differences will be observed and that proteome analysis will reveal potential reasons as to why and how cells adapt to ethanol. Since ethanol is an environmental stress, Objective 3 is designed to examine whether the proteome changes noted in response to ethanol are similar to those seen for other environmental stresses. These results will be useful in determining if the responses and regulatory mechanisms seen for ethanol-stressed cells are common to the better characterized systems already known for other stress factors (e. g., heat stress). Finally, Objective 4 is designed to explore new opportunities that exist in characterizing and quantifying proteomic differences. Together, the results from these four objectives will provide basic information that is needed in targeting specific metabolic steps and machinery for alteration, either through genetic manipulation or changes in process conditions during fermentation.

PROGRESS: 2006/01 TO 2006/12

Increased bio-fuel production in the United States is being prompted by recognition that continued use of fossil fuels has negative consequences for the environment, economy, and national security. Bio-ethanol offers promise for diversifying the national fuel portfolio, decreasing oil consumption, reducing environmental impacts, and increasing domestic markets for agricultural commodities. Nearly all bio-ethanol in the United States is currently produced using yeast fermentation of maize starch, but there are constraints to the cost-effective and long-term use of this approach. In addition, some lifecycle analyses suggest that producing bio-ethanol from corn offers little, if any, environmental advantage to petroleum-based fuels. An alternative strategy is to use thermophilic anaerobic bacteria for the conversion of cellulosic biomass to ethanol. Using cellulosic feedstocks has the potential to be economically and environmentally viable in the long-term. Clostridium thermocellum is a thermophilic anaerobic bacterium that directly converts fibrous feedstocks into ethanol. However, a technological barrier to efficient biomass conversion by this organism is its relatively low ethanol tolerance. We have selected an ethanol-resistant strain of C. thermocellum and are using state-of-the-art protein analysis techniques to characterize the molecular basis of ethanol resistance. Our initial studies utilized traditional gel-based protein separations, and we demonstrated that protein expression in the tolerant strain was dramatically different than in the sensitive wild-type. However, gel-based separations are limited by their ability to detect and resolve proteins that are high molecular weight, highly basic, or hydrophobic. With this in mind, we developed a two-dimensional liquid chromatography separation scheme coupled to tandem mass spectrometry (Multi-dimensional Protein Identification Technology; MudPIT). Our most recent work has refined this non-gel approach to provide better quantification by using a metabolic labeling strategy. Wild-type (ethanol-sensitive) cells were grown in the presence of isotopically labeled ammonium sulfate (15-N) while the ethanol-tolerant strain was grown in medium containing non-labeled ammonium sulfate (14-N). The 15-N labeled cells served as internal standards; this minimized experimental errors and provided better quantitative comparison of differences in protein levels. MudPIT analysis revealed that the expression of more than 60 proteins was significantly affected by adaptation to ethanol. These proteins take part in a wide variety of functional processes including regulation of transcription, translation, signal transduction, metabolism, transportation, post-translational modification. This is the first quantitative proteomic analysis of C. thermocellum based on the MudPIT approach. In summary, we have a reliable MudPIT analysis platform that can be used to profile the C. thermocellum proteome. These experiments will provide information needed to overcome present process limitations and to optimize microbial conversion of cellulosic biomass to alcohol, solvents and other useful products.

IMPACT: 2006/01 TO 2006/12
Continued dependence on fossil-based fuels has negative impacts on the environment, economy, and national security. The conversion of cellulosic biomass by microorganisms to bio-based products and bio-energy is a sustainable alternative. Estimates are that nearly 11 million tons of agricultural, forestry and urban biomass could be used each year for bio-fuel production in Kentucky. Bio-conversion of these feedstocks could yield 600 million gallons of ethanol, and this production could replace a significant quantity of the gasoline utilized in the state. However, there are still significant barriers that prevent the economical implementation of bio-based technologies. Our studies combine the information contained in genomic sequence databases with the emerging field of proteomics to examine the metabolism of anaerobic bacteria under industrially relevant conditions. The results of this work will be useful in designing economically relevant bio-based processes that utilize cellulosic biomass. The University of Kentucky Mass Spectrometry Facility is a partner in this project.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
Williams, T. I., Combs,J. C., Thakur,A. P., Strobel,H. J., and B. C. Lynn. 2006. A novel bicine running buffer system for doubled sodium dodecyl sulfate polyacrylamide gel electrophoresis of membrane proteins. Electrophoresis 27:2984-2995.

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Item No. 17 of 59
ACCESSION NO: 0212868 SUBFILE: CRIS
PROJ NO: LAB93884 AGENCY: CSREES LA.B
PROJ TYPE: HATCH PROJ STATUS: NEW
START: 01 OCT 2007 TERM: 30 SEP 2012

INVESTIGATOR: Boldor, D.

PERFORMING INSTITUTION:
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MICROWAVE TECHNOLOGIES FOR POSTHARVEST PROCESSING OF BIODIESEL FEEDSTOCK

NON-TECHNICAL SUMMARY: The current and global energy markets will require the use of alternative renewable fuels to a significant degree as fossil fuels prices have increased to new heights. The proposed research project will investigate the use of environmental-friendly microwave technology to extract oils and convert them into biodiesel through a transesterification reaction in which the oil is mixed with an alcohol in the presence of a catalyst. The resulting products are biodiesel and glycerol, an important chemical with multiple industrial and agricultural uses. Biodiesel can be used in most modern Diesel engines without any modifications and loss of performance. The main objectives are to extract, separate and characterize the lipid source in three oil feedstocks (rice bran, soybeans, and Chinese tallow trees seeds), to convert these oils into biodiesel, and to optimize the process such that it can be scaled to pilot and industrial level. The microwave extraction and transesterifications will be achieved in both batch and continuous mode. The use of alternative stocks for biodiesel production and adding values to some of the traditional crops grown in Louisiana (rice and soybeans) will generate significant new and additional revenue for the state's agricultural sector, spurring economic development.

OBJECTIVES: The overall objective of this research project is to foster the development of a biodiesel industry using traditional and alternative resources specific to the State of Louisiana (rice bran, soybeans, and Chinese tallow tree seeds - CTT). The economy of Louisiana is heavily dependent on the oil, gas and petrochemical industry; therefore it is exposed to fluctuations in the price of fossil fuels. While the current global environment calls for high fossil fuels prices, this is the most appropriate time to start developing the future of the energy-based economy using the abundant renewable natural resources available in Louisiana (high yield agricultural and forestry crops, favorable climate). Current technologies and feedstock for bioenergy production are not competitive with fossil fuels at low petroleum and natural gas prices. By developing cost-saving post-harvest processing methods and technologies for bioenergy production now, Louisiana will position itself at the forefront of the future renewable energybased economy. The State's economy will therefore be shielded to oscillations in fossil fuel prices due to depletion of fossil resources, political and social instability in oil-producing countries, and eventual dramatic drops in fossil fuel prices.

APPROACH: 1. To successfully separate, extract, and quantify the vegetable tallow and Stillingia oil from the CTT seeds and oil from soybeans and rice bran using batch and continuous microwave technology; 2. To characterize the composition of oil and vegetable tallow of CTT, soybeans, and rice bran; 3. To achieve microwave-assisted trans-esterification of these lipids into biodiesel; 4. To analyze the composition of the resulting streams or products and to optimize the process; 5. To scale up the extraction and trans-esterification processes to a 5 kW continuous system; 6. To estimate the feasibility and economic viability of the process.

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Item No. 18 of 59

ACCESSION NO: 0405512 SUBFILE: CRIS
PROJ NO: 5447-41000-002-01S AGENCY: ARS 5447
PROJ TYPE: USDA COOPERATIVE AGREEMENT PROJ STATUS: NEW
START: 01 MAY 2002 TERM: 30 APR 2007 FY: 2006

INVESTIGATOR: ROSENTRATER K A; MCCALLA D

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IMPROVED USES AND VALUES FOR DRIED DISTILLERS GRAINS AND C4 GRASS FEEDSTOCKS

OBJECTIVES: 1) Make, analyze, and test modified dried distillers grains and other dry mill residues. 2) Assess pretreatment and biorefining technologies.

APPROACH: 1) Develop high protein dried distillers grains using quick germ/fiber removal. 2) Develop beef rations from dried distillers grains and AFEX treated corn stover. 3) Develop ammonia fiber explosion pretreatment process for corn stover and C4 grass feedstocks. 4) Establish pretreatment laboratory for C4 grass feedstocks, corn stover, and corn fiber. 5) Establish pilot plant for C4 grass feedstocks, corn stover, and corn fiber.

PROGRESS: 2005/10 TO 2006/09
Progress Report 1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter? This project is aligned with NP 307 Bioenergy and Energy Alternatives and NP 306 Quality and Utilization of Agricultural Products. The major problems addressed by this research include depleting nonrenewable petroleum resources, making the U.S. more energy independent, providing value-added products from raw agricultural commodities, improving rural and agricultural economies, and preserving air, water, and environmental quality. This research will help resolve these problems by developing and evaluating processes and products that will increase the value of corn-to-ethanol byproducts, which are currently known as distillers dried grains (DDGS). Specifically, this projects goals include determining the potential for converting these byproduct materials into animal feeds and other value-added products, such as industrial chemicals and products. Achieving these goals will thus augment and bolster ethanol production processes and economics. In summary, this research will develop valuable coproducts from distillers dried grains currently produced in the corn-based fuel ethanol industry. For years, agricultural and rural economies have been suffering from the effects of low commodity prices. Generally speaking, the price for many agricultural commodities received by farmers has been below the cost of production. This situation has caused many farmers to go out of business which, in turn, has caused many rural economies to collapse. Furthermore, the energy security of the United States has become unstable because the U.S. increasingly imports a majority of oil from non-domestic sources. Additionally, the U.S. economy suffers when the availability, and thus the cost, of imported oil dramatically fluctuates. Research to develop value-added coproducts from the byproducts of fuel ethanol production could help ease these problems by supporting the development of the domestic bioethanol industry, by providing increased and sustainable incomes in rural economies, by the development of new bio-based products that will replace products currently made from petroleum and other non- renewable materials, and by increasing the domestic production of renewable energy which, in turn, can improve the energy security of the U. S. The majority of ethanol production facilities are currently built and operated by small companies or farm-based cooperatives that have little or no budgets for research. Thus, the successful completion of this research will directly benefit ethanol producers, farmers, and the rural communities where these industries are located. The consumer and general public will also benefit from this research through the stabilization of fuel availability as well as gasoline prices at the pump. 2. List by year the currently approved milestones (indicators of research progress) Specific tasks and deliverables that are to be accomplished by MBI International are provided in the Statement of Work which is submitted each year to ARS. Additional details regarding research can be found in the report for the parent ARS Project #5447-41000-001-00D (Fiber Extrusion to Improve Use and Production of Ethanol Byproducts). 4a List the single most significant research accomplishment during FY 2006. This project

directly contributes to the accomplishment of ARS Strategic Plan Goals #1 (Enhance Economic Opportunities for Agricultural Producers) and #2 (Support Increased Economic Opportunities and Improved Quality of Life in Rural America). The project addresses NP 307 Component I, Ethanol Coproduct Development; NP 306 Action Plan Component 2, Problem Area 2b, New Uses for Agricultural By-Products Cereals, Oilseeds, and Novel Crops; and NP 306 Component 2, Problem Area 2c, New and Improved Processes and Feedstocks Cereals, Oilseeds, and Novel Crops. During FY2006, an agreement was signed between MBI and a significant ethanol producer to develop a biorefinery utilizing the AFEX treatment process to produce succinic acid from dry mill corn fiber. 4b List other significant research accomplishment(s), if any. Completed work under a Value Added Producer Grant (VAPG) between Heartland Grain Fuels, Aberdeen, SD, and USDA Rural Development. The Heartland plant was used as a model to demonstrate the biorefinery concepts of dry corn fractionation in an ethanol plant to produce corn oil, high protein DDG, and corn germ meal as value added byproducts. Developed relationship with a significant ethanol partner to proceed with the commercial development of the AFEX Process. Using AFEX treated corn fiber as the feedstock in simultaneous saccharification and fermentation (SSF), with batch feeding, we were able to obtain solids loadings up to 34%, ethanol concentrations in the broth of 70 grams per liter, and 87% of the total glucose (from starch and cellulose) was converted to ethanol. From the broth of these fermentations we were able to precipitate a pellet that contained 75.86% of the xylan and 67.30% of the arabinose. Analysis of the precipitated pellet indicates it is 50.75% sugars, 26.3% crude protein, 5.53% ash, and 17.43% other material. The pellet sugars were 58.46% xylan and 25.83% arabinan, mostly in polymeric form. 4c List significant activities that support special target populations. The work on discovering and developing new value-added coproducts from the corn-to-ethanol process will support small farms by improving ethanol production economics and thus creating a higher demand for corn, thereby increasing profits to small farms. 4d Progress report. This report serves to document research conducted under a specific cooperative agreement between ARS and MBI International. Additional details regarding research can be found in the report for the parent CRIS #5447-41000-001-00D (Fiber Extrusion to Improve Use and Production of Ethanol Byproducts). The research conducted under this cooperative agreement is aimed at developing valuable coproducts from the distillers dried grains produced in the corn-to-bioethanol manufacturing process, as well as investigating the potential for using other lignocellulosic feedstocks for the bioethanol manufacturing process. Objectives of this cooperative agreement are to investigate the use of new technologies to enhance the value of the byproducts of ethanol manufacturing, with particular emphasis on products with animal feed value, to investigate pretreatment technologies (i.e., AFEX pretreatment) on biomass materials that could become feedstocks for fermentation into ethanol, particularly corn fiber, corn stover, switchgrass, and other warm season grasses, and to investigate the potential for these materials to impact the economy of the North Central region of the U.S. Research conducted thus far has focused on four broad areas: 1) evaluation of de-germed and de-fibered corn that generates high protein, low phosphorous DDGS streams; 2) evaluation of AFEX treated corn stover, corn fiber, and DDGS fiber; 3) the development of a bench scale AFEX treatment process to process corn fiber, corn stover, switchgrass, and DDGS fiber; and 4) the development of a pilot-scale, continuous AFEX treatment process. Research is currently underway in each of these areas. Bench-scale analysis has been completed, and the resulting data has been used to create a computer model of an AFEX-based biorefinery that is operated concurrently with a dry mill ethanol plant. To achieve biorefining, the manufacturing process includes corn fractionation, corn oil extraction, AFEX treatment of corn fiber, and subsequent fermentation into ethanol. Results from this model have been used to develop engineering designs for a biorefinery pilot plant, including process, layout, and construction plans. In order to implement these plans for a biorefinery pilot plant, a partnership base and business plan are currently being assembled for construction and operation. 5. Describe the major accomplishments to date and their predicted or actual impact. This project directly contributes to the accomplishment of ARS Strategic Plan Goals #1 (Enhance Economic Opportunities for Agricultural Producers) and #2 (Support Increased Economic Opportunities and Improved Quality of Life in Rural America). The project addresses NP 307 Component I, Ethanol Coproduct Development; NP 306 Action Plan Component 2, Problem Area 2b, New Uses for Agricultural By-Products Cereals, Oilseeds, and Novel Crops; and NP 306 Component 2, Problem Area 2c, New and Improved Processes and Feedstocks Cereals, Oilseeds, and Novel Crops. Toward these ends, the research work conducted within this project is focused on investigating and conducting research on value-added uses for residue streams resulting from the manufacture of bioethanol, and is administered under the auspices of ARS National Program #307 (Bioenergy and Energy Alternatives) and #306 (Quality and Utilization of Agricultural Products). Major accomplishments for the research project have been reported to ARS in annual reports from MBI International, and are also documented in this report. To date, the major accomplishments of this project include bench- scale analysis of sugar production from corn stover, warm season grasses, and DDGS fiber utilizing novel processing technologies (i.e., AFEX pretreatments). This research has shown that various low-value biological materials have potential viability for utilization as feedstocks for conversion into ethanol. In general, results to date have provided an essential step in assessing the feasibility of scaling up AFEX pretreatment and biorefining operations to commercial production, which have the potential to increase the profitability of current ethanol manufacturing operations, and ultimately could help support small farms by creating an increased demand for corn grain, corn stover, as well as other low-value cellulosic materials. Additionally, computer simulation models for a biorefinery pilot plant, as well as a commercial ethanol manufacturing plant have been constructed and analyzed. Furthermore, a pilot plant to implement these concepts has been designed; process, layout, and construction plans have been completed; and feasibility studies have been completed. 6. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end- user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products? During FY2006, MBI reached agreement with a commercial partner for the commercialization of the AFEX technology as a pretreatment for dry mill fractionated corn fiber. 7. List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below). 28th Symposium on Biotechnology for Fuels and Chemicals, Nashville, TN April 30 May 3, 2006 Conference Proceedings: "Separation of Glucose and Pentose Sugars by Selective Enzyme Hydrolysis of AFEX-treated Corn Stover". Hanchar, Teymouri, McCalla and Stowers. 2005 American Institute of Chemical Engineers Annual Meeting, Cincinnati, OH October 30 November 4, 2005 Technical Presentation: "Simultaneous Saccharification and Fermentation (SSF) of AFEX-treated Corn Fiber, Bagasse and CLM Using Fed-batch Technique". Teymouri, Guettler, McCalla and Stowers.

PUBLICATIONS (not previously reported): 2005/10 TO 2006/09
No publications reported this period.

Item No. 19 of 59
ACCESSION NO: 0194307 SUBFILE: CRIS
PROJ NO: MICL02047 AGENCY: CSREES MICL
PROJ TYPE: HATCH PROJ STATUS: NEW MULTISTATE PROJ NO: S-1007
START: 01 OCT 2002 TERM: 30 SEP 2007 FY: 2006

INVESTIGATOR: Lira, C.

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SCIENCE AND ENGINEERING FOR A BIOBASED INDUSTRY AND ECONOMY

NON-TECHNICAL SUMMARY: New technologies and educational programs are needed to support a biobased economy. This project will generate new technologies and educational programs to support a biobased economy.

OBJECTIVES: Objective 4: Biobased Chemicals. Objective 5: Education and Outreach.

APPROACH: Objective 4, Biobased Chemicals: Proteins will be used as biocatalysts to produce high-value products from renewable raw materials. Objective 5, Education and Outreach: Three educational and outreach tasks will be directed at identifying needed educational materials, developing those materials in distance-based delivery methods and developing a trained work force to support a biobased economy. The tasks include (1) development of an advisory board for the National Resource Center, (2), development of educational materials in high-priority topic areas, and (3) development of a national resource center for biomass education.

PROGRESS: 2006/01 TO 2006/12
The elevated pressure reactive distillation column designed by Dr. Lira and Dr. Miller is in its final stages of construction. All column components have been acquired and are being installed. The supporting

framework for the column is in place. The column is expected to be operational in February, 2007 and will be immediately put to use for two new projects that Dr.Miller and Dr. Lira and leading. One project, funded by the MEDC 21st Century Jobs Fund, is the production of succinic acid esters via reactive distillation. The second project, funded by the USDA NRI Program, is for the use of reactive distillation to produce an improved composition of biodiesel. The successful proposals submitted for these projects were significantly strengthened by the commitment of funds from the USDA 'Enabling Biorefineries' project. Mark Worden's lab is using nanofabrication methods to develop new, high-performance catalytic interfaces containing dehydrogenase enzymes, which carry out oxidation and reduction reactions. The resulting bioelectronic interfaces can use spontaneous chemical reactions to generate electricity (biological fuel cells) or use electricity to drive chemical reactions (biocatalytic reactors). Site-directed mutagenesis resulted in production of a new secondary alcohol dehydrogenase enzyme that used NADH as opposed to the much more expensive NADPH. Also, methods have been developed that allow the labile components of the bioelectronic interfaces to be regenerated. Dr. Bruce Dale's lab is working on developing integrated pretreatment, hydrolysis and fermentation systems to convert cellulosic biomass to fuels such as ethanol. Five doctoral students and a post doc are currently involved in these integrated projects. Among the important advances achieved this year are: reduction in the amount of ammonia required and concentration of ammonia required for effective application of the ammonia fiber expansion (AFEX) process, better understanding of the fundamental mechanisms that make AFEX effective, development of microplate screening methods for high throughput analysis of treated cellulosic biomass and the first demonstration that pretreated biomass (by AFEX) can be fermented by recombinant organisms without any detoxification or nutrient supplementation. Detoxification and nutrient supplementation are absolutely required for acid pretreated biomass. A major corn dry miller is now involved in scaling up and commercializing AFEX. The 2006 multi-state meeting was attended by Professor Dennis Miller.

IMPACT: 2006/01 TO 2006/12
The reactive distillation work will facilitate the simultaneous production and purification of biobased products, thereby making them more competitive with petroleum derived products. Biodiesel economics will be improved by this process. The large scale column will give us the opportunity to test concepts and develop process economics in a way that cannot be done with smaller devices. Biological fuel cell technology is improved by the nanofabrication technology. This marriage of biotechnology and nanotechnology is among the most fertile areas for advancing the use of biology to process agricultural materials for chemicals and fuel production. Finally, the work on cellulosic ethanol production is directly related to President Bush's call to reduce the cost of ethanol production from domestic energy crops such as switchgrass. The intense interest in this area is highlighted by the fact that the AFEX process is now in the early stages of commercial application.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
1. Vu, D.T.; Lira, C.T.; Asthana, N,S; Kolah, A.K. Miller D.J. Vapor-liquid equilibria in the systems Ethyl Lactate + Ethanol and Ethyl Lactate + Water, J. Chem. Eng. Data. 51, 1220-1225, (2006)
2. Brian L. Hassler, Megan Dennis, Maris Laivenieks, J. Gregory Zeikus, and Robert M. Worden (2007) Mutation of Tyr-218 to Phe in Thermoanaerobacter ethanolicus secondary alcohol dehydrogenase: effects on bioelectronic interface performance, Applied Biochemistry and Biotechnology, (in press)
3. Dale, B. E. Impacts of Ethanol on the Farm Economy. A High Growth Strategy for Ethanol. p. 31-42. Report of an Aspen Institute Policy Dialogue, published by the Aspen Institute, Queenstown, Maryland (2006)
4. Bals, B.; Dale, B. E.; Balan, V. Enzymatic Hydrolysis of Distillers Dry Grains and Solubles (DDGS) using Ammonia Fiber Expansion Pretreatment. Energy and Fuels 20, 2732-2736, (2006).
5. Saffron, C.; M., Park, J. H.; Dale, B. E.; Voice, T. C. Kinetics of Contaminant Desorption from Soil: Comparison of Model Formulations using the Akaike Information Criterion. Environmental Science and Technology 40, 7662-7667, (2006).
6. Laureano-Perez, L.; Dale, B. E.; ODwyer, J. P.; Holtzapple, M. Statistical Correlation of Spectroscopic Analysis and Enzymatic Hydrolysis of Poplar Samples. Biotechnology Progress 22, 835-841, (2006).

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Item No. 20 of 59
ACCESSION NO: 0203508 SUBFILE: CRIS
PROJ NO: MICL03414 AGENCY: SAES MICL
PROJ TYPE: STATE PROJ STATUS: NEW
START: 01 MAY 2005 TERM: 30 APR 2010 FY: 2006

INVESTIGATOR: Dale, B. E.; Lira, C. T.; Worden, R. M.; Miller, D. J.

PERFORMING INSTITUTION:
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ENABLING BIOREFINERIES

NON-TECHNICAL SUMMARY: Excessive reliance on decreasing supplies of petroleum underlies many of our national security, economic, social and environmental problems. These problems will only increase in the future, unless they are addressed and solved now. Our purpose is to enable the economic and widespread adoption of biorefineries converting plant biomass into fuels, chemicals and other products. We will develop economic, efficient processing technolgy and system wide models to promote biorefinery deployment and ensure their economic and environmental viability.

OBJECTIVES: We are in the early phases of a truly historic transition--from an economy based largely on petroleum to a more diversified economy in which renewable plant biomass will become a significant feedstock for food, fuel and chemical production. While remaining supplies of petroleum, coal and natural gas are very large, it is nonetheless obvious that the world is using these essentially nonrenewable resources at a huge and growing rate. Some experts believe that the peak rate of production of conventional oil will occur within this decade, while others predict this turning point will occur before mid century. After that point, conventional production of cheap oil will irreversibly decline. Although other sources of petroleum exist (eg, tar sands, deepwater oil), they will be more difficult and much more expensive to produce. Whatever the exact date of peak oil production, it is obvious that we are approaching a major change in the way we must provide energy and other services to the population of the world. Our overall research objective is to help develop and deploy biorefineries that will convert plant biomass into replacements for petroleum derived products. Specifically we are interested in developing new processing technologies that effectively and economically convert plant biomass into fuels, chemicals and materials. We are also developing system wide models that allow us to better understand the economic and environmental performace of biobased products. The purpose of this modeling and systems research is to help guide research efforts so that biobased products achieve their full economic and environmental potential to improve our well being.

APPROACH: Although biomass has some disadvantages compared to petroleum, its embedded energy content and costs suggest significant potential to provide raw material and energy services. We believe the disadvantages of biomass will be overcome by upgrading biomass in large, integrated processing facilities called biorefineries. Among the products derived from biomass are liquid transportation fuels, electricity and steam, a tremendous variety of chemicals, monomers and polymers, lubricants, adhesives, fertilizers as well as animal feeds. Biorefineries will first separate and react the raw materials to a relatively small range of intermediates including carbohydrates, proteins, lipids etc. These intermediates will then be upgraded by further reaction and separation steps to a very wide variety of final products. This is exactly the way we now process petroleum. This proposal supports our overall effort to refine biomass. We will develop the processing technology to effectively and efficiently convert biomass to fuels, chemicals and materials. We will also develop economic and environmental models to support biorefinery development. Specifically, our first three years of research will focus in the following areas: 1) Redox enzymes carry out oxidation and reduction reactions to convert biobased raw materials into high-value products. Industrial application of redox enzymes is limited by a many factors. The Worden lab will address these limitations. Novel redox enzymes will be isolated from the genome of Galdieria sulphuraria, a eukaryotic microbe that grows at high temperature and extremely low pH. Novel interfaces and materials that provide efficient communication between the redox enzymes, electrodes, cofactors, and other molecules will be developed in conjunction with collaborators. 2) Research funding through MAES will assist Dr. Dennis Millers laboratory in research on catalysis and separations for the biorefinery. More specifically, we will identify better catalysts for chemical conversions of biorenewables, enabling low cost, highly selective reactions to produce products that will replace existing petroleum based compounds. We also focus on reactive distillation as a new method for forming and purifying biobased products in a single process step. 3)Dr. Carl Lira's laboratory works in process design for separations in the biorenewable process stream. Process platforms based on biorenewables involve molecules and mixtures are incompletely characterized in existing literature. We will more completely characterize these components and mixtures for targeted process and separation strategies. 4) Finally, MAES funds will be used in Dr. Bruce Dale's group to model biorefinery processes using chemical engineering process simulation software such as ASPEN. Our objective is to identify the most cost sensitive areas of selected processes to better target them for research. A second modeling effort is life cycle anlysis of integrated biorefining systems to identify needed areas for environmental improvement and to better compare the environmental performance of these systems with that of petroleum refining systems.

PROGRESS: 2006/01 TO 2006/12
The elevated pressure reactive distillation column designed by Dr. Lira and Dr. Miller is in its final stages of construction. All column components have been acquired and are being installed. The supporting framework for the column is in place. The column is expected to be operational in February, 2007 and will be immediately put to use for two new projects that Dr.Miller and Dr. Lira and leading. One project, funded by the MEDC 21st Century Jobs Fund, is the production of succinic acid esters via reactive distillation. The second project, funded by the USDA NRI Program, is for the use of reactive distillation to produce an improved composition of biodiesel. The successful proposals submitted for these projects were significantly strengthened by the commitment of funds from the USDA Enabling Biorefineries project. Mark Wordens lab is using nanofabrication methods to develop new, high-performance catalytic interfaces containing dehydrogenase enzymes, which carry out oxidation and reduction reactions. The resulting bioelectronic interfaces can use spontaneous chemical reactions to generate electricity (biological fuel cells) or use electricity to drive chemical reactions (biocatalytic reactors). Site-directed mutagenesis resulted in production of a new secondary alcohol dehydrogenase enzyme that used NADH as opposed to the much more expensive NADPH. Also, methods have been developed that allow the labile components of the bioelectronic interfaces to be regenerated. Dr. Bruce Dales lab is working on developing integrated pretreatment, hydrolysis and fermentation systems to convert cellulosic biomass to fuels such as ethanol. Five doctoral students and a post doc are currently involved in these integrated projects. Among the important advances achieved this year are: reduction in the amount of ammonia required and concentration of ammonia required for effective application of the ammonia fiber expansion (AFEX) process, better understanding of the fundamental mechanisms that make AFEX effective, development of microplate screening methods for high throughput analysis of treated cellulosic biomass and the first demonstration that pretreated biomass (by AFEX) can be fermented by recombinant organisms without any detoxification or nutrient supplementation. Detoxification and nutrient supplementation are absolutely required for acid pretreated biomass. A major corn dry miller is now involved in scaling up and commercializing AFEX.

IMPACT: 2006/01 TO 2006/12
The reactive distillation work will facilitate the simultaneous production and purification of biobased products, thereby making them more competitive with petroleum derived products. Biodiesel economics will be improved by this process. The large scale column will give us the opportunity to test concepts and develop process economics in a way that cannot be done with smaller devices. Biological fuel cell technology is improved by the nanofabrication technology. This marriage of biotechnology and nanotechnology is among the most fertile areas for advancing the use of biology to process agricultural materials for chemicals and fuel production. Finally, the work on cellulosic ethanol production is directly related to President Bush's call to reduce the cost of ethanol production from domestic energy crops such as switchgrass. The intense interest in this area is highlighted by the fact that the AFEX process is now in the early stages of commercial application.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
1. Brian L. Hassler, Megan Dennis, Maris Laivenieks, J. Gregory Zeikus, and Robert M. Worden (2007) Mutation of Tyr-218 to Phe in Thermoanaerobacter ethanolicus secondary alcohol dehydrogenase: effects on bioelectronic interface performance, Applied Biochemistry and Biotechnology, (in press)

2. Dale, B. E. Impacts of Ethanol on the Farm Economy. A High Growth Strategy for Ethanol. p. 31-42. Report of an Aspen Institute Policy Dialogue, published by the Aspen Institute, Queenstown, Maryland (2006)
3. Bals, B.; Dale, B. E.; Balan, V. Enzymatic Hydrolysis of Distillers Dry Grains and Solubles (DDGS) using Ammonia Fiber Expansion Pretreatment. Energy and Fuels 20, 2732-2736, (2006).
4. Saffron, C.; M., Park, J. H.; Dale, B. E.; Voice, T. C. Kinetics of Contaminant Desorption from Soil: Comparison of Model Formulations using the Akaike Information Criterion. Environmental Science and Technology 40, 7662-7667, (2006).
5. Laureano-Perez, L.; Dale, B. E.; ODwyer, J. P.; Holtzapple, M. Statistical Correlation of Spectroscopic Analysis and Enzymatic Hydrolysis of Poplar Samples. Biotechnology Progress 22, 835-841, (2006).

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Item No. 21 of 59
ACCESSION NO: 0210467 SUBFILE: CRIS
PROJ NO: MICL03448 AGENCY: SAES MICL
PROJ TYPE: STATE PROJ STATUS: NEW
START: 01 MAY 2007 TERM: 30 APR 2012

INVESTIGATOR: Joshi, S. V.

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ECONOMIC AND ENVIRONMENTAL ASESSMENT OF BIOFUEL AND BIO-BASED MATERIALS

NON-TECHNICAL SUMMARY: A. Bio-fuels and bio-based materials hold a great promise for sustainable development because of their potential to reduce global warming effects and alleviate dependence on fossil fuels and non-renewable material sources. B. Recognizing this potential, the President's Executive Order 13134 calls for tripling of the use of bio-based products in the US economy by 2010. Similarly, the 2020 vision statement of the American chemical industry sees a bright future for bio-based products. C. However, both economic feasibility and environmental superiority of bio-based products are being hotly debated. A. The overall objective of the project is technical, economic and environmental evaluation of emerging biofuel and biobased material industries. B. The initial focus is on evaluation of the ligno-cellulosic ethanol industry along with corn-ethanol and bio diesel industries.

OBJECTIVES: The overall objective of this project is technical, economic and environmental evaluation of emerging biofuel and biobased material industries. Focus of the current research is on the evaluation of the ligno-cellulosic ethanol industry and related biofuels namely corn-ethanol and biodiesel. The aspects analyzed will include feedstock supply estimation, conversion technology assessment, supply chain and infrastructure feasibility analyses, and comprehensive environmental impact assessment under various policy scenarios.

APPROACH: The main components of the research and the analytical approach are as follows: 1. Assessment of potential supply of ligno-cellulosic materials such as agricultural residues, municipal solid waste, forest residues etc. The primary method is to build county level inventories and supply curves and aggregating to the national level. 2. Assessing potential supply of dedicated energy crops such as switchgrass. This requires modeling the switching from current cropping mix to energy crops. This analyses will be carried out using the AGMOD a multi commodity model of US agriculture developed by Dr. Ferris, Professor Emeritus, MSU. 3. Conversion technology assessment through literature review and in collaboration with engineers working in this area. 4. Assessing potential supply chain configurations. This will be done using engineering- economic modeling. 5. Environmental assessment using life cycle assessment (LCA) techniques

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Item No. 22 of 59
ACCESSION NO: 0212350 SUBFILE: CRIS
PROJ NO: MICL03462 AGENCY: SAES MICL
PROJ TYPE: STATE PROJ STATUS: NEW
START: 01 SEP 2007 TERM: 31 AUG 2012

INVESTIGATOR: Saffron, C. M.

PERFORMING INSTITUTION:
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THERMOCHEMICAL CONVERSION OF WOODY BIOMASS TO FUELS AND CHEMICALS

NON-TECHNICAL SUMMARY: The value-addition contributed by thermochemical conversion is at least partially dependent upon the rates and extents of product formation, the energy quality of the fuel, and the composition of the product stream. This project aims to elucidate and alleviate the limiting mechanisms inherent in thermochemical conversion of woody biomass.

OBJECTIVES: The project scope is to investigate the thermochemical conversion of woody biomass to fuels and chemicals. Economic models, for both a stand-alone thermochemical conversion facility and an integrated cellulosic ethanol biorefinery with thermochemical conversion, are to be formulated. The results of the economic model will be used to identify the processes or process steps that are sensitive to alterations. Beneficial alterations will be investigated to discern the nature of limiting mechanisms. A physicochemical model will be derived to describe the resultant data and assist in interpreting experimental results. A revised economic model, including information from the experimental results, will provide decision-making capability to industry regarding the deployment of thermochemical conversion.

APPROACH: The use of thermochemical conversion of woody biomass will be explored in a stand-alone and an integrated biorefinery format. Economic models will be formulated to provide insight into process limitations and to focus research effort. Process alterations that result in beneficial observations deviating from established theory will be investigated. Hypothesis formulation and experimental design will be tailored to elucidate the nature of limiting mechanisms. Physicochemical models will be formulated to describe the data and assist in interpreting experimental results. Both the stand-alone and integrated biorefinery models will be updated to reflect the scientific findings. A series of economic models will be constructed to identify the processing steps in the thermochemical conversion of woody biomass that are most sensitive to process improvements. Profitability metrics will be used to rank different processes (e.g. gasification followed by Fischer-Tropsch synthesis, gasification followed by synthesis gas fermentation to ethanol, pyrolysis oil formation, etc.) and process configurations (i.e. different methods of fractionating thermochemical products). Model results will guide experimentation in the laboratory by focusing resources on processes and process steps that are most economically sensitive to improvement. Hypothesis formulation will follow observation of limiting phenomena in the laboratory. The nature of the limiting mechanisms will be elucidated by bench-scale exploratory research--i.e. whether mass transfer rates, heat transfer rates, reaction rates, etc., are responsible for limiting the efficacy of the thermochemical routes under consideration. Spectroscopic methods, such as GC/MS, will measure the composition and quantity of the pyrolysis products. Physicochemical models will be formulated to describe the data and assist in interpreting the results. This detailed investigation is expected to result in an improved scientific understanding of the thermochemical conversion of woody biomass and of the fractionation of pyrolysis products. Further process and equipment design will be investigated in an attempt to alleviate the aforementioned limitations. In addition to the energy value of the upgraded fuel, the fractionation of value-added co-products will be considered. Revision of the economic model will commence upon interpretation of the experimental results. Integration of thermochemical conversion into cellulosic ethanol facilities (woody biomass or otherwise) will be explored to increase the product suite and energy quality of the biorefinery. Areas of focus include, but are not limited to: 1) thermochemical conversion of a fraction of the cellulosic feedstock, 2) the use of thermochemical methods as pretreatments, and 3) the thermochemical conversion of fermentation residue solids (i.e. cake solids). Profitability of the biorefinery will be estimated upon economic model formulation and is dependent upon the process material and energy balances. This biorefinery model is expected to be used as a decision-making tool for subsequent design and experimentation at the bench scale.

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Item No. 23 of 59
ACCESSION NO: 0206809 SUBFILE: CRIS
PROJ NO: MICL07709 AGENCY: CSREES MICL
PROJ TYPE: SPECIAL GRANT PROJ STATUS: EXTENDED
CONTRACT/GRANT/AGREEMENT NO: 2006-34189-17124 PROPOSAL NO: 2006-06143
START: 15 JUL 2006 TERM: 14 JUL 2008 FY: 2006 GRANT YR: 2006
GRANT AMT: $512,942

INVESTIGATOR: Dale, B. E.

PERFORMING INSTITUTION:
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BIOPROCESSING FOR UTILIZATION OF AGRICULTURAL RAW MATERIALS

NON-TECHNICAL SUMMARY: The United States is increasingly dependent on increasingly expensive imported petroleum as a raw material to produce fuels, chemicals and materials. At the same time, rural America is hard hit by a lack of industries to process our inexpensive agricultural commodities. The purpose of this project is to develop cost effective processing technology to convert inexpensive agricultural raw materials into valuable fuels, chemicals and materials.

OBJECTIVES: The MSU/MBI USDA grant funds are leveraged with other funding to accelerate the development of biobased industrial product technologies critical to the sustainability of the agricultural and rural economy. These technologies have not received adequate support from the private sector because they represent new products and processes not yet adopted by industry and the return on investment is not yet determined. MSU and MBIs missions provide for a synergistic approach to fulfill the objectives of the USDA Special Grants Program: to bring new uses for agricultural products to the stage where there is sufficient commercial interest to create new companies. MSU/MBI focus on identifying industrial and consumer uses for commodity crops, which encourages a pattern of business development that will result in stable rewarding jobs, and support communities or disadvantaged groups in the U.S. that have not employed the benefits of technology based growth. Discoveries from MSU, other universities and federal laboratories are developed and scaled up by MBI so that they are ready for commercialization. This development work provides the critical scientific and engineering information necessary to secure capital financing to construct a manufacturing facility or introduce a new process to an existing facility. Multidisciplinary teams with in depth technical expertise and strong business acumen are formed to ensure that the technology being developed fits an unmet market need. Funding from the USDA Special Grants Program enables MSU/MBI to bring basic research ideas that have demonstrated proof of principle to proof of commercial feasibility. The approach taken by MSU/MBI is a unique partnership that allows for the acceleration of new products and processes into the marketplace to create new jobs and launch new business opportunities. USDA CSREES funds will help support work in the project areas described below. Michigan State University and MBI International have several interdisciplinary teams that work on each of the project areas outlined. USDA CSREES funds are leveraged with funding from the State of Michigan, other federal agencies, industry and other State agencies to allow the consortium to significantly expand the work performed under the USDA CSREES grant. Combining funds from several sources allows the consortium to develop and demonstrate the technical and economic feasibility of a broader range of new technologies at a much faster pace.

APPROACH: The focus of this years funding will address the continuation of the following five project areas: Project 1. Developing Valuable Protein Products & Fermentable Sugars from Distillers Grain: This project has the long-term objective of producing valuable protein products and additional fermentable sugars from distillers grains. The technical approach is to treat distillers grains with the AFEX process to enhance digestibility and to assist in subsequent protein extraction and recover. The work will be done at MSU under the direction of Dr. Bruce Dale. Project 2. Design and Engineering of High Strength Compatibilized Green Structural Materials from AFEX-treated Cellulosic Materials: This project seeks to advance new fundamental knowledge through integrated research directed towards the reduction of energy use and adverse environmental impact in the development of green nanocomposites for structural applications. The work will be done at MSU under the direction of Dr. Larry Drzal. Project 3. Biomass Pretreatment: This project is aimed at development and validation of the integrated technologies that will convert low-value lignocellulosic materials into high-value products that include ethanol, specialty chemicals, biomaterials and animal feeds, i.e., a sugar-based biorefinery. This effort will occur at MBI under the direction of Dr. Mark Stowers. Project 4. Fermentation Production of Fine and Specialty Chemicals: This project is aimed at development and validation of an integrated process to produce succinic acid and butanetriol from a low cost biomass sugar stream. This effort will take place at MBI under the direction of Dr. Mark Stowers. Project 5. Identification of Commercially Attractive Biobased Technologies: This project continues the effort to bring new ideas, including broad based, competitive solicitations to industry and academia, seed projects for investigating new technological tools or approaches, and new ideas submitted by researchers into the program for consideration. This project occurs at MBI under the direction of Dr. Mark Stowers.

PROGRESS: 2006/07 TO 2007/07
Protein Extraction: Several solvents were screened to determine the best approach to removing proteins from distiller's grains. Ethanol and weak bases like ammonia were ineffective, while a strong base like sodium hydroxide had a larger effect. Extracting with 0.5M NaOH, 2mM b-mercaptoethanol, and 0.3% SDS at 70C for 90 minutes, 32% of the total protein was solubilized, while 22% of the remaining protein was solubilized during the subsequent fiber hydrolysis. However, the soluble protein was low in lysine, and the resulting hydrolysis produced low sugar yields. Attempts to solubilize protein after hydrolysis also saw low yields, with no more than 30% of the remaining protein being extracted in concentrated alkaline solutions. High Strength Materials: Both Microfibrillated Cellulose (MFC) and Cellulose Nanowhiskers (CNWs) were successfully extracted from wheat straw with yields of 34.6 % and 13.6 % respectively. The extracted MFC consisted of long slender fibrils with width ranging from 20 nm to several um. The CNWs were rod-like crystals with a width and length of 5-10 nm and 100-200 nm respectively. Both MFC and CNW reinforced polyvinyl alcohol composite films were prepared by a solvent casting technique. Excellent dispersion of the MFC and CNW in the matrices was confirmed by electron microscopy analysis of the cryo-fractured surfaces and plasma-etched surfaces. The thermal and mechanical properties of the composite films were studied and compared. MFC and CNW are now being used to produce Polylactic Acid (PLA) based biocomposites. Pretreatment: MBI successfully installed the 5-gallon reactor. The most effective AFEX conditions for corn fiber were identified. The fed batch enzyme hydrolysis method was adopted to perform high gravity (20, 30 and 40% solid loadings), obtained a relatively high glucose yield (85%) and generated hydrolysate containing as high as 135g/l of glucose. Implementing the fed batch simultaneous saccharification and fermentation (SSF) method enabled us to perform the AFEX-treated corn fiber fermentation at about 40% solid loading with final ethanol concentration of 98g/l. AFEX-treated DDG fermentation at 40% solid loading with the ethanol concentration reached 45 g/l. A mixed sugars (C5+C6) stream was generated from AFEX-treated corn fiber by enzyme and acid hydrolysis for the production of succinic acid. Fermentation: Hydrolysis of cellulosic feedstocks generates 5-and 6-carbon sugar streams, and lignin. The predominant organism used for ethanol production is S. cerevisiae, an organism that

currently cannot utilize the 5-carbon components. MBI international has a proprietary organism, A. succinogenes, capable of consuming C6 and C5 sugars simultaneously and fermenting them to succinic acid. We demonstrated the fermentation of corn-fiber derived sugars streams to succinic acid using only C5 sugars, or a mixed sugar stream of C6 and C5 sugars. Titers and yields of succinic acid production were measured. We demonstrated that >60% of the sugars in the fermentation can be biomass-derived.

IMPACT: 2006/07 TO 2007/07
Distiller's grains are a major and growing byproduct of corn dry mills. But due to the extreme conditions required to remove even a fraction of the protein from DDGS, coupled with the poor amino acid ratios obtained and low sugar yields, this extraction/hydrolysis process does not currently appear to provide a potential industrial use. Agricultural wastes such as wheat straw can be utilized to produce high strength and modulus and low density microfibrillated cellulose (MFC) and cellulose nanowhiskers (CNWs). Polylactic Acid (PLA), also derived from renewable resources such as corn starch, is a commercially available biodegradable polymer. MFC and CNW reinforced PLA nanocomposites are environmentally friendly materials with improved thermal and mechanical properties compared to neat PLA. We produced ethanol from AFEX-treated corn fiber and AFEX-treated DDGs at a level that met the technical and economical limit for industrial-scale alcohol distillation. A corn dry-mill bio-refinery that produces ~50MM gall of ethanol from starch annually could increase their product portfolio by fermenting the bran portion (~11% of corn in B-Frac operations). Assuming theoretical efficiencies of conversion this could lead to the production of an additional 3.8MM gal of ethanol (C6 sugars) and 20 MM lbs of succinic acid OR 16MM lbs of BT (C5 sugars) Alternatively, the entire bran portion (C6 and C5 sugars) could be used for succinic production, which could yield 75 MM lbs succinic acid. Furthermore, the succinic fermentation has the environmental benefit of CO2 incorporation.

PUBLICATIONS (not previously reported): 2006/07 TO 2007/07
Bals, B., Dale, B.E., Balan, V. 2006. Enzymatic hydrolysis of distillers dry grain and solubles (DDGS) using ammonia fiber expansion. Energy & Fuels. 20: 2732-2736

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Item No. 24 of 59
ACCESSION NO: 0211757 SUBFILE: CRIS
PROJ NO: MIN-12-075 AGENCY: CSREES MIN
PROJ TYPE: HATCH PROJ STATUS: NEW
START: 01 OCT 2007 TERM: 30 SEP 2010

INVESTIGATOR: Wang, P.

PERFORMING INSTITUTION:
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BIOSYNTHESIS OF METHANOL FROM BIOMASS DERIVED CARBON DIOXIDE

NON-TECHNICAL SUMMARY: The proposed synthetic strategy reflects a new venue for cellulosic and lignocellulosic liquid fuels (Fig. 7). The proposed synthetic pathway also provides a new platform for conversion of biomass carbon. Methanol can also be synthesized chemically from syngas (H2, CO2 and CO) generated by gasification of biomass. This route is an established thermo-chemical process. In the chemical industry this process is most efficiently operated starting from natural gas in petrochemical plants at a very large scale (5,000t/d). Compared to the existing thermo-chemical process, a biological CO2 ? CH3OH process will be much more selective, consume less energy, generate less pollution, cause fewer safety concerns and thus facilitate operation of smaller plants for "distributed" production of methanol.

These plants could be located where biomass can be harvested, transported and converted economically. The long-term goal of this project is to promote efficient and clean synthesis of easy-to-handle liquid fuel, methanol, from nonfermentable Minnesota biomass-derived carbon sources.

OBJECTIVES: The objectives are to investigate and develop a sequential multienzyme biocatalytic pathway for the production of methanol from CO2 (Fig. 3). CO2 may come either from the gasification of non-fermentable biomass such as lignin, from high intensity sources of CO2 such as power plants or refineries, or from the atmosphere. The biosynthesis of methanol from CO2 is the reverse process of a biological metabolic pathway.

APPROACH: The multienzyme catalytic system will couple the synthesis of methanol from CO2 with the production of dihydroxyacetone (DHA) from glycerol (Fig. 4). Glycerol, which contributes the protons needed for methanol synthesis, is a byproduct from biodiesel production from soybean oil. These two sets of reactions are coupled through the recycling of the cofactor, NAD(H) by the enzyme glycerol dehydrogenase (GDH). The processing of glycerol for production of diol is currently achieved through microbial processes. The proposed synthetic strategy will integrate two microbial pathways found in different biological sources and thus achieve one-pot synthesis of methanol and diol from renewable resources. We will explore the feasibility of the proposed concept that can be used for provisional patent filings and for future proposals for external funding. Specifically, we will (1) investigate reaction kinetics and equilibrium at different conditions including pH, substrate concentration and temperature; (2) examine the feasibility and methodology to construct the proposed mutienzyme catalysis with immobilized enzymes using meso-porous inorganic and polymer supports; and (3) study of the reaction kinetics of the immobilized multienzyme catalyst system.

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Item No. 25 of 59
ACCESSION NO: 0091840 SUBFILE: CRIS
PROJ NO: MIN-13-078 AGENCY: SAES MIN
PROJ TYPE: STATE PROJ STATUS: REVISED
START: 01 JUL 2004 TERM: 30 JUN 2009 FY: 2006

INVESTIGATOR: Gronwald, J. W.

PERFORMING INSTITUTION:
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MOLECULAR AND BIOCHEMICAL APPROACHES FOR DEVELOPING ALFALFA AS A BIOFUEL FEEDSTOCK

NON-TECHNICAL SUMMARY: There is a need to increase the production of renewal energy sources such as bioethanol. Alfalfa has potential as a cellulosic bioethanol feedstock but conversion efficiency needs to be improved. This project will address the need to improve bioconversion efficiency by generating knowledge that can be used to modify cell wall composition of alfalfa.

OBJECTIVES: Investigate the importance of the myo-inositol and nucleotide sugar oxidation pathways in the biosynthesis of cell wall matrix polysaccharides in alfalfa. Characterize the myo-inositol and nucleotide sugar oxidation pathways in transgenic alfalfa modified with genes that alter cell wall matrix polysaccharide biosynthesis.

APPROACH: Characterize the expression of UDP-glucose dehydrogenase and glucuronic acid 1-P pyrophosphorylase in alfalfa. Compare expression of UDP-glucose dehydrogenase and glucuronic acid 1-P pyrophosphorylase with changes in cell wall structure and composition in developing alfalfa stems.

PROGRESS: 2006/01 TO 2006/12
A large proportion of plant cell walls consists of matrix polysaccharides (pectin and hemicellulose). In alfalfa, the amount and structure of these polysaccharides influence the efficiency of converting cell wall biomass into ethanol. The myo-inositol oxidation pathway plays a key role in the synthesis of cell wall matrix polysaccharides in plants and UDP-sugar pyrophosphorylase (USP) is an important protein in this pathway. In earlier research conducted with the model plant Arabidopsis, we identified the gene that makes the USP protein. Our research also provided the first report that USP plays a critical role in cell wall synthesis in pollen. In current research to better define the role of USP in cell wall development, we prepared an antibody to the purified Arabidopsis USP protein. Research conducted with the antibody indicated that there are two isoforms of USP in Arabidopsis tissues. Immunoprecipitation assays conducted with the antibody indicated that USP is responsible for UDP-glucuronic acid pyrophosphorylase activity measured in Arabidopsis tissue extracts. These results provide evidence that USP serves as the terminal enzyme of the myo-inositol pathway and therefore plays a key role in cell wall synthesis. We have also examined the role of USP in cell wall formation in developing soybean embryos. Both gene expression and enzyme activity of USP increased during the growth of soybean embryos suggesting that USP plays an important role in cell wall synthesis. In future research, we plan to use gene silencing to block USP in developing soybean embryos and determine whether this extra carbon will be redirected toward the synthesis of more seed oil and protein. The results of this research have advanced understanding of an important metabolic pathway that regulates the formation and composition of plant cell walls. This new knowledge will be used to plan future research to modify alfalfa cell walls and increase the potential of alfalfa as a bioenergy crop. This knowledge will also be used to develop molecular approaches to redirect carbon into metabolic pathways that increase soybean seed oil and protein content.

IMPACT: 2006/01 TO 2006/12
There is a need to develop renewable energy sources to decrease dependence on imported fossil fuels and reduce the negative impact of fossil fuel use on the environment. In Minnesota, alfalfa offers considerable potential for development as a cellulosic feedstock for ethanol production. Basic knowledge generated by this research project will be used to develop transgenic alfalfa varieties that exhibit enhanced potential for cellulosic ethanol production. Knowledge regarding the regulation of cell wall synthesis in developing soybean seeds will be used to increase oil and protein content.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
1. Litterer, L.A., Schnurr, J.A, Plaisance, K.L., Storey, K.K., Gronwald, J.W., Somers, D.A. 2006. Characterization and expression of Arabidopsis UDP-sugar pyrophosphorylase. Plant Physiology and Biochemistry 44:171-180.
2. Schnurr, J.A., Storey, K.K., Jung, H.G., Somers, D.A., Gronwald, J.W. 2006. UDP-sugar pyrophosphorylase is essential for pollen development in Arabidopsis. Planta 224:520-532.
3. Litterer, L.A., Plaisance, K.L., Schnurr, J.A., Storey, K.K., Jung, H.G., Gronwald, J.W., Somers, D.A. 2006. Biosynthesis of UDP-glucuronic acid in developing soybean embryos: possible role of UDP-sugar pyrophosphorylase. Physiologia Plantarum 128:200-211.

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Item No. 26 of 59
ACCESSION NO: 0093725 SUBFILE: CRIS
PROJ NO: MIN-14-064 AGENCY: CSREES MIN
PROJ TYPE: HATCH PROJ STATUS: REVISED
START: 01 OCT 2007 TERM: 30 SEP 2012 FY: 2006

INVESTIGATOR: Runge, C. F.

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TRADE, ENVIRONMENT AND AGRICULTURAL SCIENCE IN THE GLOBAL COMMONS

NON-TECHNICAL SUMMARY: This project focuses on international trade conflicts in agriculture, sustainability of bioduels and the role of plant biotechnology to break out of the food-fuel dilemma. The purpose is to provide trade policy recommendations, analyze biofuels policy and propose solutions to the food-fuel dilemma.

OBJECTIVES: 1. Trade: to evaluate proposals to advance the Doha Round of multilateral trade negotiations, with specific attention to 2007 farm bill alternatives and proposals for reform. 2. Environment: to analyze the environmental impacts of biofuels on (a) land use in the U.S. and developing countries; (b) consequent water pollution impacts; (c) cellulosic and non-food alternatives as raw inputs; and (d) environment/trade interactions related to biofuel imports to the U.S. 3. Agriculture: to re-examine the role of plant biotechnology in the food-fuel debate over biofuels, both (a) in terms of improved productivity of food cropping; (b) reduced environmental impacts of planted crops; and (c) developing non-food inputs to the biofuels sector.

APPROACH: 1. Trade: evaluation of proposals through interviews with key participants in the trade and agricultural policy sphere, including joint collaboration with private sector, foundation, government representatives and colleagues (eg., Johnson, Tutweiller, Pearson, Senauer, Tiffany, Taff). Work with Johnson and Tutweiller will focus on Doha Round and Farm Bill alternatives, using interviews with government and private sector actors in the legislative and negotiating process. Consultation with Pearson will be on trade policy issues. Pearson is currently Chairman of the Federal Trade Commission. Work with Senauer and Tiffany will focus on biofuels and related trade matters, analyzing impacts of the 54 cent-per-gallon tariff on imported ethanol from federal trade data. Data will be drawn from interviews and federal trade statistics sources. Work with Taff will involve use of his energy simulation model to analyze alternative scenarios. 2. Environment: analysis of (a) shifts in land use from other crops to corn and soybeans and (b) their environmental consequences for soil loss and water pollution; (c) comparative consequences of various cellulosic and non-food alternatives; (d) environmental impacts of substituting imported biofuels for domestic sources, both in the U.S. and in exporting countries (eg., Brazil). Data will be drawn from U.S. Geological Survey and USDA sources as well as World Resources Institute data bases. The shift in land use from other crops to corn and soybeans will utilize USDA crop-reporting data on shifts in crop acreage. Comparative consequences for cellulosic and non-food alternative inputs will include cost analysis, environmental effects on land and water resources, and international environmental implications of shifts in land uses. 3. Agriculture: to re-examine the 2004-2005 inventory of the global diffusion of plant biotechnology undertaken for the Council on Biotechnology Information (CBI) with specific reference to laboratory experiments, field trials and commercialization of traits specific to the challenges posed by biofuels. These traits include (a) yield and quality improvements in food crops currently used as stocks (corn, soybeans, rapeseed, canola, oil palm) and (b) traits related to environmental impacts of alternatives stocks (tree crops, grasses). The extension of the 2004-2005 inventory will employ APHIS data sets on registration and field trials, but would focus on biofuel-related traits, including enzyme properties allowing more rapid conversion to ethanol or biodiesel fuels. Also considered would be biotech crops with a combination of these traits and more environmentally benign traits.

PROGRESS: 2006/01 TO 2006/12
Research continues into U.S. and international aspects of international trade and the food system. In addition, the new stream of research focusing on school feeding programs has generated journal article acceptances, three M.S. theses, and a pending book proposal. This is consistent with the U of M's initiative on Healthy Foods, Healthy Lives and has provided economic analysis of the choices made by school food administrators. In addition, I have published a journal article on intellectual property rights in an international context in World Development, an article in The American Interest on trade, a new entry for

Agricultural Economics in The New Palgrave Dictionary of Economics (first published as a CIFAP Working Paper), and an assessment of the role of the federal Production Tax Credit for wind farms.

IMPACT: 2006/01 TO 2006/12
Decisions by state and federal policy makers concerning trade, agriculture and intellectual property policy, and school feeding program decisions at the local, state and federal level.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
1. Agriculture of Corruption. The American Interest (Winter-Jan./Feb. 2007): 70-78.
2. Exclusion, Inclusion and Enclosure: Historical Commons and Modern Intellectual Property. World Development 34(10)(2006): 1713-1727.
3. Re-examining the Production Tax Credit for Wind Power: An Assessment of Policy Options (with Douglas Tiffany). St. Paul, MN: Fresh Energy. March 1, 2006.
4. Agricultural Economics: A Brief Intellectual History. Center for International Food and Agricultural Policy (CIFAP) Working Paper WP06-1, June 2006. Department of Applied Economics, University of Minnesota.
5. Grainger, Corbett A. 2005. Measuring Innovation in a High School Cafeteria: A Case Study of Hopkins, Minnesota. Masters Plan B Paper.
6. Wagner, Barbara J. 2006. Demographic, Budgetary and Regulatory Factors Constraining the Nutritional Quality of Foods Served in the National School Lunch Program. Masters Thesis.
7. Agiwal, Swati. 2006. Financial Analysis of School Lunch: Hopkins, Minneapolis and Saint Paul School Districts. Masters Plan B Paper.

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Item No. 27 of 59
ACCESSION NO: 0087768 SUBFILE: CRIS
PROJ NO: MIN-43-068 AGENCY: CSREES MIN
PROJ TYPE: HATCH PROJ STATUS: REVISED
START: 01 OCT 2005 TERM: 30 SEP 2010 FY: 2006

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LIGNIN BIOSYNTHESIS, BIODEGRADATION AND DERIVATIVE PLASTICS

NON-TECHNICAL SUMMARY: This project seeks to rectify three fundamentally misconceived ideas that have long plagued the lignin field. The manner in which lignins are non-randomly assembled in plant and wood cell walls will be elucidated; the correct enzymatic basis for lignin biodegradation will be established; and formulations for useful thermoplastics with high (above 80 percent) contents of simple lignin derivatives will be developed. The results are expected to play important roles in the development of processes that will be used in biorefining plant and woody materials on a commodity scale.

OBJECTIVES: This project constitutes an umbrella for three different areas of lignin research. In regard to lignin biosynthesis, the mode of dehydrogenative monolignol polymerization will be examined in the presence of species that could act as templates for assembling the macromolecules being formed. Plausible template species include high molecular weight lignin components and proline-rich proteins from plant/wood cell walls. The work will be focused upon the working hypothesis that the final step in lignin biosynthesis could involve the transcription and replication of macromolecular configurations through template polymerization processes. Compatible molecular contours among the interacting substructures involved in the replication process have already been identified through molecular modeling. Preliminary molecular orbital calculations have suggested that the nonbonded orbital interactions between the aromatic rings in lignins can be equivalent in strength to two or more hydrogen bonds. As far as lignin biodegradation is concerned, an enzyme has been isolated in the project leader's group from the white-rot fungus Trametes cingulata that can completely depolymerize high molecular weight softwood kraft lignin components without requiring a cofactor or mediator. Since lignin depolymerase is naturally produced by white-rot fungi in extremely low concentrations, the enzyme will be expressed at much higher levels in a suitable host organism. The stability and catalytic activity of the recombinant enzyme will be improved, and the extent of its glycosylation will be reduced (so that it is not counterproductively lost through adsorption on surfaces). Finally, the utility of the improved recombinant lignin depolymerase will be documented in facilitating the conversion of renewable lignocellulosic plant materials into feedstocks for further bioprocessing. In creating new lignin-based polymeric materials, softwood kraft, organosolv and milled wood lignins will be methylated and blended with 15% target levels of miscible low-glass-transition-temperature polymers to create thermoplastics that can compete with polystyrene and other commodity plastics. Building on recent insights gained in the project leader's research group, the new kraft lignin-based polymeric materials will pave the way for developing thermoplastics from the organosolv and milled wood lignins, which will share common features with corresponding biorefinery byproduct lignins. These new thermoplastics will be unique among polymeric materials in that their mechanical properties will rest on supramacromolecular complexes containing many thousands of individual components. The integrity of these huge entities will need to be preserved as the content of the low-glass-transition-temperature polymeric plasticizer in the blend is systematically minimized. This will be accomplished by reducing both the lignin-lignin and plasticizer-plasticizer interactions at the molecular level, while adjusting the lignin-plasticizer interactions to the threshold required for blend homogeneity.

APPROACH: The studies of lignin biosynthesis seek to delineate how lignin template species can determine the configurations of macromolecular dehydropolymerisates formed from monolignols. The possibility that the primary structures of lignin chains are transcribed from proline-rich cell-wall proteins will be investigated. Upon formation at the surface of such a protein, a progenitorial lignin chain is expected to undergo replication through a template polymerization mechanism. The lignin templates employed will vary according to chain length, frequencies of different interunit linkages, monomer residue configuration, and methylation of their aromatic hydroxyl groups. The effects of these template characteristics upon monolignol dehydropolymerization will be examined with respect to the molecular weights and radii of gyration of the dehydropolymerisates formed, the identities of the interunit linkages, template compatibility with coniferyl and sinapyl alcohol monomers, and their preference for monolignols or the corresponding radicals. Molecular orbital calculations will be employed to estimate the strengths of the dominant intermolecular nonbonded orbital interactions involved in these processes. The lignin biodegradation study will have a profound impact on efficient biomass utilization in biorefineries. De novo peptide sequencing of the lignin depolymerase will be used to identify the most closely related gene(s) in Phanerochaete chrysosporium, whose genome has been completely sequenced. These genes are not expected to be among those previously identified as coding for ligninolytic enzymes in white-rot fungi. The lignin depolymerase gene will be cloned and expressed in a suitable host organism. Through directed evolution strategies and classical protein engineering, the stability and catalytic activity of the recombinant enzyme will be improved. To minimize the adsorption of the protein onto surfaces during industrial applications, the extent of its glycosylation will be reduced. The utility of the improved recombinant lignin depolymerase will also be documented in enhancing enzyme-catalyzed polysaccharide degradation in lignocellulosic substrates. In regard to lignin-based plastics, formulations based on the methylated Organosolv and milled wood lignin derivatives will be different from previously created alkylated kraft lignin-based thermoplastic blends. Some exploratory work will be needed to discover what types of low-glass-transition-temperature polymers act as plasticizers in forming homogeneous blends with the two former lignin derivatives. To improve plasticizer efficacy in these new polymeric materials, the intermolecular plasticizer-plasticizer and lignin-lignin interactions have to be reduced without decreasing the plasticizer-lignin interactions below the threshold required for blend miscibility. This will be accomplished by (i) judicious manipulation of the configuration of the low-glass-transition-temperature polymer, and (ii) introduction of particular low molecular weight compounds that synergistically enhance plasticization of the resulting blends by interacting preferentially with the lignin components.

PROGRESS: 2006/01 TO 2006/12

A complete hypothetical mechanism has been developed for the replication of primary structure during lignin biosynthesis. It has been proposed that the necessary information is encoded in (relatively rigid) double-stranded lignin templates dynamically poised in a state of single-strand elongation and compensating double-strand dissociation at the advancing front of every lignifying domain. The most significant progress in understanding template structure during this reporting period was made with density functional theory using the new M05-2X hybrid meta exchange-correlation functional that is parametrized for kinetics, thermochemistry and non-covalent interactions. Computations for guaiacyl complexes involved in 8-O-4', 8-5' and dibenzodioxocin lignin substructures have yielded estimates of ~7-9 kcal/mol for the stabilization energies between the interacting aromatic rings that maintain the double-stranded template structure. The latter are all approximately parallel, but cofacially offset from one another, with perpendicular spacings of ~3.4 angstroms. Such interaction energies can be equivalent in their effects to two or more hydrogen bonds, and thus should be sufficient to ensure that the primary structure of a parental chain may be replicated during lignin biosynthesis through an endwise dehydropolymerization process. It is noteworthy that, compared to MP2/6-31G(d) level wave function theory, the more accurate M05-2X/6-31+G(d,p) density functional theory requires far fewer computational resources, and yet the results from both methods have been quite comparable. Significant progress has been made in the work devoted to the isolation, characterization and production of the lignin depolymerizing enzyme, lignin depolymerase. Two lignin depolymerase enzymes from Trametes cingulata had been previously evaluated according to their ability to depolymerize soluble high molecular weight kraft lignin components. Both enzymes have been subjected to tryptic digestion after a lengthy iso-electric focusing procedure that separates the extracellular fungal enzymes from one another and the residual lignin substrate. The resulting polypeptides have been analyzed by electrospray ionization-ion trap mass spectrometry. Using the PEAKS software package from Bioinformatics Solutions Inc., many of the resulting fragmentation patterns have successfully yielded reliable de novo sequence information. More significantly, characteristic posttranslational modifications of certain oligopeptides have provisionally identified the type of mechanism that lignin depolymerase employs. Moreover, in certain cases, sets of primers constructed from the polypeptide sequences have facilitated specific PCR amplification of polynucleotide sequences in the cDNA library obtained from T. cingulata cultures under conditions where lignin biodegradation was taking place.

IMPACT: 2006/01 TO 2006/12
Interest in effective ways of handling lignins during the large-scale biorefining of plant materials (including wood) has been steadily growing. The lignins must be removed efficiently and utilized in the most appropriate way as the plant polysaccharides are degraded and converted to liquid fuels and organic chemicals. Both removal and utilization will be facilitated by a clearer understanding at the molecular level of, respectively, the first step in lignin biodegradation and the structural characteristics of the biopolymer chains formed during lignin biosynthesis. The entire project is focused on implementing these goals.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
Chen, Y.-r. and Sarkanen, S. 2006. From the macromolecular behavior of lignin components to the mechanical properties of lignin-based plastics. Cellulose Chem. Technol. 40(3-4): 149-163.

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Item No. 28 of 59
ACCESSION NO: 0195065 SUBFILE: CRIS
PROJ NO: MINW-2003-06003 AGENCY: CSREES MINW
PROJ TYPE: SPECIAL GRANT PROJ STATUS: NEW
CONTRACT/GRANT/AGREEMENT NO: 2003-34291-13283 PROPOSAL NO: 2003-06003
START: 15 SEP 2003 TERM: 14 SEP 2006 GRANT YR: 2003
GRANT AMT: $464,361

INVESTIGATOR: Nagel, J. L.

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PRODUCER ALLIANCE INFRASTRUCTURE & AGRICULTURAL DIVERSITY IN THE NORTHERN GREAT PLAINS

NON-TECHNICAL SUMMARY: There are ongoing concerns in the region regarding the future of agriculture and farm and ranch profitability in the Region. The purpose will be to increase agricultural diversity and commercial links to the global marketplace in the Region. The project will focus on concerns regarding the future of agriculture and rural communities in the Northern Great Plains region in an effort to identify opportunites for success in the Region.

OBJECTIVES: This 5-state project has one primary goal and four objectives, each focusing on concerns regarding the future of agriculture and rural communities in the Northern Great Plains region. The primary goal is to bring together the many resources of the Region into a collaborative effort to address shared rural economic, environmental, energy and community issues within the 5 state region. The first objective of this project is to establish a NGP working group of the existing producer alliances within the Region. The second objective is to bring together the work being done at several land grant universities and other research institutions in the Region on the traceability of agriculture products and the use of sustainable agriculture and land managment practices to develop a regional strategy for gaining marketplace advantage for producers and processors in the Region thru implementation of traceability systems and sustainable agriculture practices. The third objective is to further development of opportunities in use of biomass and biofuels as energy sources. The fourth objective is to complete Renewing the Countryside books for South Dakota and Nebraska. NGP and GPISD will continue to link past and ongoing USDA, DOE, DOL, and private foundation funded projects with the project. This includes work in agriculture diversity-especially vegetable production and processing, eBusiness training, leadership development, international education programs, Powering the Plains, sustainable agriculture, and renewable energy development. NGP and GPISD will also continue their extensive outreach and education efforts related to the work they are doing under this project.

APPROACH: The specific research and development activities include on-going interaction with existing producer allliances, completing an asset assessment of current infrastructure in each state, creating a regional asset/baseline analysis of activities to meet alliance needs, completing a strategic work program with alliances, design the establishment of a complete traceability system for the products typically grown in the region, research the critical components of a system of traceabiltiy for producer alliances, identify what new products could be developed from native species which will also foster a more healthy and diverse ecosystem, identify the current scientific base of knowledge and what scientific gaps exist, research the potential for cellulose as a feedstock for ethanol production, expand a website resource to include success stories and sustainable models, produce a commercially successful book that conveys a message of hope and possibility in rural communities, and develop a program that compares stories of success from the northern plains with those of climatologically similar countries in Europe.

PROGRESS: 2003/10 TO 2004/09
Objective 1:Producer Alliances-NGP continutes to promote on-going interaction between state alliances. FarmConnect (the MN alliance) continues to serve as a model for other states. Funding for specific projects rather than cooperation between producers is an on-going concern. Objective 2:Traceability Systems-NGP sponsored a study program of leading edge agricultural developments in Europe. State ag officials, farmers, representatives of non-government organizations, NGP board members & staff participated. From this programs two publications listed below were produced. Objective3:Biomass Energy Development-GPISD continues to investigate cellulosic bio-fuels, power development and native grass biofuels. GPISD staff provided leadership to the Bush Foundation supported Powering the Plains initiative. A report on the potential of Native Species is nearing completion. NGP has concluded that there is need for current, regional information about native floara & fauna as it pertains to adding value to farm income. Objective 4:Renewing the Countryside-Contacts have been made with RTC staff in Minneapolis. There is a continuing pursuit of funding for South Dakota and Nebraska as Northwest Area Foundation has chosen to no longer provide financial assistance. Objective 5:NGP Regional Authority-NGP convened several

meetings of the Governor's Alternates to the Authority. A regional work plan,identification of 'distressed counties' criteria, and management of the Authority were the primary focus of this group.

IMPACT: 2003/10 TO 2004/09
1. Several commodity groups have started education programs in traceability and many are using NGP produced materials. NGP has been asked to develop and participate in several traceability demonstration projects. 2. A Northern Tier Hydrogen Fuels program has been started as a result of the bio-fuels work. 3. A national initiative for Renewing the Countryside has been started and books are being prepared in several states. 4. NGP is helping the University of Minnesota-Crooksotn develop a Sustainability Institute.

PUBLICATIONS (not previously reported): 2003/10 TO 2004/09
1. Orngard, S. and Joannides, J., 2003. Renewing the Countryside Iowa, www.renewingthecountryside.org
2. Bergan,S.,Crabtree,B., Hoffman,L., Gast,J.,Glassheim,E.,Nagel,J., Ricord, B., Gopal,R., Hulse,D., Johnson, C., 2003. Renewing the Countryside North Dakota,www.renewingthecountryside.org
3. Wagner, G.L. and Glassheim, E. 2003. Traceability of Agricultural Products,www.ngplains.org
4. Glassheim, E., Nagel, J., and Roele, C. D., 2004. The New Marketplace in European Agriculture:Environmental and Social Values with the Food Chain,www.ngplains.org

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Item No. 29 of 59
ACCESSION NO: 0200095 SUBFILE: CRIS
PROJ NO: MISZ-065390 AGENCY: CSREES MISZ
PROJ TYPE: SPECIAL GRANT PROJ STATUS: EXTENDED
CONTRACT/GRANT/AGREEMENT NO: 2004-34158-14682 PROPOSAL NO: 2004-06324
START: 15 AUG 2004 TERM: 14 AUG 2007 FY: 2006 GRANT YR: 2004
GRANT AMT: $736,009

INVESTIGATOR: Hopper, G. M.; Leightley, L. E.; Shepard, J. P.

PERFORMING INSTITUTION:
FOREST PRODUCTS
FOREST AND WILDLIFE RES CENTER
MISSISSIPPI STATE, MISSISSIPPI 39762

WOOD UTILIZATION RESEARCH PROGRAM 2004

NON-TECHNICAL SUMMARY: Economic and utilization research of southern wood resources is directed at improving and extending the use of these resources, adding value and economic return to forest management, developing a better understanding of the contribution of forestry and forest products industries to our economy, improving costs of producing wood products, improving performance of wood in residential housing and furniture construction, and reducing environmental impacts of wood production.

OBJECTIVES: The objectives of this research are to administer a continuing program of research and technical assistance to improve the utilization and values of southern timber resources, to strengthen existing efforts in timber harvesting and wood utilization and to support new research initiatives in these areas.

APPROACH: Utilization of Wood Based Materials in Housing: Synthesize a series of functional water repellent materials for wood in the laboratory and identify their long-term water repellency for use in oriented strand board, particle board and decking lumber. Evaluate water repellent treatments for wood which could reduce the amount of biocide needed to protect wood from biodegradation. Evaluate the durability of new engineered wood products, such as Pressed Scrim Lumber and new methods of increasing service life. Determine the microbial breakdown of biocide treated wood and subsequent fungal colonization of the wood. New Manufacturing Systems for Wood Based industries: Develop a device for detecting knots in logs moving at speed through electrode sensors. Develop a real-time after press radio frequency device to detect moisture content (MC) and specific gravity. Pursue advanced research into the application and implementation of lean manufacturing to increase the American furniture industry's productivity, competitiveness and profitability. Fiber and Chemicals from Wood: Use flash pyrolysis to convert cellulosic materials into BioOil. Separate and identify chemical products in the BioOil. Potential supply and demand for bio-oil as an alternative fiber output for forests in Mississippi will be examined. The trade-offs in managing timber resources for bio-oil as compared to traditional timber commodities, such as sawtimber, will be evaluated. The development of an alternative fiber market will improve the production efficiencies and competitiveness in the international and domestic markets. Timber Harvesting and Wood Utilization in Mississippi: A thorough review of forestry and agricultural literature will be conducted to examine the origins and scientific basis for adopting commonly accepted BMPs in Mississippi thereby improving the understanding of the economic costs and benefits and the relative effectiveness of BMP practices at a watershed-scale. A methodology that will document and identify the relative contributions of sediment from various land-use practices and physical features in the landscape will be developed.

PROGRESS: 2006/01 TO 2006/12
Five new biocides have been developed, tested, and proven effective for use in engineered wood composites. Two parallel pull flow assembly lines have been designed and implemented in two furniture factories. Two compounds have been synthesized as potential water repellent and stabilization agents for wood composites, but neither were found to be improvements over currently used industrial wood treatments. A waterborne water repellent made from tall oil rosin was tested using southern pine sapwood. Detection of fungi associated with the accelerated soil contact decay test method was completed on 432 below-ground samples. Bio-oils from pine and oak species have been fractionated into water soluble and non-soluble components. Char characteristics have been analyzed and the potential utilization of the chars as a substitute for activated carbons for metals has been tested. A real-time after-press RF device and device simulation analysis on different variable effects on signal responses has been completed. A comprehensive literature review on sediment production as a function of forest management practices has been completed, and a welfare analysis has been conducted using a Muth-type equilibrium desplacement model. Total graduate student FTE is 5.50.

IMPACT: 2006/01 TO 2006/12
Manufacturers of engineered wood products have been provided with five new alternatives for biocides, all of which showed no practical negative impacts on the products that were treated. The implementation of parallel pull flow assembly lines has increased factory productivity by approximately 50%, reduced direct labor and factory floor space by roughly 15%, reduced assembly cycle time by 33%, and increased units per worker by 73%. Synthesis of a new series of potential functional water repellents has provided the research community with a platform molecule from which new water repellents and wood adhesives can be developed in the future. The development of improved, cost effective water repellents would be very beneficial to consumers concerned with protecting above-ground wood from warping, splitting, and checking. Results from investigations of microbial populations associated with the accelerated soil contact decay test method will reduce the time to evaluate preservative treatments from years to months. The production and analysis of chemicals and fuels from lignocellulosic feed stocks is key to the development of alternative fuels from woody biomass in the Southern U.S. A non-destructive, real-time method for monitoring and locating specific gravity and MC can supply crucial information needed to control and improve manufacturing of wood composite products. Evaluations of welfare distribution suggest that future tech and financial assistance for forestry BMPs should target those who have experienced considerable financial losses: landowners and loggers.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
1. Barnes, H. M., Slay, R. A., Seale, R. D., Linsey, G. B. 2006. Treatability of steam pressed scrim lumber (SPSL). Proceedings, American Wood-Preservers Association 102:68-72.
2. Barnes, HM, RJ Murphy. 2006. Effect of vapor boron treatment on some properties of wood strand and fiber composites. Composites: Part A 37(2006):1402-1405.
3. Kirker, G.T., M.L. Prewitt and S.V. Diehl. 2006. Effects of Chlorothalonil (CTN) and Butylated Hydroxy Toluene (BHT) on Microbial Communities involved in deterioration of wood using T-RFLP:I Accelerated laboratory decay study. IRG/WP 06-20332

4. Kirkpatrick, J. W., Barnes, H. M. 2006. Copper naphthenate treatment for engineered wood composite panels. Bioresource Technology 97(15):1959-1963.
5. Kirkpatrick, J. W., Barnes, H. M. 2006. Treatments for engineered wood composites - a review. International Research Group on Wood Protection, Document No. IRG/WP 06-40323, 21 pp.
6. Kirkpatrick, J. W., Barnes, H. M. 2005. Biocide treatments for engineered composite panels. Proceedings of the American Wood-Preservers Association 101:92 (Technical Abstract).
7. Leightley, L. and P. H. Steele. 2005. Energy and chemicals from wood biomass - an industry of the future for Mississippi. Tree Talk 27(4):15-16. The Mississippi Forestry Assoc., Jackson, MS.
8. Mohan, D., C. U. Pittman and P. H. Steele. 2006. Single, binary and multi-component adsorption of copper and cadmium from aqueous solutions on Kraft lignin - a biosorbent. Journal of Coloid and Interface Science, Issue 297, p 489-504.
9. Mohan, D., C. U. Pittman and P. H. Steele. 2006. Pyrolysis of wood -biomass for bio-oil: a critical review. Energy and Fuels 20(3):848-889
10. Schultz, T.P., D.D. Nicholas, G.T. Kirker, M.L. Prewitt, S.V. Diehl. 2006. Effect of the antioxidant BHT on reducing depletion of chlorothalonil wood after 54 months of ground-contact exposure.
11. Steele, P. H., J. E. Cooper, B. Mitchell. 2006. Identifying knot wood in green and kiln-dried southern pine lumber with dielectric scanning. Five pages in Proceedings, Digital Imaging IX, and ASNT Topical Conference. Mashantucket, CT. July 24-26, 2006.
12. Steele, P. H. and J. E. Cooper. 2006. Utilization of electrical impedance tomography to detect internal anomalies in southern pine logs. In Review of Quantitative Nondestructive Evaluation Vol. 14, ed. By D. O. Thompson and D. E. Chimenti. American Institute of Physics 0-7354-031200/06. 1942 pp.
13. Sun, C. 2006. Welfare effect of forestry Best Management Practices in the US. Canadian Journal of Forest Research.

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Item No. 30 of 59
ACCESSION NO: 0210809 SUBFILE: CRIS
PROJ NO: MOR-2007-02131 AGENCY: CSREES MO.R
PROJ TYPE: NRI COMPETITIVE GRANT PROJ STATUS: NEW
CONTRACT/GRANT/AGREEMENT NO: 2007-35504-18256 PROPOSAL NO: 2007-02131
START: 01 SEP 2007 TERM: 31 AUG 2010 GRANT YR: 2007
GRANT AMT: $425,000

INVESTIGATOR: Angenent, L. T.; Scholten, J. C.; Cotta, M. A.; Dien, B. S.

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MIXED COMMUNITY BIOREACTORS TO CONVERT (LIGNO) CELLULOSIC FEEDSTOCKS INTO THE LIQUID BIOFUEL BUTANOL

NON-TECHNICAL SUMMARY: Energy independence is a high priority for the United States given existing trends of rising worldwide energy demand. Agricultural feedstocks in the US have potential as a considerable energy source for liquid fuels, such as ethanol and butanol, alongside realizing a carbon neutral transportation economy. The microbial breakdown and subsequent solubilization of polymeric cellulosic compounds is seen as the rate-limiting step in bioprocessing of agricultural feedstock. This makes evolutionary sense, because lignocellulosic materials form the scaffolds for plant metabolism and must resist microbial attack. However, some evolutionary biosystems, such as insect (e.g., termites) and some mammalian (e.g., ruminants) intestinal tracts, have created environments for a diverse microbial community to thrive by extracting energy from lignocellulose and passing it to the host. We will mimic

these evolutionary biosystems in the laboratory and will break down lignocellulosic feedstocks into butyrate in bioreactors. The produced butyrate solution will then be fed to a second bioreactor with a pure-culture of Clostridium sp. that will convert butyrate into the liquid biofuel - butanol. Producing butanol from agricultural feed stock is important, because converting agricultural waste into biofuels enhances economic opportunities for agricultural producers and rural life in general. In addition, producing carbon-neutral biofuels also protects and enhances the US natural resources and environmental quality.

OBJECTIVES: The overall goal of the proposed study is to improve the conversion efficiency of lignocellulosic feedstock (i.e., fractionated corn fiber) into a liquid biofuel (i.e., butanol) by understanding mixed culture microbial processing. We propose to convert corn fiber into butyrate with a mixed culture, first-stage bioprocess, and subsequently produce butanol from a butyrate-rich substrate in a second-stage bioprocess. The supporting objectives are: 1. to ascertain the effects of pretreatment on the molecular structure and microbial break down efficiency and rate for a lignocellulosic feedstock; 2. to manipulate a diverse community bioprocess to produce predominantly butyrate; and 3. to optimize the secondary bioprocess for butanol production from a mixed substrate.

APPROACH: To validate the perceived need to pretreat before microbial hydrolysis, pretreated (three different pretreatment methods) and nonpretreated fractionated corn fiber (i.e., corn fiber) particles will be studied by solid-state nuclear magnetic resonance (NMR) to investigate changes on the molecular level. In addition, the efficiency and rate of microbial breakdown for these materials in our primary bioprocess will be tested by long-term (one year) bioreactor studies with anaerobic sequencing batch reactors (ASBRs). Biodegradation efficiency will also be studied by removing partly degraded corn fiber from the bioreactors for molecular inquiry. The key to bioprocessing with a mixed culture is to generate a predominant fermentation end product that is not likely to be further degraded. We will investigate how several operating tools can be used to shift bacterial fermentation towards butyrate as the predominant and desired end product. Molecular techniques that do not rely on bacterial cultivation and modeling approaches will aid in understanding our experimental results. Some butyrate-producing clostridia can shift their metabolic pathway towards acetone-butanol fermentation. This shift occurs during the initiation of sporulation at low pH and is called solventogenesis. Butyrate-rich effluent from the first-stage bioprocess will be fed to the second-stage, pure-culture Clostridium sp. solventogenic bioprocess. Some carbohydrate must be supplemented to provide the metabolic energy to convert butyrate into butanol. An experimental and modeling approach will be used to maximize butanol generation, minimize supplementary carbohydrate addition, and test for unknown effects due to the diverse composition of the butyrate-rich feed.

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Item No. 31 of 59
ACCESSION NO: 0192708 SUBFILE: CRIS
PROJ NO: MONK-2002-00424 AGENCY: CSREES MONK
PROJ TYPE: SMALL BUSINESS GRANT PROJ STATUS: NEW
CONTRACT/GRANT/AGREEMENT NO: 2002-33610-11881 PROPOSAL NO: 2002-00424
START: 15 MAY 2002 TERM: 14 NOV 2002 GRANT YR: 2002
GRANT AMT: $80,000

INVESTIGATOR: Mcgrath, S.

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INNOVATIVE ENZYMATIC REACTOR FOR PRODUCTION OF ALTERNATIVE FUELS

NON-TECHNICAL SUMMARY: The USA produces 20 billion pounds of plant oils and 11 billion pounds of animal fats and recycled greases annually. The animal fats represent a potential biodiesel output of 1.5 billion gallons compared to the estimated production of 230 million gallons in 2000. Based on developing a truly competitive technology that can cost effectively convert fats to biodiesel, it will be possible to capture a substantial portion of this untapped market. Concerns related to feeding animal by-products (already banned in Europe) will increase the incentive to convert fats to biodiesel and further strengthen the market potential of the technology. Conservatively estimating the potential as 20% market share after 5 years, will give access to a total of 300 million gallons of biodiesel with a value of roughly $300 million per annum at current fuel prices.

OBJECTIVES: The Phase I project will demonstrate the feasibility of an innovative reactor for the production of alterantive fuels with complete conversion of free fatty acids coupled with continuous recovery of products. Key features of the continuous process for the conversion of agricultural by-products to biodiesel are: Ability to utilize low cost agricultural by-products such as vegetable oils, tallow, and yellow grease to develop a value-added industrial biodiesel production process; Continuous recovery of products to eliminate end-product inhibition for the process and shift thermodynamic equilibrium to the product direction. The novel reactor will be used to address all the major limitations of the contemporary processes enabling cost competitive production of biodisel, without generation of any waste by-products. Biodiesel is a nontoxic, biodegradable replacement for petroleum diesel. Currently, biodiesel is produced by a process called transesterification. The vegetable oil or animal fat is first filtered, then preprocessed with alkali to remove free fatty acids. It is then mixed with an alcohol (usually methanol) and a catalyst (usually sodium or potassium hydroxide). The oils' tri-glycerides react to form esters and glycerol, that are then separated from each other and purified. This is an expensive, complex process. The novel process that will result out of this Phase I project circumvent these issues and provides for a process that produces a high quality biodiesel and glycerol product independent of the feedstock composition or moisture content. No waste products are produced in the one-step process. This project will result in a step change in technology development enabling the low cost production of biodiesel from a variety of feedstocks including vegetable oils, (soya, rape and canola), tallows and yellow grease.

APPROACH: See Objectives.

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Item No. 32 of 59
ACCESSION NO: 0188612 SUBFILE: CRIS
PROJ NO: NEB-29-011 AGENCY: CSREES NEB
PROJ TYPE: SPECIAL GRANT PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2001-34233-10499 PROPOSAL NO: 2002-06133
START: 01 SEP 2001 TERM: 31 AUG 2004 FY: 2004 GRANT YR: 2002
GRANT AMT: $59,863

INVESTIGATOR: Hanna, M. A.

PERFORMING INSTITUTION:
INDUSTRIAL AGRICULTURAL PRODUCTS CENTER
UNIVERSITY OF NEBRASKA
LINCOLN, NEBRASKA 68583

INDUSTRIAL AGRICULTURAL PRODUCTS CENTER

NON-TECHNICAL SUMMARY: The viability of production agricuture is dependant upon good markets for the commodities. This project examines the viability of using agricultural commodities including biomass to produce biopolymer, biofuel and biopower. The research will document feedstock resources, conversion technologies, and compatibility of multiple uses of feedstocks.

OBJECTIVES: The project objectives are to enhance research efforts in the areas of biopolymers, biofuels and biopower.

APPROACH: Composites of starch acetate and degradable synthetic polymers, including polycaprolactone (PCL) and Eastar Bio Copolyester (EBC) will be prepared by reactive extrusion. A twin-screw extruder with co-rotating mixing screws (Model CTSE-V, C. W. Brabender, Inc.) will be used to conduct the extrusions. Thin sheets will be prepared using a pair of take-off rolls (Model SE 606, C. W. Brabender, Inc.). Foams also will be made using an extruder. Alcohol will be used as the blowing agent. Molds will be manufactured for injection and compression-molding articles from the composites. Thermal properties of the composites will be determined using differential scanning calorimetry (DSC) and a thermal conductivity probe. Physical characteristics of the composites such as expansion, density, and water resistance will be determined. Mechanical properties including tensile strength, elongation, and spring index will be studied using an Instron universal testing machine. Micrographs of the composites will be obtained using a scanning electron microscope (SEM) to study the morphological characteristics of the samples. Biodegradation tests will be conducted in laboratory composting chambers. Temperature and humidity of the chambers can be adjusted. Rates of degradation will be monitored by gas chromatography. Efforts in the area of biofuels will be primarily in the area of biodiesel, as opposed to ethanol, etc. The continuous transesterification for producing biodiesel, referred to in the Progress Report above, will allow for sequential addition of the alcohol-catalyst solution. Our goal is to reduce the mole ratio of methanol to triglyceride from the 6:1 that is typically used to 4 or 5:1 and to achieve complete reaction in a matter of seconds with no mechanical mixing as is typically used. The independent variables will include the mole ratio of methanol or ethanol to triglyceride, the use of sodium hydroxide versus potassium hydroxide as the catalyst, and the volume flow rate of the reactants through the system. Soybean oil and beef tallow will be the triglyceride feedstocks used. The dependent variable will be the time required to complete the transesterification reaction. Efforts in the area of biopower will concentrate on availability of feedstocks and gasification as a method of converting biomass to a synthesis gas. A number of biomass feedstock summaries have been prepared in recent years. The data for Nebraska will be compiled. From this, projections will be made regarding the sustainability of biopower generation in Nebraska. Concurrently, gasification of selected materials such as Eastern red cedar trees will be evaluated.

PROGRESS: 2001/09 TO 2004/08
The biopolymers research effort included a more comprehensive look at the requirements of acetylating starch. We included the personnel from a stach company in our evaluations and interpretations. Our efforts included lab scale testing with 600ml reactors through pilot scale testing with a reactor that can process 100 kg batches. We have made numerous batches for in-house extrusions for the purpose of characterizing starch acetate as a biopolymer for use in packaging and insulation foams. Extrusions have been done with our laboratory scale C.W. Brabender extruders as well as commercial extruders at three different companies. Our commercialization efforts continue. Our in-house characterization of starch acetate has included the extrusion of starch acetate with numerous other ingredients with the goal of enhancing the functional properties of the extrudates. The other ingredients included other biopolymers such as PLA and Mater-Bi; fibers such as cellulose, corn fiber and oat fiber. Our efforts have resulted in numerous pubications and follow up research grant proposals to agencies such as USDA-CSREES, the Nebraska Corn Board and several companies. The biodiesel research efforts led to an understanding of the relative solvencies of ethanol, biodiesel and diesel fuels. Our data were used to develop a phase diagram that clearly shows the porportions of each that can be mixed to form stable fuel blends. This will be published soon. The combination of our biofuels and biopower research efforts and the interest in the private sector to get involved in the production of biofuels, led us to a proposal to develop a map of the bioresources in Nebraska that could be used to identify the best location for processing facilities. We received a Value-Added Processing Grant in conjunction with the Nebraska Soybean Association to begin the development of the resources map.

IMPACT: 2001/09 TO 2004/08
The research on characterization of starch acetate as a basis for expanded foam production supports our commercialization efforts in the same area. Our patent is still pending. We are using the research results from both the starch acetate and the biodiesel efforts to prepare competitive grants proposals to futher our

understanding of these topics and to position ourselves better for commercialization. A biodisel proposal has been submitted to the NSF and another to USDA-CSREES. Another two proposals have been submitted to USDA-CSREES on related starch biopolymer characterization and utilization. We currently have two funded projects that are spin offs of this project

PUBLICATIONS (not previously reported): 2001/09 TO 2004/08

1. Guan, J.J. and M.A, Hanna. 2003. Post extrusion steaming of starch acetate foams. Trans. of the ASAE. 46(6):1613-1624.
2. Guan, J. and M.A. Hanna. 2004. Functional properties of extruded foam composites of starch acetate and corn cob fiber. Ind. Crops and Products. 19(3):255-269.
3. Guan, J., K. Eskridge and M.A. Hanna. 2004. Functional properties of extruded acetylated starch-cellulose foams. J. of Polymers and the Environment. 12(3): 113-121.
4. Guan, J., Q. Fang and M.A. Hanna. 2004. Selected functional properties of extruded starch acetate-natural fiber foams. Cereal Chemistry. 81(2):199-206.
5. Guan, J., Q. Fang and M.A, Hanna. 2004. Functional properties of extruded starch acetate blends. J. Polymers and the Environment. 12(2):57-63.
6. Guan. J., Q. Fang and M.A. Hanna. 2004. Marcomolecular characteristics of starch acetate extruded with natural fibers. Trans. of ASAE. 47(1):205-212.
7. Seker. M., H. Sadikoglu, M. Ozdemir and M.A. Hanna. 2003. Cross-linking of starch with reactive extrusion and expansion of extrudates. International J. Food Properties. 6(3):473-480.
8. Seker. M., H. Sadikoglu and M.A. Hanna. 2004. Properties of cross-linked starch produced in a single-screw extruder with and without a mixing element. J. of Food Process Engineering. 27(1):47-63.

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Item No. 33 of 59
ACCESSION NO: 0186027 SUBFILE: CRIS
PROJ NO: NHR-2000-01888 AGENCY: CSREES NH.R
PROJ TYPE: NRI COMPETITIVE GRANT PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2001-35504-10041 PROPOSAL NO: 2000-01888
START: 15 DEC 2000 TERM: 30 JUN 2003 FY: 2003 GRANT YR: 2001
GRANT AMT: $103,000

INVESTIGATOR: Wyman, C. E.

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FUNDAMENTALS OF WATER-ONLY HYDROLYSIS FOR ADVANCED BIOMASS PRETREATMENT

NON-TECHNICAL SUMMARY: Biological processing of abundant lignocellulosic biomass sources such as agricultural residues and ultimately dedicated crops to fuels and chemicals in advanced biorefineries would create major new markets for agriculture and rural manufacturing. Such processes are powerful routes to sustainable production of organic fuels and chemicals on a large scale with unparalleled potential for environmental, economic, and strategic benefits. Enzyme-based technologies offer high product yields vital to achieving these ends, but pretreatment needed to obtain such yields is the typically the most expensive single step with significant impacts on other costs. Recently, technologies based on flow of water through biomass have been shown to promise significant cost reductions but suffer from some important limitations. The overall objective of this proposal is to better understand the mechanisms that differentiate

these advanced processes from conventional water-only technologies and build a foundation to improve pretreatment technologies. Because short-chained oligomer intermediates could be a key to explaining such differences, novel models will be developed that focus on the kinetics of oligomer formation, mass transfer, and reaction. Their predictions will be compared to those for currently applied models and data from the literature. New data will then be collected for water-only pretreatment and used to assess the applicability of the various models. The goal is to provide new data, models, insight, and mechanisms that will facilitate the development and commercialization of lower

OBJECTIVES: Overall Goal of the Proposed Project. The overall goal of the project is to understand factors that will help explain differences in performance between uncatalyzed steam heating and flowthrough hot water pretreatment technologies and provide a platform for developing innovative systems that can realize the unique advantages of each while overcoming their distinctive limitations. We propose to develop rate models to predict the performance of these uncatalyzed systems and determine how well typical homogeneous models can predict hydrolysis of solids and sugar accumulation and degradation versus new models based on mass transfer coupled to reaction. Particular attention will be focused on understanding the role of oligomers in hemicellulose hydrolysis, a subject that has received little attention in the past for uncatalyzed systems. The following objectives are the focus for this project: Objective 1: Design new models to integrate mass transfer effects into hemicellulose hydrolysis kinetic pathways, assess how reaction rate control is influenced by the relative rate constants, and compare to literature results. Particular focus will be on modeling the relationship among formation of oligomers in solid biomass, their mass transfer into the liquid phase, and reaction of these oligomers to monomers. These tools will be compared to conventional homogeneous reaction models and alternative models based on continued reaction in the solid. The models will also be used to define experimental conditions. Objective 2: Perform kinetic experiments to measure how reactor performance and sugar and oligomer recovery change with reaction and recovery conditions. Experiments will be run to determine how well commonly applied first order homogeneous kinetic models explain hemicellulose hydrolysis under varying conditions and to provide sugar monomer and oligomer data that will allow us to determine what role, if any, mass transfer plays in hemicellulose hydrolysis. This element will focus on novel approaches to capture oligomers and monomers at reaction conditions for batch systems. Objective 3: Characterize the solid and liquid fractions for hemicelluose hydrolysis run under various reaction conditions and product collection strategies and determine important interactions. This portion of the project will focus on characterizing the sugars and oligomers in the liquid phase and the carbohydrate composition in the residual solids. These results will support closure of material balances and provide information on kinetic irregularities between reaction rate predictions based on sugar recovery versus those based on residual solid profiles. Objective 4:Apply kinetic models to estimate appropriate rate constants and mass transfer coefficients based on performance data from experiments. The models will be calibrated based on experiments from Objective 2 and 3 and applied to project the reasons for differences in performances between flowthorugh and batch systems.

APPROACH: The focus of the project is on understanding hemicellulose hydrolysis processes that use no added chemicals. The feedstock will be primarily sugar cane bagasse, and the proposed project will be carried out in four elements, corresponding to the project objectives. In the first element, we will build off a recently developed kinetic model to evaluate hemicellulose hydrolysis pathways to undissolved oligomers, dissolved oligomers, monomers, and degradation products. We will also extend existing homogeneous models for hemicellulose hydrolysis with and without consideration of oligomeric intermediates to understand important differences with models that include mass transfer. The results from these models will provide insight to design out experimental systems and approaches to analyze the data to differentiate between these mechanisms. The second element will be directed at running reactor experiments at different conditions to capture liquid and solid phase and develop a new picture of hydrolysis kinetics. One set of experiments will use small tubes heated to reaction temperature by a fludized sand bath followed by quick pressure release through a valve system into an attached tube and rapid removal and quenching of the tubes in ice water. We will then conduct screening experiments to compare the oligomer profiles for different approaches to separate solids and liquid while hot from the reactor configuration above. In one decompression will be through a fritted disk or fine screen to allow passage of only dissolved material without solids. In the second experiments, the material will be discharged into a large volume of water at various run times to determine whether longer chained oligomers can be recovered before they reprecipitate back on the biomass. Another set of batch reaction experiments will focus on separation of some of the liquid from the solid at various times, continuing the reaction for a longer time, and then quenching the system. Data will be developed in the third element to provide new information on the time dependent sugar and oligomer profiles for hemicellulose hydrolysis solid and liquid samples recovered in different approaches. In particular, we seek to learn how the concentration of sugars and oligomers in the liquid phase varies with time, temperature, and recovery methods. The fourth element will analyze the data from these experiments to develop relationships among the concentrations of various sugars and oligomers, time, temperature, reactor configuration, and other factors. As part of this approach, the application of neural networks will be explored to analylze the complex data expected. However, particular focus will be on applying the most promising models identified in element 1. These will be calibrated based on a few sets of experiments such as the various modes of batch heating, and the model predictions will be compared to observed performance for a different set of experiments to determine which best describes these experimental results.

PROGRESS: 2001/10 TO 2002/09
This research project focused on three fundamental factors vital to understanding hemicellulose hydrolysis in just water: temperature transients, solids concentration, and biomass acetylation. A cylindrical heat conduction model was developed to predict experimental heating profiles and coupled with conventional first-order hydrolysis reaction kinetics to demonstrate that temperature transients can significantly impact performance. This information was used to devise a dimensionless expression relating the rates of reaction and heat conduction to guide design of experimental systems and to evaluate reaction rate differences for published studies. Modeling for experiments with hydrolysis of deactelyated biomass at varying acetic acid loadings showed that rate constants for xylan hydrolysis, xylooligomer hydrolysis, and monomer degradation all increased with acid concentration. It was observed that adding higher amounts of acetic acid than released from biomass could not replicate yields without deacetylation. A simple, first-order reaction model for xylan hydrolysis to oligomers that subsequently form monomers that in turn degrade was found to reasonably fit experimental data for different initial solids concentrations with 0.5 in. diameter tubular batch reactors. In addition, a one-step, first-order model was found to fit experimental profiles for the hydrolysis of bound acetyl groups to free acetic acid. However, our data and models showed that there were statistically significant differences in yields of hemicellulose sugars for solids concentrations of 5 and 20 percent with higher sugar yields obtained at lower solids loadings. Such variations are contrary to widely applied first order kinetic models. Although solids concentration affected yields, no evidence was found to support the hypothesis that oligomer solubility limits are responsible for this behavior. In addition, the heat transfer/kinetic model demonstrated that poor heat transfer at higher solids loadings could not solely account for the observed differences in yields at different solids concentrations. Modeling of data for varying solids concentrations revealed that the xylan and oligomer hydrolysis rates increased while monomer degradation rates decreased with solids loading. Therefore it was concluded that the increased concentration of acetyl groups present at higher solids concentrations is not the primary cause of observed differences in yields as a function of solids concentration. Overall, this project suggests that current models do not accurately represent the mechanism of hemicellulose hydrolysis. Plausible explanations for the solids-loading dependent yields include reduced mass transfer at higher solids loadings, increased chemical reactions of soluble sugars with other components at higher solids concentrations, and other than first-order order reactions being involved. However, additional experiments such as batch autohydrolysis of completely deacetylated material at various solids loadings are needed to determine the effects of increasing the concentrations of other components released during hydrolysis without increasing acid concentration.

IMPACT: 2001/10 TO 2002/09
Conversion of abundant cellulosic biomass into fuels and commodity chemicals provides a unique path for sustainable, large-scale production of organic fuels, chemicals, and materials with improved energy security, reduced trade deficits, and limited net greenhouse gas emissions while disposing of problematic solid wastes and creating rural employment. Biological conversion promises the low costs required for large impacts, but the pretreatment step essential to high yields is still too expensive. This project seeks to improve the understanding of differences in pretreatment by hemicellulose removal in just water for seemingly similar conventional uncatalyzed steam heating and flowthrough hot water methods and provide a platform for developing low cost innovative systems that can realize the unique advantages of each while overcoming their important limitations.

PUBLICATIONS (not previously reported): 2001/10 TO 2002/09

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PROJ NO: NHR-2004-00952 AGENCY: CSREES NH.R
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LIGNIN BLOCKERS FOR LOWER COST ENZYMATIC HYDROLYSIS OF PRETREATED CELLULOSE

NON-TECHNICAL SUMMARY: Biomass is unique for sustainable production of organic products with unparalleled environmental, economic, and strategic benefits possible. Cellulosic biomass is abundant and inexpensive, with cellulosics at under $40/ton potentially contributing less than $0.40/gallon of ethanol, as an example, and the challenge is low processing costs with high yields. Application of modern biotechnology has made dramatic progress, with enzymatic processing now costing about the same as for corn. However, less expensive cellulase enzymes are still needed for major impact. Recently, we showed that proteins we term lignin blockers lower enzyme use in an apparently different way than surfactants studied before, and our goal is to develop this novel technology to significantly reduce enzyme costs for converting cellulose to fuels and chemicals. This research will identify new blockers; characterize interactions of cellulase and promising blockers; evaluate blocker effectiveness with enzymatic hydrolysis alone and with fermentation; devise novel processes to capitalize on blockers; and develop models to better understand coupled hydrolysis-blocker performance. The new data and models will define a path to significantly lower the cost of cellulose hydrolysis by enzymes and compliment recent developments in cellulase production and pretreatment to facilitate emergence of a beneficial industry. This project will develop technology that promises to substantially reduce the cost of biologically converting cellulosic biomass into fuels and chemicals such as ethanol.

OBJECTIVES: The primary goal is to more fully develop lignin blocker technology for biological conversion of pretreated cellulosic biomass to glucose that can be converted to ethanol and a range of other products either biologically or chemically. In particular, we seek to understand and apply lignin blockers to reduce enzyme loadings and costs for enzymatic digestion of pretreated cellulose to glucose. The first objective of the research is to screen different soluble proteins and other promising compounds not yet considered with pretreated biomass to define a library of promising lignin blockers that could reduce cellulase loadings and costs. A second objective is to measure cellulase and blocker adsorption and desorption when applied with different lignin blockers and cellulase addition strategies and pretreatment conditions. The work will include examining adsorption and desorption of cellulase and promising lignin blockers identified alone and in combination to quantify the capacity of biomass for each and determine whether lignin blockers are best added before cellulase or can be added with or after cellulase. The next objective is to define the impact of the most promising lignin blockers on enzymatic hydrolysis of pretreated cellulose to determine how performance of the system is influenced by amounts of lignin blocker, cellulase, cellulose, and lignin; temperature; pH; glucose accumulation; beta-glucosidase supplementation; and ingredient addition strategies. Because we believe lignin blockers will be particularly effective in continuous cellulose hydrolysis, we also plan to determine how batch versus continuous operation impacts performance. Another objective is to investigate the performance of the most promising lignin blockers when used with pretreated cellulose in simultaneous saccharification and fermentation (SSF) to define the impact on performance versus cellulase use because SSF eliminates equipment and speeds rates, yields, and concentrations of ethanol production while inhibiting invasion by unwanted organisms. We will focus on how SSF performance is influenced by amounts of lignin blocker, cellulase, cellulose and lignin; temperature; pH; glucose accumulation; beta-glucosidase supplementation; ingredient addition strategies; and fermentation configuration. Our final objective is to develop models to relate enzymatic hydrolysis rates and yields to concentrations of lignin blockers and cellulase; the cellulose, lignin, and other component content of pretreated biomass; process conditions; and the use of other ingredients (e.g., supplemental beta-glucosidase). This research element will focus on improving the understanding of how adsorption and desorption of lignin blockers and cellulase are influenced by processing conditions and how they in turn affect the performance of hydrolysis systems and use that information to project pathways to further improve performance

APPROACH: Corn stover is the primary feedstock for this project because it represents a major biomass source; a reliable, controlled supply is available; and we have extensive experience with its pretreatment and enzymatic hydrolysis. In the first element of research, alternative lignin blockers will be rapidly screened using cellulose and pretreated stover to define a library of compounds beyond those we found initially that promise reduced cellulase loadings and costs. Cellulase and blocker adsorption and desorption will be measured in the second element for different lignin blocker and cellulase addition strategies and pretreatment conditions. These experiments will include characterization with cellulose, lignin, and pretreated stover and determining the influence of the order of ingredient addition and treatment conditions. We also plan to apply special inactive cellulases to better understand how lignin blockers and cellulase interact at hydrolysis temperatures. This effort will include evaluating how hydrolysis process variables affect capture of cellulase and lignin blockers. The third research element will define the impact of the most promising lignin blockers on enzymatic hydrolysis of pretreated cellulose. This portion will focus on how performance is influenced by amounts of lignin blockers and cellulase, cellulose and lignin content, temperature, pH, glucose accumulation, and ingredient addition strategies. Because cellulase is strongly inhibited by accumulation of glucose and particularly cellobiose, the enzymatic hydrolysis rate will be followed with changes in solids concentration and beta-glucosidase supplementation. A valuable part is to explore whether lignin blockers are particularly effective for enzymatic hydrolysis of pretreated biomass in continuous fermentations. A fourth research element will investigate the effect of the most promising lignin blockers on performance of the simultaneous saccharification and fermentation (SSF) system. Overall, many practitioners favor SSF for ethanol production because it eliminates equipment and enhances rates, yields, and concentrations of ethanol production despite requiring somewhat lower temperatures. Thus, we will also measure how SSF performance is influenced by amounts of lignin blockers and cellulase, cellulose and lignin concentrations, temperature, pH, glucose accumulation, beta-glucosidase supplementation, fermentative organism choice, ingredient addition strategies, and fermentation configuration. We will explore whether continuous SSF further enhances the benefits of lignin blockers. In the final element, we will model how lignin blockers, cellulase, and beta glucosidase interact with cellulose, lignin, and pretreated biomass to better understand what factors appear to govern their effects. We desire to relate hydrolysis performance to adsorption characteristics of enzymes and blockers to gain a better appreciation for operating conditions that will realize the greatest benefits. We also plan to develop models to describe the effect of blockers on cellulose hydrolysis for SSF with regular and cellobiose fermenting yeast.

PROGRESS: 2005/10 TO 2006/09
In this study, the interactions between cellulase enzyme and the different components of biomass were determined by developing adsorption data and calculating adsorption isotherms. Our results showed that

BSA treatment of dilute acid pretreated corn stover reduced cellulase adsorption by substrate. We also showed that this phenomenon is more evident when the substrate is treated at 50C than at 4C. Lignin showed a high adsorption capacity for cellulase. These results confirm that cellulase has a higher mass affinity than either ß-glucosidase or BSA for Avicel cellulose, dilute acid pretreated corn stover, and corn stover lignin. It also proved that ß-glucosidase and BSA show very little affinity for cellulose and higher affinity for pretreated corn stover and lignin, demonstrating their tendency to adsorb to the lignin fraction. In general, the adsorption of protein by the substrates increased with temperature. Tween 80 and BSA were both evaluated as additives for hydrolysis of pretreated corn stover on the basis of how much enzyme they could displace for the same extent of conversion. The results showed that BSA and Tween 80 both improved hydrolysis of dilute acid pretreated corn stover and that this benefit was most dramatic at low enzyme loadings. Specifically, the need for ß-glucosidase supplementation could be significantly reduced by use of additives, reducing enzyme costs through significantly lower addition of ß-glucosidase. For example, the most dramatic results were that an enzyme loading of just 5 FPU/g glucan was adequate to achieve a glucan conversion of as high as 85% in the presence of additives while three times that amount of cellulase (15 FPU/g glucan) was required without BSA. For AFEX pretreated corn stover, Tween 80 performed slightly better than acid pretreated corn stover although additional work is required to reveal the underlying mechanism. The conversion of glucan in pretreated corn stover at 120 hours by 5 FPU/g glucan of cellulase was improved by up to 20% by the presence of 10 mg/mL of either BSA or Tween 80. BSA treatment decreased adsorption of cellulase and ß-glucosidase on pretreated corn stover, pointing out that BSA decreased adsorption of enzyme on the lignin fraction. Application of kinetic models to this project also support this mechanism. Both versions of the model used herein resulted in elevated values for the reaction rate constant for the conversion of cellobiose to glucose by ß-glucosidase, confirming our last report that there is more ß-glucosidase remaining in the liquid fraction of the hydrolysis mixture. In other words, there is less unproductively bound ß-glucosidase. Our attention will now focus on evaluating the effect of lignin blockers on continuous SSF of pretreated corn stover, and a mathematical model is being developed to predict the performance of continuous SSF and point out opportunities to realize high ethanol yields at very low cost.

IMPACT: 2005/10 TO 2006/09

Cellulosic biomass must be pretreated to realize high sugar yields via enzymatic digestion and achieve competitive costs for production of large market fuels and commodity chemicals with powerful societal benefits. However, pretreatment is the most expensive step followed closely by the cost of enzymatic hydrolysis of pretreated substrate and costs of the enzymes. A better understanding of factors that control substrate-enzyme interactions is invaluable to identifying low cost paths. Addition of non catalytic proteins such as BSA has been shown to enhance cellulose hydrolysis by enzymes or lower the amount of enzyme required to realize a particular conversion, potentially lowering costs. Thus, we are determining interactions among substrate features, enzymes, and BSA to better understand mechanisms by which these additives influence hydrolysis and identify promising routes to reduce enzyme use and speed rates. Adsorption and desorption profiles were measured to better clarify the interaction of BSA, cellulase, and beta-glucosidase with cellulose, pretreated corn stover, and lignin from corn stover. This data indicates that BSA adsorbs much more strongly on lignin than cellulose, thus reducing non productive enzyme binding to lignin and enhancing performance. Thus, our results confirm that treatment of pretreated biomass with inexpensive protein would reduce costs significantly. In addition, application of a new restart protocol suggests that the slow down in rate with enzymatic digestion is due to changes in enzyme effectiveness and not cellulose reactivity.

PUBLICATIONS (not previously reported): 2005/10 TO 2006/09

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COORDINATED DEVELOPMENT OF LEADING BIOMASS PRETREATMENT TECHNOLOGIES

NON-TECHNICAL SUMMARY: Leading technologies will be applied to prepare low cost lignocellulosic biomass for conversion to a variety of products that would open up major new markets for agricultural feedstocks. The effort will be performed by a Biomass Refining Consortium among Auburn Univ, Dartmouth College, Michigan State Univ, the National Renewable Energy Lab, Purdue Univ, and Texas A&M Univ. Optimum pretreatment conditions will be defined for ammonia explosion, aqueous ammonia recycle, controlled pH, dilute acid, lime, and uncatalyzed technologies based on the extensive experience of this team. The resulting fluid and solid streams will be fully characterized, and the data will be used to accurately close material balances. In addition, energy balances will be estimated. The fermentability of the liquids and digestibility of the solids by cellulase enzyme wil be assessed and used to guide definition of pretreatment conditions. Thermochemical hydrolysis of the cellulose rich solids will also be evaluated to provide data on this optional approach to cellulose use. Economic assessments will be applied to estimate the cost of pretreatment by each approach on a consistent basis. Priority will be given to insuring accurate data is developed for corn stover, but a hardwood and switchgrass will also be included to the extent possible to apply accurate analysis. The comparative studies will be guided by an agricultural and industrial advisory board, and the results widely disseminated.

OBJECTIVES: The overall goal of the proposed project is to develop comparative information on leading pretreatment operations for production of sugars from the hemicellulose and cellulose fractions of biomass for fermentation or chemical reaction to a wide range of high volume commodity products. In particular, corn stover will be used initially as a representative feedstock, and material balances will be acurately closed to describe the fate of the key biomass constituents for each pretreatment approach. Comprehensive analysis will be made of the biomass source and liquid and solid products from pretreatment to support closing of material balances and characterization of the key reactions. The cellulose rich solids from each will also be processed to glucose, and the liquid hydrolyzate fermented to provide important data for commercial use and to assess the relationship among pretreatment system, conditions, effects on the biomass substrate, and subsequent downstream processing. Similar data will be gathered subsequently on a hardwood energy crop and switchgrass, as time permits gathering the comprehensive data vital to the project. The overall results will improve these pretreatment technologies further, provide valuable comparative data to industry on each technology, provide information to accelerate near-term scale-up and application by reducing risk, and develop new insight into biomass hydrolysis that will help define advanced processes that further reduce costs. The following specific objectives have been defined for the proposed work: Objective 1: Apply each of the leading pretreatment technologies to recover hemicellulose sugars and prepare biomass for cellulose hydrolysis. Objective 2: Monitor the recovery, reactions, and fate of lignin, hemicellulose, cellulose, and protein for each of these pretreatment technologies, and gather comprehensive data to characterize these components. Objective 3: Develop and accurately close material balances for each of the pretreatments and calculate energy balances for each. Objective 4: Hydrolize pretreated solids with enzyme and acid and evaluate the fermentability of liquid hydrolyzate for the most promising conditions for each pretreatment technology. Objective 5: Compare performance among systems on a consistent basis and publish and otherwise widely disseminate the results. Also, work with individuals, groups, and firms to apply these results commercially.

APPROACH: This effort will be a cooperative project among Auburn Univrsity, Dartmouth College, Michigan State University, Purdue University, and Texas A&M university, along with non reimbursed participation by the National Renewable Energy Laboratory (NREL). Each of the academic institutions will be responsible for pretreating biomass by technologies for which they have substantial development experience and capabilities. In addition, each of these laboratories will apply established analytical methodologies to capitalize on existing capabilities and insure results on a consistent basis. NREL will provide facilities and guidance and help in the assessment and application of the results. The first priority will be to use corn stover for all tests to provide a common basis for comparison followed by a hardwood and switchgrass, as possible, while meeting the comprehensive dtaa gathering vital to this project. Furthermore, a panel of industrial and agricultural experts will meet regularly with the team to guide the project and serve as extension agents. In the first element of the project, each team will pretreat biomass to recover sugars and prepare cellulose for subsequent hydrolysis. Auburn University will apply liquid ammonia recycled percolation (ARP); Dartmouth College will employ acid catalyzed and uncatalyzed technologies; Michigan State University will utilize ammonia fiber extrusion (FIBEX); Purdue University will run controlled pH pretreatments; and Texas A&M University will work with lime based processes. Each team will apply conditions based on their extensive experience. To meet the second objective, each of the collaborators will be responsible for specific evaluations using available instrumentation. Particular attention will be given to the effects of the various pretreatments on hemicellulose, cellulose, lignin, and protein. A key component of objective 3 is to accurately determine the fate of key constituents for each pretreatment technology. Material balances are challenging for biomass systems because of the heterogeneity of the material, its complex structure, and side reactions. However, because high yields of sugars are central to product revenues, it is critical to determine yields accurately, and substantial effort will be devoted to this objective. A key measurement is the determination of the yield of glucose from cellulose for objective 4 because this, together with hemicellulose sugar recovery, is central to obtaining revenues in a commercial context. Thus, enzymatic digestibility will be evaluated for each pretreatment based on established procedures. The fermentability of the liquid hydrolyzates to ethanol will also be determined to allow comparison of technologies. In addition, the digestibility of cellulose from each pretreatment will be determined with dilute sulfuric acid.

PROGRESS: 2002/10 TO 2003/09
The project is on schedule. The Agricultural and Industrial Advisory Board was expanded some to 16 members, and the Team met twice with them to obtain feedback. The Team held conference calls to coordinate throughout the year. Corn stover was used as the feedstock for all tests. Auburn University reduced liquid rates to 3.3 mL/g of stover for Ammonia Recycled Percolation (ARP) pretreatment. Auburn also developed a new SAA pretreatment method at moderate conditions of <100C and atmospheric pressure. Advantages are the process is simple, most hemicellulose is retained, and process energy and equipment costs are low. It was also proven that recombinant E. coli can efficiently utilize both the glucan and the xylan in treated corn stover. Dartmouth College achieved 90% total sugar yields for batch dilute acid pretreatment followed by enzymatic hydrolysis at high loadings. The optimum conditions for these steps were not the same, and enzymes enhanced xylose recovery. Models were devised to predict high

temperature pH and describe oligomer and monomer release. Hemicellulose removal increased significantly with water flow through biomass, contrary to customary predictions, and possible mechanisms were developed. A novel periodic-flow was developed to reduce water use but enhance performance. Michigan State University optimized ammonia fiber explosion (AFEX) to convert cellulose and hemicellulose to glucose and xylose and realized near theoretical yields of glucose and about 80% yields of xylose. Over 90% yields of glucose resulted at lower enzyme loadings of 7.5 IU/gram glucan. MSU also provided infrared, fluorescence, and X ray diffraction analysis for all team members. MBI International is pursuing scale up and commercialization of AFEX with interested parties. Purdue University optimized their controlled pH process at 190C with pH control at 4-7 through liquid buffer capacity to minimize monosaccharides and avoid their degradation to inhibitors that affect fermentation. Controlled pH pretreatment of stover resulted in 87% of glucan and 78% of xylan being hydrolyzed at a cellulase loading of 15 FPU/g pretreated corn stover, and 88% of the glucose and xylose was converted to ethanol by a recombinant yeast developed at Purdue. Texas A&M University treated corn stover with lime and air at 25-55C for up to 16 weeks. The optimal conditions were 4 weeks with 0.1 g of lime/g biomass at 55C. At an enzyme loading of 15 FPU/g glucan, 92.4% of glucan and 78.4% of xylan were hydrolyzed. At an enzyme loading of 60 FPU/g glucan, 97.1% of glucan and 81.0% of xylan were hydrolyzed. This lime/air treatment has been licensed to Terrabon, LLC who is raising $2 million to construct a demonstration plant. The National Renewable Energy Laboratory (NREL) participated through Department of Energy funding and continued to store and supply corn stover and cellulase. Process and economic models were also updated for each pretreatment with performance and digestibility data from each researcher. In addition, NREL conducted comparative simultaneous saccharification and fermentation studies on samples from each pretreatment process.

IMPACT: 2002/10 TO 2003/09
Cellulosic biomass is a unique sustainable resource for making organic fuels and chemicals. Very low greenhouse gas emissions is a powerful attribute, but cellulosic biomass use would also enhance energy security, reduce trade deficits, create rural employment, and dispose of waste. Cost and availability of cellulosics are attractive, and the primary need is to reduce processing costs. Pretreatment is essential for biological conversion, and the project is developing comparable information on leading pretreatments that would open up major new agricultural markets. Data and projected costs are compared and widely disseminated, and opportunities are sought to accelerate commercial applications. An Agricultural and Industrial Advisory Board gains insight and provides guidance in regular meetings. The project gives significant momentum to biomass processing as well as important educational benefits. Key findings are emerging through this project. First, each pretreatment provides highly digestible cellulose, with about 90% of the cellulose converted to glucose. The project also shows that hemicellulase activity in enzyme increases hemicellulose sugar yields, particularly for high pH pretreatment technologies. The initial economic analysis has projected similar costs for all processes, but further integration and optimization is ongoing to more fully capture the unique features of each pretreatment. A number of papers, patent applications, and presentations resulted, and several companies are working with participants to commercially apply pretreatment technologies.

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10. Kim S, Thanakoses P, Holtzapple MT. 2003. "Lime pretreatment," AIChE Annual Meeting, San Francisco, CA, November (proceedings and oral presentation).
11. Kim TH, Lee YY. 2003. "Pretreatment of corn stover by soaking in aqueous ammonia," AIChE Annual Meeting, San Francisco, CA, November (proceedings and oral presentation).
12. Laureno-Perez L, Teymouri F, Dale B, Alizadeh H. 2003. "Understanding factors that limit enzymatic hydrolysis of biomass: Characterization of pretreated corn stover," AIChE Annual Meeting, San Francisco, CA, November (proceedings and oral presentation).
13. Liu C, Wyman CE. 2003. "Comparsion of batch, stop-flow-stop, and flowthrough pretreatment of corn stover," AIChE Annual Meeting, San Francisco, CA, November (proceedings and oral presentation).
14. Lloyd TA, Wyman CE. 2003. "Overall sugar yields from corn stover via thermochemical hemicellulose hydrolysis followed by enzymatic hydrolysis," AIChE Annual Meeting, San Francisco, CA, November (proceedings and oral presentation).
15. Mosier N, Hendrickson R, Kim Y, Zeng M, Dien B, Welch G, Wyman CE, Ladisch MR. 2003. "Optimization of controlled pH liquid hot water pretreatment of corn fiber and stover," AIChE Annual Meeting, San Francisco, CA, November (proceedings and oral presentation).
16. Teymouri F, Laureano-Perez L, Dale B, Alizadeh H. 2003. "Ammonia fiber explosion (afex) for pretreatment of corn stover: recent research results," AIChE Annual Meeting, San Francisco, CA, November (proceedings and oral presentation).
17. Wyman CE, Dale B, Eggeman T, Elander R, Holtzapple M, Ladisch M, Lee YY. 2003. "Comparison of selected results for application of leading pretreatment technologies to corn stover," AIChE Annual Meeting, San Francisco, CA, November (oral presentation).

18. Wyman CE, Eggeman T. 2003. "Pretreatment of lignocellulosic biomass: update on biomass refining cafi studies," AIChE Annual Meeting, San Francisco, CA, November (oral presentation).
19. Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY, Eggeman T, Wyman CE. 2003. "Comparative data from application of leading pretreatment technologies to corn stover," 25th Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO, May (oral presentation).
20. Hendrickson R, Mosier NS, Ladisch MR. 2003. "Generation of coproducts derived from a modified hot water pretreatment of corn stover," 25th Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO, May (poster).
21. Kim TH, Lee YY. 2003. "Pretreatment of corn stover by low-liquid ammonia percolation process," 25th Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO, May (poster).
22. Kim TH, Lee YY. 2003. "Effect of lignin on enzymatic hydrolysis of cellulose," 25th Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO, May (poster).
23. Liu C, Wyman CE. 2003. "Impact of fluid velocity and contact time on corn stover pretreatment in a flowthrough reactor," 25th Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, Colorado, May 5 (poster).
24. Kim YM, Mosier NS, Hendrickson R, Goetz J, Ladisch MR. 2003. "Hydrolysis of oligosaccharides using strong cation exchange catalyst and cellulase enzymes," ACS National Meeting, New Orleans, March 25 (poster).
25. Dale B, Wyman CE, Elander RT, Holtzapple MT, Ladisch MR, Lee YY. 2002. "Recent activities of the Consortium for Applied Fundamentals and Innovation," AIChE Annual Meeting, Indianapolis, IN, November 4 (presentation).
26. Kim TH, Sunwoo C, Lee YY. 2002. "Pretreatment and fractionation of corn stover by aqueous ammonia," AIChE Annual Meeting, Indianapolis, IN, November (proceedings and oral presentation).
27. Liu C, Wyman CE. 2002. "Evaluation of a flowthrough reactor for corn stover pretreatment," AIChE Annual Meeting, Indianapolis, IN, November (proceedings and oral presentation).
28. Mosier NS, Hendrickson R, Welch G, Ladisch CM, Ladisch MR. 2002. "Design and scale-up of corn fiber and corn stover pretreatment for fuel ethanol production," AIChE Annual Meeting, Indianapolis, IN, November.
29. Liu C, Wyman CE. 2002. "Comparison of batch and flowthrough biomass pretreatment systems for biological production of fuels and chemicals," 12th European Biomass Conference and Exhibition, Amsterdam, The Netherlands, June (poster and proceedings paper).

30. Dale BE, Laureano-Perez L. 2002. "Spectroscopic characterization of pretreated corn stover," 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, April (poster).
31. Kim TH, Lee YY. 2002. "Fractionation of corn stover by a two-stage percolation process," 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN (poster).
32. Liu C, Wyman CE. 2002. "Effect of flow rate on the dissolution of hemicellulose, lignin, and total mass for pretreatment of corn stover in a flowthrough reactor," 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May (poster).
33. Lloyd TA, Wyman CE. 2002. "Application of a depolymerization model for predicting thermochemical hemicellulose hydrolysis," 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May (presentation).
34. Teymouri F, Dale BE. 2002. "Effects of pretreatment on the activity of enzymes in plants: cellulase enzymes and ribulose diphosphate carboxylase," 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, May (presentation).

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Item No. 36 of 59
ACCESSION NO: 0193616 SUBFILE: CRIS
PROJ NO: NYC-143346 AGENCY: SAES NY.C
PROJ TYPE: STATE PROJ STATUS: EXTENDED
START: 01 OCT 2002 TERM: 30 SEP 2012 FY: 2006

INVESTIGATOR: Brady, J. W.

PERFORMING INSTITUTION:
FOOD SCIENCE
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USE OF COMPUTER MODELING AND MD SIMULATIONS

NON-TECHNICAL SUMMARY: The objective of this project is to develop a systematic understanding of how water affects the properties of biological molecules, and also of how such solutes affect the behavior of water. The project will use computer simulations to determine information which cannot be gained by other means, and where possible, the simulations will be tested by direct comparison with experimental values.

OBJECTIVES: The objectives of this project are to characterize the way in which biopolymer structure and conformation determine function. A major goal is to understand the way in which biological solutes affect the properties of solvent water and also the way in which aqueous solvent influences the conformation and functional properties of biological solutes. The major focus of the studies will be on proteins, including cellulases; carbohydrates, including cellulose; lipids; and nucleotides. The detailed structuring which biological solutes impose on solvent water will be characterized from MD simulations, and this structuring will then be correlated with the specific structural features of the solute molecule, in an effort to develop a general model of how particular molecules will affect solvent and in turn be affected by solvation. Another goal will be to characterize the accuracy of the simulations by comparing the calculated results to data obtained from various experiments, including NMR and neutron diffraction experiments.

APPROACH: In this project molecular mechanics calculations will be used to model the conformational behavior and aqueous solvation of a series of biological molecules. Conformational energy calculations and molecular graphics methods will be used to study the preferred structures and conformations of a variety of biological molecules and biopolymers. Molecular Dynamics simulations will be used to model the way in which atoms and molecules respond to assumed forces arising from empirical force fields by solving the classical Newtonian equations of motion for the systems. The trajectories produced from such computer simulations can then be analyzed to compute observable properties as the appropriate average over the time series of atomic coordinates. A particular focus of this project will be to calculate solvent density around biological solutes and to examine the effects of solvation on biopolymer conformation. Various experimental techniques will also be used to subject the calculated properties to experimental verification. The most important of these will be first difference neutron diffraction experiments on specially synthesized subject molecules which are specifically labeled by isotopic substitution at particular sites.

PROGRESS: 2006/01 TO 2006/12
Our large-scale molecular dynamics simulations of cellulase enzymes interacting with cellulosic substrate have been continued and extended during this reporting period. We have focused on the CBH I cellulase from Trichoderma reesei, a quite complex multi-domain molecular machine. In addition, simulations have been conducted on a system consisting of just the cellulose binding domain (CBD) of the CBH I cellulase bound to cellulose. Another simulation which has been initiated consists of the entire CBH I molecular complex docked onto a crystalline cellulose substrate as before, but with one of the cellulose chains broken and fed into the active site binding tunnel to determine what effect the presence of the substrate in the active site has on the dynamics of the system. Since the microfibril models used in our previous simulations are actually too large, we have also built and equilibrated new smaller microfibrils with more realistic dimensions to use as substrates in these simulations. All of these simulations are still proceeding.

IMPACT: 2006/01 TO 2006/12
The impact of this work will be in helping to improve the industrial conversion of cellulose into monosaccharides for use as a feedstock in the production of ethanol for fuel. Bio9mass conversion to reduce the US's dependence on imported fuels is a major national goal and has been mentioned in the State of the Union address twice in a row. The limiting factor in cellulose hydrolysis is the insolubility of the substrate, and understanding the interaction of water with this substrate and how to disrupt the microcrystalline structure would be of great significance in optimizing the process. In addition, developing an understanding of the enzymatic mechanism would also be of great use in designing improved activity in mutants through site-directed mutagenesis.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
Matthews, J.F., C.E. Skopec, P.E. Mason, P. Zuccato, R.W. Torget, J. Sugiyama, M.E. Himmel and J.W. Brady. 2006. Computer Simulation Studies of Microcrystalline Cellulose IB. Carbohydr. Res. 341, 138(2006).

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Item No. 37 of 59
ACCESSION NO: 0211017 SUBFILE: CRIS
PROJ NO: NYZ-2340-02-005 AGENCY: CSREES NY.Z
PROJ TYPE: NRI COMPETITIVE GRANT PROJ STATUS: NEW
CONTRACT/GRANT/AGREEMENT NO: 2007-35504-18341 PROPOSAL NO: 2007-02176
START: 01 SEP 2007 TERM: 31 AUG 2010 GRANT YR: 2007
GRANT AMT: $499,299

INVESTIGATOR: Stipanovic, A. J.; Winter, W. T.; Driscoll, M. S.

PERFORMING INSTITUTION:
CHEMISTRY
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ELECTRON BEAM & X-RAY IRRADIATION OF LIGNOCELLULOSIC BIOMASS - SYNERGIES W/ BIODELIGNIFICATION & HEMICELLULOSE REMOVAL IN REDUCING RECALCI.

NON-TECHNICAL SUMMARY: It is well established that the production of biobased fuels and chemicals from lignocellulosic biomass will require the application of a ôpretreatmentö technology to reduce the recalcitrance of the composite cellular structure of woody plants. In this study, we will demonstrate the utility of commercially available electron beam generators in ôloosening upö the solid state structure of woody biomass to enhance the rate of conversion to sugars, ethanol and other bioproducts. The purpose of this study is to evaluate the impact of electron beams as a pretreatment to make lignocellulosic biomass easier to convert to biobased products.

OBJECTIVES: Objective 1- Biomass Selection: A group of potential feedstocks suitable for a lignocellulosic-based biorefinery will be procured, dried and ground into chips. A sub-sample will be subjected to a sequential biodelignification / hot water hemicellulose extraction protocol using pilot-scale facilities following prior work at SUNY-ESF. Feedstocks will include a northeastern hardwood, fast-growing plantation grown willow (also a hardwood), a common softwood species used for papermaking, corn stover, and switchgrass. In addition, we will evaluate cellulosic resources such as recycled paper pulp and paper mill fines or sludge as feedstocks without biodelignification and hemicellulose extraction, since these represent model systems for cellulose itself. The chemical composition of these feedstocks will be determined by thermogravimetric analysis (TGA; percent hemicellulose, cellulose, lignin and ash), Nuclear Magnetic Resonance Spectroscopy (NMR) and standard TAPPI methods for lignin. Objective 2 - High Energy Radiation Treatment: In collaboration with our industrial partner, Radiation Dynamics Inc. (RDI; member of the IBA Group), we will design an experimental protocol to optimize the impact of high energy radiation treatment on the solid state morphology of woody biomass in an effort to reduce the recalcitrance of these substrates. Adjustable parameters will include the type of radiation (electron beam or x-ray), radiation intensity and flux, duration of irradiation, total dose, etc. Other potential variables include irradiation in the dry state, oxygenated atmospheres, or irradiation of biomass slurries in water. Each woody biomass feedstock will be irritated in the un-pretreated control state and after the biodelignification / hemicellulose extraction pretreatment sequence described above. We will also, perform preliminary process economics calculations based on an optimum radiation dose. Objective 3. Biomass Characterization: Before and after radiation treatment, each biomass and cellulose substrate will be characterized to determine the impact of irradiation on the composition, solid state morphology and potential processibility to biobased products. In additional to the compositional analysis mentioned above, other measurements will include: solid-state crystallinity by x-ray diffraction and CP/MAS 13C NMR, available surface area, and potential degree of conversion to reducing sugars and ethanol by using standard NREL methods. In addition, the cellulose samples will be subjected to molecular weight analysis by Size Exclusion Chromatography in a novel cellulose solvent system to determine the extent of depolymerization associated with the radiation treatment.

APPROACH: In this study, we propose to use to use high energy electrons and x-rays to partially depolymerize and decrystallize lignocellulosic biomass thereby reducing its recalcitrance. Samples will be exposed to an electron beam with doses from 1 to 1000 kGy with and without additional treatments such as a high oxygen environment to facilitate degradation. Further, exposure profiles will include a high dose rate electron beam and a lower dose rate x-ray beam to determine if this parameter has an effect on the degradation of the cellulose. Electron energies will also be varied from about 500 keV to 3 MeV with the possibility of going as high as 10 MeV if there appears to be a strong energy response. RDI will also provide Monte Carlo calculations to estimate the electron energy and beam power required for both the experimental irradiation and for high throughput industrial processing. In the later phases of the study we hope to evaluate a continuous feedstock supply system involving a conveyor belt or a pumped slurry configuration where the biomass is suspended in water. The impact of electron beams will be studied with and without other biomass pretreatments such as biodelignification and hot water extraction of hemicellulose to deteremine if these approaches are synergistic in reducing recalcitrance.

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Item No. 38 of 59
ACCESSION NO: 0406585 SUBFILE: CRIS
PROJ NO: 5445-21000-008-04R AGENCY: ARS 5445
PROJ TYPE: USDA INHOUSE PROJ STATUS: TERMINATED
START: 01 AUG 2002 TERM: 31 AUG 2005 FY: 2004

INVESTIGATOR: HANSON J D; BERDAHL J D; KRUPINSKY J M; LIEBIG M A; FRANK A B

PERFORMING INSTITUTION:
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BIOFUEL FEEDSTOCK DEVELOPMENT

OBJECTIVES: The objectives for this project are as follows: 1) To compare and characterize liquid fuels biomass production potential of switchgrass monocultures to native warm-season grass mixtures from western Minnesota to central North and South Dakota; and to identify most environmentally sustainable methods of production. 2) To identify the region in the Northern Plains most ripe for profitable production of native grasses for use in cellulosic liquid fuels conversion.

APPROACH: The primary purpose of this research is to compare liquid fuel biomass production potential of switchgrass monocultures to native warm-season grass mixtures from western Minnesota into central North and South Dakota. Yield and feedstock quality data will be used to characterize the best management practices and cultivars, and also to help determine the geographic scope of a viable cellulosic bio-ethanol industry in the Northern Plains. Additional attention will be paid to evaluating changes in soil quality and carbon storage, habitat development and species density and other ecological concerns.

PROGRESS: 2002/08 TO 2005/08
4a What was the single most significant accomplishment this past year? This report serves to document research conducted under a reimbursable agreement between ARS and the Department of Energy involving biomass production, management practices, and chemical composition of biomass from monocultures and mixtures of three native warm-season grasses. This research is associated with parent CRIS 5445-2110000-008-00D Integrated Forage, Crops, and Livestock Systems for the Northern Great Plains. Switchgrass is considered a valuable bioenergy crop with significant potential to sequester soil organic carbon. A project was conducted by ARS researchers at the Northern Great Plains Research Laboratory to evaluate soil carbon stocks within established switchgrass stands and nearby cultivated cropland on farms throughout the northern Great Plains and northern Cornbelt. Soil organic carbon was greater in switchgrass stands than cultivated cropland at soil depths of 0 to 2, 12 to 24, and 24 to 36 inches, and especially so at the deeper soil depths where treatment differences were 7.74 and 4.35 Mg/ha (or 6900 lbs/ac and 3900 lbs/ac) for the 12 to 24 and 24 to 36 inch depths, respectively. Consequently, switchgrass appears to be effective at storing soil organic carbon not just near the soil surface, but also at depths below 12 inches where carbon is less susceptible to loss. 4b List other significant accomplishments, if any. Understanding the impacts of mycorrhizial fungi and glomalin on the production of potential perennial biofuels crops such as switchgrass and big bluestem can help producers develop efficient management systems. The role of native and introduced mycorrhizial inoculums on switchgrass production under ambient and elevated CO2 levels and the impact of nitrogen and drought stress on the above- and below-ground biomass and ethanol production potential in big bluestem and switchgrass are being evaluated. Information from these experiments will provide producers with management options for producing biofuel crops. Understanding potential yield reducing in switchgrass is important if its potential as a biofuel is to be realized. Scientists at Mandan, ND, Corvallis, OR, and Beltsville, MD collaborated in the identification of Bipolaris oryzea, a common fungal pathogen on rice, as the causal agent of a leaf-spot disease on switchgrass. This pathogen has the potential to reduce yields of switchgrass under intensive plantings for biomass production, particularly if susceptible varieties were seeded over a wide area. 4d Progress report. Some perennial grasses have the potential to be converted into ethanol providing an alternative energy source as well as economical and environmental benefits to agricultural producers. The objective of this research has been to identify cultivars adapted to the region and limitations to germination and establishment, evaluate the ability of switchgrass to preserve

the soil resource and store carbon, and identify other perennial grasses with ethanol production potential. From this research, Sunburst switchgrass was identified as the cultivar with the greatest biomass potential for the northern half of the Northern Great Plains, while Trailblazer was the most productive cultivar for southern half of this region. Optimum germination temperature is approximately 77F and optimum pH is 6.0. An evaluation of switchgrass stands and nearby croplands in the northern Great Plains and northern cornbelt, indicated switchgrass stands tended to store more carbon at depths below 12 inches where it is less susceptible to loss back into the atmosphere. The impact of native and introduced mycorrhizial fungi on biomass production under elevated and ambient CO2 concentrations is currently being studied. Additional research suggested that big bluestem might have a higher ethanol producing potential than switchgrass. Herbaceous bioenergy crops may be an important component in integrated systems in the Northern Great Plains. This research provides producers with information to maximize the potential of bioenergy crops.

PUBLICATIONS (not previously reported): 2002/08 TO 2005/08
No publications reported this period.

Item No. 39 of 59
ACCESSION NO: 0211174 SUBFILE: CRIS
PROJ NO: ND01463 AGENCY: CSREES ND.
PROJ TYPE: HATCH PROJ STATUS: PENDING NEW
START: 01 OCT 2007 TERM: 30 SEP 2012

INVESTIGATOR: Pryor, S. W.

PERFORMING INSTITUTION:
AGRICULTURAL AND BIOSYSTEMS ENGINEERING
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USE OF NORTHERN GREAT PLAINS AGRICULTURAL RESOURCES FOR BIOENERGY AND BIOPRODUCT DEVELOPMENT

NON-TECHNICAL SUMMARY: Economic, environmental, and political concerns regarding national petroleum usage has driven an increased interest in biobased fuels and other bioproducts. The USDA and USDOE have agreed to strongly support the increased use of biofuels, biopower, and bioproducts to reduce national dependence on oil imports in the coming decades. The purpose of this project is to increase the technical and economic viability of using Northern Great Plains agricultural resources for energy and high-value bioproducts. Specific areas to be explored include: alternative starch crops for integration with the corn ethanol industry, characterization and pretreatment of mixed feedstocks for cellulosic ethanol production, and utilization of canola meal for adhesives, plastics, and composites.

OBJECTIVES: The first project objective is to evaluate and increase the technical viability of bioenergy productions using agricultural residues, dedicated energy crops, and other biomass resources to produce sustainable and economically viable fuels for transportation, heating, or electrical generation. The second project objective is to increase the technical and economic viability of commercial and industrial bioproducts either independently or as coproducts of related bioenergy production processes. Accomplishments related to this objective are also expected to affect the first objective through the economic impacts of coproduct development on bioenergy processing.

APPROACH: Although bioenergy and bioproduct development could encompass a wider range of projects, the following procedures outline initial projects falling under these categories. Initial work in the area of bioenergy will include feedstock development for the starch ethanol industry and feedstock analysis and pretreatment for cellulosic ethanol production. Feedstock development for the starch ethanol industry will involve testing the technical feasibility of co-fermenting pea starch with corn in a dry-grind ethanol facility. Fermentation rates and yields will be examined when corn is replaced by pea starch at levels of 10, 30, and 50%. Initial work on cellulosic ethanol development will include CRP biomass characterization and optimization of pretreatment conditions for mixed feedstocks to maximize fermentable sugar production. All feedstock characterization will be completed using established NREL protocols for feedstock preparation and determination of extractives, structural carbohydrates, and lignin. Grasses and agricultural residues will be pretreated for cellulosic ethanol production, hydrolyzed with cellulase enzymes, and measured for glucose and xylose content as a percentage of theoretical yields. These data will be used in conjunction with individual biomass species and biochemical compositional analysis to predict sugar yields from pretreated mixed feedstocks. Mixed biomass feedstocks will also be treated and analyzed as described above to test if sugar yields from pretreated mixed feedstock are equal to the sum of sugar yields from the pretreated individual single species biomass samples. Initial work in the bioproducts area will focus on the utilization of canola meal proteins for production of industrial products such as adhesives, plastics, or composite materials. Tasks falling under this objective include: protein separation, characterization, modification, and utilization. Canola oil will be extracted and canola meal proteins will be purified to varying degrees for characterization and use for industrial bioproducts. Canola meal and protein characterization will include analysis of protein, carbohydrate, fat, and fiber content of canola meal produced from various oil extraction techniques. Protein characterization will be based on electrophoretic pattern and amino acid composition. The fiber component will be characterized based on length-to-width ratio and strength properties. Protein functionality will be characterized in terms of solubility, viscosity, emulsification, and foaming properties and the relationship between these properties and product performance will be explored. Various techniques will be tested for protein denaturation and functional group modification to modify industrial properties of canola meal and or canola proteins. Among the most promising methods for protein modification for adhesive formulation are treatment with alkali, sodium dodecyl sulfate, and sodium dodecyl benzene sulfonate. Modifications will be carried out to determine their effects on product functionality described above.

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Item No. 40 of 59
ACCESSION NO: 0187998 SUBFILE: CRIS
PROJ NO: NDW-20010045 AGENCY: CSREES ND.W
PROJ TYPE: OTHER GRANTS PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 00-38819-8989-R PROPOSAL NO: 2001-03154
START: 01 JUN 2000 TERM: 31 MAY 2002 FY: 2003 GRANT YR: 2001
GRANT AMT: $241,891

INVESTIGATOR: Aulich, T. R.

PERFORMING INSTITUTION:
ENERGY & ENVIRONMENTAL RES CTR
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NATIONAL ALTERNATIVE FUELS LABORATORY PHASE 11

NON-TECHNICAL SUMMARY: A new high-performance, economical fuel is needed replace lead-containing aviation gasoline; new economic sources of octane are needed for the U.S. gasoline pool; and new easily biodegradable solvents are needed to replace hazardous chlorinated solvents. This project will 1) help commercialize AGE85 (an ethanol-based alternative to aviation gasoline) by developing an ASTM fuel specification for AGE85, and 2) demonstrate performance, environmental, and economic benefits of ethanol-based acid esters as octane-providing fuel additives and solvents.

OBJECTIVES: 2000-03900. Help commercialize aviation-grade E85 (AGE85), an EERC-formulated ethanol-based aviation fuel approved for use in certified aircraft by the U.S. Federal Aviation Administration (FAA), through 1) developing an American Society of Testing and Materials (ASTM) specification for AGE85, and 2) working with the University of North Dakota School of Aerospace Sciences (UND Aerospace), one of the two largest civilian pilot-training facilities in the U.S., to attain

FAA approval of AGE85 for the UND fleet of about 90 aircraft. Help increase commercial renewable fuels and chemicals production and utilization through establishment of partnerships with industry, agriculture, and environmental groups to 1) research and publicize beneficial emissions effects of ethanol-blended and other renewable-content fuels, 2) develop, evaluate, and help commercialize new fuel additives and fuel formulations, and 3) evaluate and help commercialize new biomass processing technologies and biobased products. Continue EERC leadership of the Red River Valley Clean Cities Coalition (RRV Coalition).

APPROACH: Commercialization of AGE85 will be advanced by 1) assembling an ASTM-approved task group for drafting an AGE85 fuel specification, and leading the task group through the democratic, iterative ASTM fuel specification development process, and 2) assisting UND Aerospace through setting up a safe, reliable, fuel storage and dispensing system that meets all materials compatibility requirements; ensuring that all aircraft fuel system components meet all materials compatibility requirements; development and performance of fuel analysis and property determination quality assurance procedures for bulk-delivered and on-site-stored AGE85; development of preflight fuel inspection and testing procedures; performance of analytical and experimental testing as required by FAA directives; and researching and reporting of fuel-related issues as required to meet FAA directives. To help commercialize renewable fuels and chemicals, partnerships like recent and ongoing collaborations with the Minnesota Corn Research and Promotional Council, South Dakota Corn Utilization Council, American Coalition for Ethanol, and American Lung Association of Minnesota will be established to 1) perform on-road emissions testing with systematically adjusted gasoline and diesel fuel formulations containing ethanol and other renewable fuels, 2) conduct and report laboratory and engine tests that demonstrate performance and emissions benefits of ethanol-based esters and higher-weight alcohols as gasoline octane providers, and 3) conduct and report laboratory tests to demonstrate performance, economic, and environmental advantages of using ethanol-based esters and other fermentation-derived chemicals as solvents, plasticizers, and polymer feedstocks. Leadership of the RRV Coalition will be provided by 1) coordinating alternative fuel (ethanol, biodiesel, natural gas, and others) vehicle acquisition efforts for regional public and private fleets, 2) continuing regular publication of the RRV Coalition newsletter, The Valley Drive, 3) maintaining, improving, and updating the RRV Coalition Website, 4) coordinating establishment of alternative fuel infrastructure like E85 and biodiesel refueling facilities, 5) designing and promoting alternative fuels educational activities (including Advancing the Choice workshops) throughout the RRV Coalition region of eastern North Dakota, northwestern Minnesota, and southern Manitoba, Canada, and 6) providing technical assistance to local initiatives for alternative fuels commercialization, including the Fargo biodiesel initiative as well as the activities of the North Dakota Corn Growers Association geared toward increased ethanol production, distribution, and use.

PROGRESS: 2000/06 TO 2002/05
As a result of National Alternative Fuels Laboratory (NAFL) work on developing aviation ethanol (AvE), the U.S. Federal Aviation Administration (FAA) approved a multi-partner project to demonstrate the safety, performance, economic, and maintenance benefits of AvE versus lead-containing aviation gasoline. Industry partners include Phillips Petroleum and Textron Lycoming. The NAFL initiated work with Phillips to optimize the AvE formulation and develop, at FAA request, an AvE fuel specification that garners approval from the American Society of Testing and Materials. The NAFL-developed on-road, on-board-vehicle exhaust emissions analysis system was used in an ongoing partnership with the American Lung Association of Minnesota (ALAMN) to evaluate air quality effects of ethanol-blended gasolines based on emissions of total hydrocarbons, carbon monoxide, and oxides of nitrogen. Results of winter and summer tests were published on the ALAMN website at www.alamn.org, and demonstrate the emissions benefits of ethanol and low sulfur content in gasoline. A project was conducted to optimize formulation of a blend of ethanol, biodiesel, and petroleum diesel based on performance, fuel economy, and emissions. Adding ethanol to diesel fuel is gaining prominence as a method to achieve significant reductions in exhaust emissions of particulates and oxides of nitrogen, but keeping ethanol and diesel together in solution requires an emulsifier or co-solvent. The NAFL is evaluating the use of biodiesel as a co-solvent for ethanol and diesel. The project showed the viability of a blend of 50% biodiesel-50% ethanol, which, when added to petroleum diesel at a concentration of 10%, ensures against ethanol phase separation at a temperature as low as 0 degrees F. Also being evaluated is the viability of the NAFL-developed dual fermentation (DFB) concept, which would split the sugar solution fed to either a corn- or biomass-based ethanol plant into two process streams: one for ethanol production and one for production of a carboxylic acid like lactic, propionic, butyric, or succinic. Each of these carboxylic acids can then be combined with ethanol, via an NAFL-developed direct esterification process based on the reaction used to produce

biodiesel, to yield ethyl esters with high value as industrial feedstocks, solvents, and gasoline octane providers. Based on work to date, application of the direct esterification approach to production of ethyl lactate appears to offer significant advantages over other high-profile approaches to recovery and purification of fermentation-derived lactic acid. The NAFL also initiated a project with a commercial biomass pyrolysis company to optimize a fast pyrolysis-based process for production of ethanol from cellulosic biomass. Key to the success of the effort will be maximizing pyrolysis yields of anhydrosugars, which can be converted to yeast-fermentable six-carbon sugars utilizing NAFL-developed hydrolysis techniques.

IMPACT: 2000/06 TO 2002/05
The National Alternative Fuels Laboratory (NAFL) program is working to develop and optimize processes to enable product diversification options for corn-based ethanol plants, and pyrolysis-based processes to enable economic production of ethanol from cellulosic biomass. Commercialization of these processes will provide product diversification and value-added opportunities for existing and future ethanol plants, and help make lower-cost biomass-based ethanol a reality. The NAFL is also working to assess and demonstrate the commercial viability, performance, environmental benefits, and safety of 1) ethanol-based, biodiesel-containing aviation ethanol, 2) ethanol-blended, low sulfur-content gasoline, 3) oxygenated diesel fuel that utilizes biodiesel as a co-solvent for ethanol, and 5) ethyl esters for use as solvents, industrial chemical feedstocks, and gasoline octane providers. Commercialization of these fuels and fuel additives will 1) eliminate the last commercial fuel still permitted by the U.S. Environmental Protection Agency to contain toxic lead (aviation gasoline), 2) significantly expand ethanol and biodiesel demand, since the current general aviation fuel market is about 400 million gallons per year and rising (its historic high was about 700 million gallons in the early 1980s), and current U.S. diesel fuel consumption is about 40 billion gallons per year, 3) improve air quality, 4) reduce fuel costs, and 5) increase U.S. energy independence and national security.

PUBLICATIONS (not previously reported): 2000/06 TO 2002/05
1. Olson, E., Sharma, R., and Aulich, T. 2002. Ester Fuels and Chemicals from Biomass. Accepted for publication in: Proceedings of the 24th Symposium on Biotechnology for Fuels and Chemicals, April 28-May 1, 2002, hosted by Oak Ridge National Laboratory.
2. Olson, E., Sharma, R. 2001. Direct Esterification of Ammonium Carboxylates with Ethanol and Methanol. Provisional Patent Application, Serial No. 60/344,934. Filed with U.S. Patent Office December 31, 2001.
3. Helder, D., Behnken, J., and Aulich, T. 2000. Design of Ethanol-Based Fuels for Aviation. American Society of Automotive Engineers Paper No. 00GATC-38.

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Item No. 41 of 59
ACCESSION NO: 0191656 SUBFILE: CRIS
PROJ NO: NDW-2002-06205 AGENCY: CSREES ND.W
PROJ TYPE: OTHER GRANTS PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2002-38819-01906 PROPOSAL NO: 2002-06205
START: 01 JUL 2002 TERM: 31 DEC 2003 FY: 2003 GRANT YR: 2002
GRANT AMT: $275,184

INVESTIGATOR: Aulich, T. R.

PERFORMING INSTITUTION:
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NATIONAL ALTERNATIVE FUELS LABORATORY - PHASE 12

NON-TECHNICAL SUMMARY: Lower-cost methods are needed for production of ethanol from non-corn kernel "biomass" resources, and production of biodiesel from a wide variety of oil seed crops, used cooking oils, and tallow. New economic sources of octane are needed for the U.S. gasoline pool, cleaner diesel fuels are required to reduce air pollution, and new easily biodegradable solvents are needed to replace hazardous chlorinated solvents. This project will 1) pursue development of a low-cost thermal process for converting biomass (like corn stalks, wood waste, and wheat and rice straw) into viable ethanol feedstocks, 2) optimize a catalyst used to make biodiesel that will reduce or eliminate expensive water washing requirements, 3) investigate renewable gasoline and diesel fuel additives and formulations based on engine performance and exhaust emissions, and 4) pursue development of new processes that will provide product diversification and value-added opportunities for existing and future ethanol plants.

OBJECTIVES: Lower the cost of biomass ethanol production by developing a new thermal pyrolysis process to convert cellulose and hemicellulose to fermentable sugars without consumption of expensive acids, enzymes, or other chemical or biological agents. Lower the cost of biodiesel production by developing and optimizing a high-efficiency non-sodium hydroxide esterification catalyst that reduces or eliminates the need for washing sodium out of esterified fuel product. Continue leadership of the Red River (of the North) Valley Clean Cities (RRVCC) Coalition. Help increase commercial renewable fuels and chemicals production and utilization through establishment of partnerships with industry, agriculture, and environmental groups to 1) research and publicize beneficial emissions effects of ethanol- and other renewable-content fuels, 2) develop, evaluate, and help commercialize new fuel additives and fuel formulations, and 3) develop, evaluate, and help commercialize new biomass processing technologies and biobased products.

APPROACH: Development of a thermal pyrolysis process for hydrolysis of biomass to fermentable sugars will build on recent Energy & Environmental Research Center (EERC) work with effluents from commercial sugar beet pulp dryers, and work performed by Weyerhauser Corporation, Crown Zellerbach, and the University of Waterloo in Ontario, Canada. The process will utilize a low-pressure, high-flow-rate gas to effect rapid removal of "anhydrosugar" pyrolysis products from the heated zone, thereby preventing their destruction, and a solid acid catalyst for anhydrosugar hydrolysis, which will eliminate problems associated with neutralization and loss of liquid acids. A bench-scale pyrolysis reactor will be used in tests with up to five different biomass feedstocks. Development and optimization of a new catalyst for biodiesel production will be based on recent EERC work on sunflower oil esterification. Laboratory experiments will evaluate a series of systematically adjusted catalyst configurations based on reaction conditions required to effect complete conversion of triglycerides to methyl esters, while leaving a sufficiently small amount of catalyst residue to negate the need for water washing. Leadership of the RRVCC Coalition will be provided by 1) coordinating alternative fuel (ethanol, biodiesel, natural gas, and others) vehicle acquisition efforts for regional public and private fleets, 2) continuing regular publication of the RRVCC Coalition newsletter, The Valley Drive, 3) maintaining, improving, and updating the RRVCC Coalition Website, 4) coordinating establishment of alternative fuel infrastructure like E85 and biodiesel refueling facilities, 5) designing and promoting alternative fuels educational activities (including "Advancing the Choice" workshops) throughout the Coalition region of eastern North Dakota, northwestern Minnesota, and southern Manitoba, Canada, and 6) providing technical assistance to local initiatives for alternative fuels commercialization. To help commercialize renewable fuels, chemicals, and related processes, partnerships like recent and ongoing collaborations with the Minnesota Corn Research and Promotional Council, South Dakota Corn Utilization Council, North Dakota Soybean Council, American Coalition for Ethanol, and American Lung Association of Minnesota will be established to 1) perform on-road and engine-dynamometer testing to demonstrate performance and emissions benefits of new gasoline and diesel fuel formulations containing ethanol, biodiesel, and other renewable fuels (including an oxygenated diesel that uses biodiesel as a co-solvent for ethanol), 2) conduct and report laboratory tests on optimizing processes for production of ethanol-based "esters" for use as gasoline octane providers and as chemical and polymer feedstocks, and 3) conduct and report laboratory tests to demonstrate the viability of processes that can be integrated with commercial conventional and upcoming biomass ethanol production processes.

PROGRESS: 2002/07 TO 2003/12
In partnership with Ensyn Renewables, Inc., of Boston, the University of North Dakota Energy & Environmental Research Center (EERC) is working to develop a fast pyrolysis-based biorefinery (FPB)

concept. The FPB is based on the Ensyn-developed rapid thermal process (RTP), which is commercially employed at three plants in Wisconsin for fast pyrolysis conversion of woody biomass to a bio-oil for specialty chemical and fuel applications. The EERC is working with Ensyn to tailor the RTP for conversion of wood and other biomass resources to a bio-oil optimally suited as a biorefinery input, with maximized levels of anhydrosugars that can be converted to fermentable six-carbon sugars using an EERC-developed low-temperature catalytic hydrolysis process that does not require the use of liquid acids or enzymes. In recent optimization work with an anhydrosugar-rich Ensyn bio-oil fraction, the EERC process has been demonstrated to achieve near-100% conversions of anhydrosugars to glucose. In other work related to the FPB concept, EERC researchers developed a process for extraction (from fast pyrolysis bio-oil) of a specific compound and conversion of the compound to a high-value additive utilized as a dispersant in most latex paints produced today. Current project effort is being directed toward economic optimization and commercialization of both the anhydrosugar and paint dispersant precursor conversion processes. EERC researchers compared three major Twin Cities (Minneapolis-St. Paul) 10%-ethanol-blended (E10) gasoline brands based on predicted and actual tailpipe emissions and demonstrated that gasoline sulfur content has a significant impact on automobile exhaust emissions of hydrocarbons, carbon monoxide, and oxides of nitrogen. The investigation utilized U.S. Environmental Protection Agency (EPA) MOBILE6.2 vehicle emission modeling software and average fuel chemistry data for Twin Cities 87-octane Holiday, BP, and SuperAmerica summer- and winter-blend fuels to predict exhaust emissions from a typical 2002 model-year car. Average fuel chemistry data inputs to the EPA model were based on analysis of E10 samples collected at the pump from randomly selected Twin Cities gasoline retailers. Actual emissions data were acquired in on-the-road tests utilizing an EERC-developed on-board-vehicle emissions analysis system. Both the predicted and actual emissions data illustrated the importance of reduced sulfur content to lower exhaust emissions. Reports on the National Alternative Fuels Laboratory-conducted Twin Cities gasoline surveys are accessible through the American Lung Association of Minnesota (ALAMN) Web site. A series of nonsodium-hydroxide catalysts were evaluated based on conversion of soybean oil triglycerides to fatty acid methyl ester (FAME) biodiesel. One of the catalysts consistently generated FAME yields of 99%. A precipitation-based method was evaluated for separation of FAME and residual catalyst. The method was consistently demonstrated to effect catalyst removal to a level of 40 parts per million.

IMPACT: 2002/07 TO 2003/12
A catalytic process was developed that enables producing fermentable sugars from a bio-oil generated via fast pyrolysis (heating under a deficiency of oxygen) of waste wood. Commercialization of the process will enable economic production of ethanol and other biobased products from cellulosic biomass. Progress was made on development of an alternative (to sodium hydroxide) and potentially lower-cost catalyst system for production of FAME biodiesel from vegetable oil triglycerides. Unlike biodiesel production with conventional sodium hydroxide catalyst, which requires water washing for excess catalyst removal from the biodiesel product, the alternative catalyst under development will be removable via precipitation and filtration. In a study conducted in partnership with the ALAMN, the impact of low-sulfur, 10%-ethanol-blended gasoline (E10) on reducing automobile tailpipe emissions was demonstrated. ALAMN is using the study findings to advocate for wider use of lower sulfur-content E10 as a viable strategy for improving air quality in the Minneapolis-St. Paul metropolitan region.

PUBLICATIONS (not previously reported): 2002/07 TO 2003/12
Olson, E., Aulich, T., Sharma, R., Timpe, R. 2003. Ester fuels and chemicals from biomass. Appl. Biochem. Biotechnol. 105-108:843-851.

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Item No. 42 of 59
ACCESSION NO: 0195206 SUBFILE: CRIS
PROJ NO: NDW-2003-06204 AGENCY: CSREES ND.W
PROJ TYPE: OTHER GRANTS PROJ STATUS: TERMINATED

CONTRACT/GRANT/AGREEMENT NO: 2003-38819-02014 PROPOSAL NO: 2003-06204
START: 01 JUL 2003 TERM: 30 JUN 2004 FY: 2004 GRANT YR: 2003
GRANT AMT: $280,835

INVESTIGATOR: Aulich, T. R.

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NATIONAL ALTERNATIVE FUELS LABORATORY - PHASE 13

NON-TECHNICAL SUMMARY: Lower-cost methods are needed for production of ethanol from non-corn kernel biomass resources, and production of biodiesel (a diesel fuel additive or replacement made from vegetable oils or animal fats). A non-lead-containing, high performance aviation fuel is needed to help ensure long-term availability of fuel for the general aviation industry. New, domestically produced biobased fuels and products, along with economically viable methods for their production, are needed to ensure national security, improve our environment, and sustain economic growth. This project will 1) pursue development of new high-value products (including pharmaceutical building blocks) from bio-oils generated via high-temperature-processing of biomass feedstocks like corn stover, wheat straw, prairie grass, and waste wood and paper, 2) optimize a new, lower-cost process for producing biodiesel, 3) help commercialize an ethanol-based aviation fuel for piston engine aircraft via demonstrating fuel compatibility with aircraft fuel system materials of construction, 4) pursue development of fueling stations and increased fleet utilization of ethanol-based and biodiesel-containing fuels, and 5) work with ag and environmental groups to commercialize and increase utilization of biobased fuels and non-fuel products and biomass processing technologies.

OBJECTIVES: Develop new high-value products (including pharmaceutical building blocks) from "bio-oils" generated via fast pyrolysis of lignocellulose biomass resources like corn stover, wheat and barley straw, switch grass, waste wood and paper, and others. Optimize an innovative catalytic process to improve the efficiency and reduce the cost of producing biodiesel. Help commercialize aviation-grade ethanol (AGE), a high performance alternative to lead-containing aviation gasoline, through collaborating on a recently initiated effort to develop an American Society of Testing and Materials (ASTM) fuel specification for AGE. Increase renewable fuels and chemicals commercial production and utilization through establishment of partnerships with industry, agriculture, and environmental groups to develop new biobased fuels and products and biomass processing technologies. Continue leading the Red River Valley Clean Cities Coalition (RRVCC) in developing partnerships and region-wide initiatives to establish commercial E85 and biodiesel fuel stations and increase renewable fuels utilization.

APPROACH: Pyrolysis product development will focus on characterization of bio-oils generated from straw, stover, grass, and wood, identification of potential products, and development of effective and economic extraction and conversion technologies based on solid acid catalysis methods. Bio-oil samples will be generated by NAFL partner Ensyn Technologies, Inc., of Ottawa, Canada, using the Ensyn bench-scale fast pyrolysis reactor. Based on preliminary characterization of wood-generated bio-oils, product development efforts will focus on levoglucosan-derived pharmaceutical building blocks, sugar esters, furans, and non-fermentable sugars. Biodiesel process improvement efforts will utilize an NAFL-developed non-alcohol alkylating agent in a column reactor packed with a solid acid catalyst. Different reaction conditions and catalysts will be evaluated based on fatty acid methyl (or ethyl) ester yield, operational cost, and co-product purity and recovery. The effort to help commercialize AGE will focus on establishing AGE compatibility with materials used in general aviation fuel systems via tests conducted according to ASTM and Society of Automotive Engineers procedures. To help commercialize and increase utilization of renewable fuels, chemicals, and related biomass processing technologies, partnerships like recent and ongoing collaborations with the Minnesota Corn and Soybean Research and Promotion Councils, South Dakota Corn Utilization Council, American Coalition for Ethanol, and American Lung Association of Minnesota will be established to 1) perform on-road and engine dynamometer testing to demonstrate performance and emissions benefits of new gasoline and diesel fuel formulations containing ethanol, biodiesel, and other renewable fuels (including an oxygenated diesel that uses biodiesel as a co-solvent for ethanol), 2) conduct and report laboratory tests on optimizing processes for production of ethanol-based esters for use as polymer feedstocks, chlorinated solvent replacements, and chemical intermediates, and 3) conduct and report laboratory tests to demonstrate the viability of process improvements that can be integrated with commercial conventional and upcoming biomass ethanol production processes. Leadership of the RRVCC Coalition will be provided by 1) coordinating alternative fuel (ethanol, biodiesel, and others) vehicle acquisition efforts for regional public and private fleets, 2) continuing regular publication of the RRVCC Coalition newsletter, The Valley Drive, 3) maintaining, improving, and updating the RRVCC Coalition Website, 4) coordinating establishment of alternative fuel infrastructure like E85 and biodiesel refueling facilities, 5) designing and promoting alternative fuels educational activities (including Advancing the Choice workshops) throughout the Coalition region of eastern North Dakota, northwestern Minnesota, and southern Manitoba, Canada, and 6) providing technical assistance to local initiatives for alternative fuels commercialization.

PROGRESS: 2003/07 TO 2004/06
In partnership with Ensyn Renewables, Inc., the Energy and Environmental Research Center (EERC) is developing a fast-pyrolysis biorefinery (FPB) concept. The FPB is based on the Ensyn pyrolysis process that is commercially employed at four plants in Wisconsin for conversion of biomass to a bio-oil for chemical and fuel applications. EERC has developed processes that enable 1) converting bio-oil anhydrosugars to fermentable 6- carbon sugars via a low temperature catalytic hydrolysis, and 2) converting bio-oil hydroxyacetaldehyde (HA) to high value chemicals. Using Ensyn bio-oil fractions, the EERC hydrolysis has achieved near 100 percent conversions of anhydrosugars to glucose, and a provisional patent was filed for the process. Using HA-rich bio oil fractions, EERC has developed a simple reaction sequence for producing large-market alkanolamines. EERC developed and demonstrated a transesterification process that enables utilization of soapstocks and other high free fatty acid-content vegetable oil processing coproducts and animal fats as biodiesel feedstocks. In tests with commercial vegetable oil coproducts, the process, which utilizes a non-alcohol alkylating agent and an acid catalyst, has been demonstrated to effect fatty acid conversions of up to 99 percent. EERC investigated the compatibility of aviation-grade ethanol (AGE) with a comprehensive array of materials used in piston engine aircraft fuel systems. The investigation utilized metals, elastomers, plastics, rubbers, composites, adhesives, and other materials to compare AGE to leaded aviation gasoline based on fuel immersion-generated impacts on corrosion, solubilization, swelling, shrinkage, flexibility, and shear strength. Results of the investigation, which showed a few possibly significant differences between AGE and aviation gasoline, were reported to an American Society of Testing and Materials (ASTM) task force working on development of an ASTM specification for AGE. EERC compared three major Minneapolis and St. Paul 10 percent ethanol gasoline (E10) brands, E85 (a blend of 85 percent ethanol and 15 percent gasoline), and nonoxygenated gasoline based on predicted and actual tailpipe emissions, and demonstrated that gasoline sulfur content has a significant impact on automobile exhaust emissions of hydrocarbons, carbon monoxide, and oxides of nitrogen. The investigation utilized EPA MOBILE6.2 vehicle emission modeling software and average fuel chemistry data for 87-octane summer-blend fuels to predict exhaust emissions from a typical 2002 model year car. Average fuel chemistry data inputs to the EPA model were based on analysis of E10 samples collected at the pump from randomly selected Minneapolis and St. Paul gasoline retailers. Actual emissions data were acquired in tests utilizing a 2002 flexible-fuel Ford Taurus and a chassis dynamometer. Both the predicted and actual emissions data illustrated the importance of reduced sulfur content to lower exhaust emissions. Of the five fuels evaluated in the dynamometer testing, the E85 generated the lowest grams-per-mile tailpipe emission of carbon dioxide.

IMPACT: 2003/07 TO 2004/06
A catalytic process was developed for producing alkanolamines from bio-oil. Because alkanolamines are derived from petroleum and natural gas and represent an annual market of 3.3 billion pounds, commercialization of the process will enable replacement of oil with biomass in chemical markets and improve biorefining economics. Progress was made on an economic process for producing methyl esters from low value feedstocks high in free fatty acids. Commercialization of the process will result in improved biodiesel economics. Some of the most critical questions on replacing leaded aviation gasoline with aviation-grade ethanol (AGE) derive from materials compatibility concerns. The EERC investigation on AGE materials compatibility provides definitive empirical evidence regarding how key aircraft material properties are impacted by AGE immersion. The EERC report on the investigation will help expedite prosecution of an American Society of Testing and Materials specification for AGE, which was requested by the U.S. Federal Aviation Administration. In a study conducted with the American Lung Association of

Minnesota (ALAMN), the impact of low sulfur, 10 percent ethanol gasoline (E10) on reducing automobile tailpipe emissions was demonstrated, as was the impact of E85 (85 percent ethanol and 15 percent gasoline) on reducing tailpipe carbon dioxide emissions. ALAMN is using the study findings to advocate wider use of lower sulfur content E10 and E85 as a viable strategy for improving air quality in the Minneapolis and St. Paul metropolitan region.

PUBLICATIONS (not previously reported): 2003/07 TO 2004/06
1. Olson, E.S.; Sharma, R.K.; Aulich, T.R. 2004. The Higher-Alcohols Biorefinery: Improvement of the Catalyst for Ethanol Conversion. Appl. Biochem. Biotechnol. 113-116, 913-932.
2. Olson, E.S.; Sharma, R.K.; Aulich, T.R. 2004. The Higher Alcohols Biorefinery II: Conversion of Ethanol to High Cetane Diesel Additives. In proceedings of the 26th Symposium on Biotechnology for Fuels and Chemicals, Chattanooga, TN, May 9-12, 2004.
3. Aulich, T.R. 2003. Hydrogen, Ethanol, and Energy Security. Ethanol Today, November 2003.

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Item No. 43 of 59
ACCESSION NO: 0199475 SUBFILE: CRIS
PROJ NO: NDW-2004-06202 AGENCY: CSREES ND.W
PROJ TYPE: OTHER GRANTS PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2004-38819-02182 PROPOSAL NO: 2004-06202
START: 01 JUL 2004 TERM: 30 JUN 2005 FY: 2005 GRANT YR: 2004
GRANT AMT: $251,229

INVESTIGATOR: Aulich, T. R.

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NATIONAL ALTERNATIVE FUELS LABORATORY - PHASE 14

NON-TECHNICAL SUMMARY: Lower-cost methods are needed for production of fuel and chemical intermediates from lignocellulosic biomass. Lower cost biodiesel feedstocks and methods for their conversion to biodiesel are needed. Better methods for extracting value from wind energy and utilization of ethanol plant-generated carbon dioxide are needed. This project will 1) optimize and evaluate methods for production of chemical intermediates from bio-oil generated by pyrolysis of biomass, 2) optimize a non-alcohol-based esterification process for producing biodiesel from low-value vegetable oil coproducts known as soapstocks, and 3) optimize and evaluate a process that utilizes electricity and carbon dioxide for production of urea fertilizer.

OBJECTIVES: The first objective is to develop new high-value products from bio-oils generated via fast pyrolysis of wood, corn stover, wheat and barley straw, switch grass, and other lignocellulose biomass resources. The secod objective is to optimize a non-alcohol-based esterification process for production of fatty acid methyl esters from low-value, high free fatty acid-content vegetable oil refining coproducts known as soapstocks. The third objective is to optimize and evaluate the economics of a low-severity (ambient temperature and pressure) electrochemical synthesis-based process for production of urea fertilizer from wind-generated electricity and ethanol plant-generated carbon dioxide.

APPROACH: Pyrolysis product development will focus on development of processes for producing chemical intermediates from wood- and switch grass-derived bio-oil fractions rich in hydroxyacetaldehyde.

Bio-oil samples will be generated by project partner Ensyn Renewables, Inc., of Boston, using the Ensyn bench-scale fast pyrolysis reactor. Biodiesel-from-soapstock process development will optimize the non-alcohol-based esterification process based on catalyst type and configuration, reaction temperature and pressure, and reactor residence time. Work will also include development of waste water-minimization methods, and methods for removal of soapstock-contributed contaminants. The urea production process development effort will comprise laboratory-scale work to improve the efficiency of the electrochemical synthesis by utilizing new cathode materials and solid-polymer electrolyte technologies.

PROGRESS: 2004/07 TO 2005/06
The National Alternative Fuels Laboratory (NAFL) at the University of North Dakota Energy & Environmental Research Center is optimizing a low-temperature electrochemical process for producing urea fertilizer from ethanol coproduct carbon dioxide (CO2), coal combustion emissions of nitrogen oxides (NOx), and electricity. NAFL researchers designed and fabricated an electrochemical cell and used it to produce urea from reagent-grade feedstocks. Project activities are currently focused on electrode catalytic optimization for improved process efficiency. In partnership with Ensyn Renewables, Inc., the NAFL is developing the fast-pyrolysis biorefinery based on the commercial Ensyn process for conversion of biomass to bio-oil. The NAFL developed processes for low-temperature catalytic hydrolysis of bio-oil anhydrosugars to fermentable 6-carbon sugars, and converting bio-oil hydroxyacetaldehyde (HA) to high value alkanolamines. A glucose stream generated from bio-oil via the hydrolysis process was submitted to the University of Minnesota Biotechnology Institute for evaluation as fermentation ethanol feedstock. Using HA-rich bio oil fractions, the NAFL developed a simple reaction sequence for producing large-market alkanolamines. In response to a request from a major chemical manufacturer, the NAFL is working with Ensyn to evaluate the life cycle economics of an alkanolamines process based on utilization of the HA-based reaction sequence. The NAFL developed and demonstrated a low-temperature, solid acid-catalyzed transesterification process that enables utilization of unrefined vegetable oil and other lower-value materials as biodiesel feedstocks. In tests with crude soybean oil, the process consistently achieved 100% conversion of fatty acids to methyl esters. Current research is focused on catalyst optimization to reduce residence time, pressure, and methanol-to-fatty acid molar ratio requirements. As a component of an ongoing effort to commercialize aviation-grade ethanol (AGE) as an alternative to leaded aviation gasoline, the NAFL initiated long-term soak tests to evaluate a series of corrosion inhibitors for performance with aircraft-grade aluminum alloys. Results will be presented to the American Society of Testing and Materials (ASTM) Ethanol Aviation Fuel Development Task Force at the December 2005 ASTM meeting. In collaboration with the American Lung Association of Minnesota, the NAFL assessed the greenhouse gas reduction potential of E85. The assessment comprised measurement of tailpipe emissions from a 2004 E85-capable Ford Explorer Sport Trac running on E85 and gasoline, and analysis of fuel life cycle CO2 emissions data reported by Argonne National Laboratory. The investigation showed that if the approximately 5 million E85-capable vehicles on the road today were to burn corn-based E85 instead of gasoline, this would effect a CO2 emission reduction of 20 million tons per year. With lignocellulosic biomass-based E85, the reduction would be 31 million tons per year. To put these potential impacts in perspective, a typical 500-megawatt coal-fired power plant generates about 3.8 million tons of CO2 per year.

IMPACT: 2004/07 TO 2005/06
Commercialization of the NAFL electrochemical urea synthesis would result in cheaper fertilizer, provide a market for ethanol plant-generated CO2, and turn power plant NOx emissions into a revenue stream. Operating the process with wind-generated electricity would enable extracting value from wind energy without the need for additional transmission capacity, and promote rural economic development on Great Plains. Because petroleum-derived alkanolamines are an annual market of 3.3 billion pounds, commercialization of the NAFL HA-to-alkanolamines process will enable replacement of oil with biomass in chemical markets and improve biorefining economics. Commercialization of the NAFL biodiesel process will enable utilization of unrefined vegetable oils and other cheaper feedstocks, which will improve biodiesel economics. Concern has been raised regarding the possibility of aluminum corrosion due to long-term AGE exposure. The NAFL soak tests will assess the corrosion potential of AGE and evaluate the effectiveness of commercial corrosion inhibitors. Resolving the aluminum corrosion issue is critical to achieving an ASTM specification for AGE, and, as stipulated by the U.S. Federal Aviation Administration, an ASTM specification is critical to AGE commercialization. NAFL data on the greenhouse gas emission reduction impact of E85 are being publicized by ALAMN to promote E85 and, in conjunction with higher

gasoline prices, have contributed to increasing Minnesota E85 consumption from 705,000 gallons in 2001 to 4.3 million gallons for the first 8 months of 2005.

PUBLICATIONS (not previously reported): 2004/07 TO 2005/06
1. Olson, E.S., Sharma, R.K., Timpe, R.C. and Aulich, T.R. 2005. T.R. High-Cetane Diesel Fuels from Biomass. Appl. Biochem. Biotechnol. In review.
2. Olson, E.S. Parallel Processing Biorefineries. 2005. Prepr. Pap. Am. Chem. Soc., Div. Fuel Chem. 50 (2), 681-682.
3. Olson, E.S., Sharma, R.K., Timpe, R.C. and Aulich, T.R. 2005. High-Cetane Diesel Fuels from Biomass. In Proceedings of the 27th Symposium on Biotechnology for Fuels and Chemicals.

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Item No. 44 of 59
ACCESSION NO: 0206950 SUBFILE: CRIS
PROJ NO: NDW-2006-06229 AGENCY: CSREES ND.W
PROJ TYPE: OTHER GRANTS PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2006-38819-03470 PROPOSAL NO: 2006-06229
START: 01 JUN 2006 TERM: 31 MAY 2007 GRANT YR: 2006
GRANT AMT: $261,313

INVESTIGATOR: Aulich, T. R.

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NATIONAL ALTERNATIVE FUELS LABORATORY - PHASE 16

NON-TECHNICAL SUMMARY: To reduce U.S. dependence on oil and reduce carbon dioxide emissions, lower-energy and lower-cost methods are needed for production of ethanol from corn and lignocellulosic biomass, and economically competitive methods are needed to produce polymers from lignocellulosic biomass. In addition to its use as a fuel, ethanol has potentially higher value as a chemical intermediate that could be used to produce products normally derived from petroleum. Methods are needed for extracting value from carbon dioxide generated as an ethanol coproduct, and better methods are needed for extracting value from wind energy and for producing urea fertilizer, the cost of which is primarily dependent on the increasing cost of natural gas. This project will optimize and evaluate 1) methods for improving the energy efficiency of ethanol production from corn and lignocellulosic biomass, 2) processes for producing polymers from bio-oil generated by heating lignocellulosic biomass in a deficiency of oxygen, 3) a process for converting ethanol to butanol and other chemical intermediates that is compatible with integration at corn- and lignocellulose-based ethanol production facilities, and 4) a process that uses electricity (ideally generated from wind) and ethanol coproduct carbon dioxide to produce urea fertilizer.

OBJECTIVES: The objectives are to are to optimize and evaluate 1) improved energy efficiency technologies for ethanol production from starch and lignocellulosic feedstocks based on the use of self-flocculating yeast and an innovative bioreactor design, 2) methods for utilizing hydroxyacetaldehyde extracted from bio-oils (generated via biomass pyrolysis) as feedstock for producing polyesters, polyurethanes, and polyamides, 3) a low-temperature, low-pressure process for converting ethanol to butanol and other higher alcohols that is compatible with integration at corn- and lignocellulose-based ethanol production facilities, and 4) an electrochemical synthesis that utilizes ethanol coproduct carbon dioxide, nitric oxide, and electricity (ideally generated from wind) to produce urea fertilizer.

APPROACH: Ethanol process efficiency improvement will focus on generating a clear beer, optimizing a membrane-based pervaporation process for dehydration of the clear beer, and increasing the ethanol concentration in lignocellulose feedstock-derived fermentation broth via beer recycle. Polymer process development will focus on utilization of hydroxyacetaldehyde in reactions similar to those employed in production of polyesters, polyurethanes, and nylon. Ethanol-to-higher alcohols process development will focus on optimization of a catalyst system based on yield, durability, and reaction temperature, all of which will impact operational cost. Urea process development will utilize a recently designed and fabricated electrochemical reactor system to optimize the urea synthesis using 1) reagent-grade carbon dioxide, and 2) carbon dioxide recovered from an ethanol plant.

PROGRESS: 2006/06 TO 2007/05
The National Alternative Fuels Laboratory (NAFL) at the University of North Dakota Energy & Environmental Research Center developed and is optimizing electrochemical processes for producing nitrogen-based fertilizers from nitric oxide (NO), carbon dioxide, hydrogen, and electricity. An electrochemical cell was designed, fabricated, and used to produce ammonium nitrate (AN), ammonia, and urea from reagent-grade feedstocks. Data from these lab-scale experiments were utilized to assess the economics of the processes based on the use of NO recovered from electric utility coal combustion emissions and carbon dioxide generated as a coproduct of fuel ethanol production. The economic assessment indicated good potential economic viability for the AN process, primarily because of its low hydrogen input requirement. Ongoing AN process optimization work is focused on improving the catalytic activity of the electrodes utilized to drive the process. NAFL developed and is optimizing a catalyst system for converting ethanol to butanol and other alcohols via a low-severity process that is compatible with integration at current and new ethanol production facilities. Using a carbon-based catalyst, a 40% conversion of ethanol to butanol was achieved. NAFL developed new synthetic methods for producing polymer products by substituting petroleum-derived ethylene oxide with hydroxyacetaldehyde (HA), a constituent of bio-oil generated via fast pyrolysis of lignocellulose. NAFL experiments indicated that HA can undergo a variety of reactions at its hydroxyl and aldehyde functionalities to yield large-market polyglycolamine products including latex paint dispersants and polymer-flood enhanced oil recovery agents. NAFL pursued development of a low-energy ethanol production route based on a continuous fermentation process that uses self-flocculating yeast and a specially designed suspended-bed airlift bioreactor (SBAB). A self-flocculating yeast strain was selected and acquired from the USDA Culture Collection, and two SBAB fermenters for ethanol continuous fermentation were designed and constructed. Fermentation study was focused on cellulosic ethanol, with emphasis on development of a beer recycle process. Beer recycle is crucial for improved cellulosic ethanol economics because the low density of cellulosic biomass typically translates to a low-concentration sugar solution and subsequent low-ethanol titer, which results in a high per-gallon ethanol dehydration cost. Typical ethanol concentration in a cellulose fermentation broth is about 4%, versus starch-based ethanol titers that can range from 12%-17%. Initial testing utilized purchased cellulose and cellulase enzyme to yield an 8.5% glucose concentration hydrolysate, which was fermented to yield a clear beer with an ethanol concentration of 4%. This clear beer was recycled through a second hydrolysis to yield an ethanol concentration of 8%. Attempts to achieve a third recycle were unsuccessful because of a reduction of cellulase activity by ethanol. During this project, an invention was conceived that comprises a method for the electrochemical production of ammonium nitrate from nitric oxide.

IMPACT: 2006/06 TO 2007/05
Commercialization of the NAFL electrochemical ammonium nitrate synthesis would result in cheaper fertilizer and turn coal-fired power plant NOx emissions into a revenue stream. Operating the process with wind-generated electricity would enable extracting value from wind energy without the need for additional transmission capacity and would promote rural economic development on the Great Plains. Because butanol is an excellent gasoline oxygenate with fuel properties that complement (rather than compete with) those of ethanol, commercialization of the NAFL low-severity process for conversion of ethanol to butanol represents a valuable product diversification option for current corn-based and future cellulosic ethanol plants. Because petroleum-derived ethylene oxide-based polymer production processes encompass large markets, commercialization of HA-based processes to produce these polymers will enable the replacement of oil with biomass in chemical markets and improve biorefining economics. The commercial viability of the HA-based processes will depend to a large extent on the cost of lignocellulosic pyrolysis-based HA

production and, ultimately, how this cost compares with the cost of ethylene oxide. Thus, HA-based products with the best potential viability are likely to be those with a sales price of greater than $0.50/lb.

PUBLICATIONS (not previously reported): 2006/06 TO 2007/05

Liu, C.; Hu, B.; Chen, S.; Glass, R.W. Utilization of Condensed Distillers Solubles as Nutrient Supplement for Production of Nisin and Lactic Acid from Whey. Appl. Biochem. Biotechnol. 2007, 137-140 (1), 875-884.

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Item No. 45 of 59
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PROJ NO: OKL02461 AGENCY: CSREES OKL
PROJ TYPE: SPECIAL GRANT PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2001-34447-10302 PROPOSAL NO: 2001-03143
START: 01 JUL 2001 TERM: 30 JUN 2003 FY: 2003 GRANT YR: 2001
GRANT AMT: $841,883

INVESTIGATOR: Huhnke, R. L.; Bellmer, D. D.; Bowser, T. J.; Epplin, F. M.; Lalman, J. A.; Taliaferro, C. M.

PERFORMING INSTITUTION:
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BIOMASS-BASED ENERGY RESEARCH

NON-TECHNICAL SUMMARY: Development of advanced alternative liquid transportation fuels, especially ethanol, will continue to be a national priority for the foreseeable future. This project involves the integrated activities of the Oklahoma/Mississippi Consortium (Oklahoma State University, The University of Oklahoma and Mississippi State University) in addressing most important issues in the conversion of biomass to liquid fuel utilizing a unique bioconversion process. Research conducted by this Consortium is reflected through five primary research projects: Feedstock Development, Biomass Gasification and Syngas Conditioning, Syngas Fermentation, Microbial Catalyst Development, and Economics.

OBJECTIVES: Determine optimal procedures for stand establishment and sustained biomass production for selected species with potential as dedicated feedstock crops. Develop and optimize gasification technology for syngas production through improved mass and energy balance measurement systems and techniques. Assess the impact of syngas composition on chemical productivity and cell growth within bench-scale fermenters. Determine the effect of medium components on the fermentation of syngas to ethanol. Examine the enzyme activities responsible for ethanol production. Determine the cost of system components (production, harvest, storage, transportation, processing, gasification, bioconversion, and ash disposal) for one or more potential feedstocks. Evaluate the potential demand for anticipated products (i.e. ethanol, ash, and change in air quality).

APPROACH: For selected species and varieties with a use history, studies will be initiated to determine the effects of fertilization (kind, rate, and date) and harvest (frequency, timing, cutting height) variables on biomass yield and chemical compositions. Operating conditions of gasifier at OSU will be optimized to produce desired syngas products with minimum waste. CO, H2, CO2 and CH4 production will be maximized while O2, tar, and ash content are minimized. Gasification system optimization involves changing the physical and operational parameters of the equipment, based on results of gasification experiments, researchers' experience and knowledge obtained from the literature. Studies will be initiated that will use syngas compositions of various constituents and relative concentrations. Kinetic parameters will be measured that include the CO, CO2, and H2 uptake rates, cell growth rate and concentration, ethanol/acetic acid ratio, yield of products relative to CO consumption, and the production rates of ethanol, acetic acid, butanol, and CO2. Manipulation of medium components to improve the conversion of syngas to ethanol will be examined. Primary measurements of organic acids and alcohols will be by gas chromatography, while ethanol concentrations will be confirmed using an alcohol dehydrogenase assay. Production coefficients will be developed after consultation with production scientists and used to develop input-output coefficients, while enterprise budgets will be used to determine production costs of dedicated agricultural biomass production activities. Historical data on product quantities and prices will be used to construct demand models representing the markets for the various products produced by the gasification process.

PROGRESS: 2001/07 TO 2003/06
Feedstock Development: Differences were detected in concentrations of chemical constituents in biomass samples of Kanlow switchgrass either harvested at 30-day intervals during winter (standing cured biomass) or at different maturity stages (preboot, full heading, end-of-season) during the growing season. One notable difference was significantly ($P<0.05$) lower levels of K in mature (heading stage and later), relative to immature, biomass because of the potential positive impact of lower K concentration when switchgrass feedstock is gasified or used in co-firing. Breeder and Foundation class plantings were made of bermudagrass breeding line LCB84X 16-66 in preparation for its release as a commercial cultivar. Gasification: Switchgrass has consistently been gasified to produce 14 - 19% CO, 3 - 5% H2, 4-5% CH4, 15 - 18% CO2 and 50-60% N2, and trace components (dry basis values). These values were obtained at a gasifier temperature of 650 to 800 degrees C and a biomass-to-air ratio of 0.27 to 0.29. A mass balance of the system has been successfully conducted. 5 to 7% of the biomass feedstock is removed from the system as ash. The ash contains about 50% carbon. Using a pilot-scale fluidization chamber, biomass fluidization studies were conducted to characterize behavior of the biomass in the gasifier. Results showed that both minimum and complete fluidization velocities were more sensitive to sand particle size as compared to the percent biomass level in a sand-biomass mixture. It was demonstrated that clean syngas could be produced and then stored at 100 psig with oxygen content of less than 1200 ppm. Fermentation: Syngas generated from biomass has been fermented. The bacteria were initially grown on compressed gases with similar composition as the generated gas. The gas source was then switched to the generated gas once the cell concentration remained at steady state for at least one day. The introduction of producer gas does not kill the cells. Cell growth stops. The cell decline observed in the chemostat agrees very well with a model suggesting no growth and loss of non-growing cells from the exit stream of the bioreactor. These results demonstrate that cell recycle is necessary. Microbial Catalysts: Clostridium carboxidovorans (P7) and Clostridium ragsdalei (P11) were deposited with the American Type Culture Collection and the Deutsche Sammlung von Mikroorganismen und Zellkulturen. Both P7 and P11 produce ethanol from CO in ammonium-free medium, eliminating a major medium cost. Hydrogenase, carbon monoxide dehydrogenase, formate dehydrogenase, and alcohol dehydrogenase are important enzymes for the conversion of CO to ethanol. The ability to measure these enzyme activities in whole cells is useful for monitoring fermentations and determining possible conversion bottlenecks.

IMPACT: 2001/07 TO 2003/06
The ultimate goal of our research is to move our unique bioconversion process to commercialization. Feedstocks for this process will include low-cost perennial grasses and crop residues, resulting in another possible source of farm income.

PUBLICATIONS (not previously reported): 2001/07 TO 2003/06
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2. Das, M.K., Fuentes, R.G., and Taliaferro, C.M. 2003. Genetic variability and trait relationship in switchgrass. Crop Sci. (In Press).
3. Datar, R., Shenkman, R., Cateni, B.G., Huhnke, R.L., and Lewis, R.S. 2003. Ethanol from Biomass-generated producer gas. Proceedings of the Annual AIChE Meeting, San Francisco, CA.

4. Fuentes, R. G. and Taliaferro, C. M. 2002. Biomass yield stability of switchgrass (Panicum virgatum L.) cultivars. p. 276-282. IN J. Janick & A. Whipkey (eds.). Trends in New Crops and New Uses. Proceedings 5th Natl. Symposium on New Crops and New Uses, Strength in Diversity, 10-13 Nov. 2001, Atlanta, GA. ASHS Press, Alexandria, VA. USA.
5. Krushna, P., Bowser, T.J., Bellmer, D.D., and Huhnke, R.L. 2003. Fluidization characteristics of sand and mixtures of chopped switchgrass and sand. ASAE 036084. American Society of Agricultural Engineers, St. Joseph, MI.
6. Rajagopalan, S., Datar, R.P., and Lewis, R.S. 2002. Formation of ethanol from carbon monoxide via a new microbial catalyst, Biomass and Bioenergy, 23: 487-493.

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Item No. 46 of 59
ACCESSION NO: 0207135 SUBFILE: CRIS
PROJ NO: OKL02593 AGENCY: CSREES OKL
PROJ TYPE: HATCH PROJ STATUS: NEW
START: 01 OCT 2006 TERM: 30 SEP 2011

INVESTIGATOR: Wilkins, M. R.

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RENEWABLE FUELS FROM OKLAHOMA BIOMASS

NON-TECHNICAL SUMMARY: The United States imports over 50f its petroleum from foreign sources, which threatens the nation's energy security. This project seeks to develop and improve processes to utilize cellulosic biomass grown in Oklahoma to produce fuel ethanol. Ethanol is a renewable, locally-produced fuel that can help reduce imports of petroleum from foreign sources.

OBJECTIVES: The overall objective is to identify technologies and process conditions that will most efficiently convert feedstocks that can be grown in Oklahoma into sugars that can be used to produce fuel ethanol. Individual objectives are to: (1) identify pretreatments that maximize ethanol production from an enzymatic hydrolysis process using lignocellulosic feedstocks; (2) evaluate thermotolerant microorganisms for ethanol production using SSF and lignocellulosic materials; and (3) evaluate non-senescent sorghum as a feedstock for ethanol production in Oklahoma.

APPROACH: Switchgrass and sorghum bagasse will be tested as feedstocks for ethanol production. Three pretreatments, liquid hot water, steam explosion, and alkaline, will be used to determine the best pretreatment for increasing cellulose reactivity while reducing yeast inhibition. Five thermotolerant Kluyveromyces marxianus yeast strains will be tested to determine their suitability for use in an ethanol process using switchgrass and/or sorghum bagasse. These yeasts will be tested at temperatures ranging from 40-45 degrees C. These temperatures are closer to the optimal temperatures for cellulase enzymes than temperatures generally used for ethanol fermentation (30-37 degrees C), allowing for faster hydrolysis times and/or lower enzyme loadings. Additionally, non-senescent sorghum, a variety of sorghum that delays senescence after grain production allowing the plant to store sugars such as glucose and sucrose in the stalk, will be grown and harvested on test plots in Bixby, OK. The grain will be harvested and starch in the grain will be hydrolyzed to glucose using alpha-amylase and gluco-amylase enzymes. Also, the stalks will be pressed to express a juice containing glucose, sucrose, and other sugars. Both the hydrolyzed starch and juice will be fermented using Saccharomyces cerevisiae, the K. marxianus strains previously mentioned, and Zymomonas mobilis. Several temperatures and pH values will be tested to evaluate the best microorganism and fermentation conditions for maximum ethanol production.

PROGRESS: 2006/10 TO 2007/09
OUTPUTS: Experiments were conducted to evaluate hydrothermolysis as a pretreatment for preparation of switchgrass for enzymatic hydrolysis. Hydrothermolysis involves heating a slurry of biomass and water under pressure to keep the water in liquid form. Three temperatures (190, 200 and 210 degrees C) and three hold times (10, 15 and 20 minutes) were evaluated. After pretreatment, the remaining insoluble solids were recovered by filtration and hydrolyzed by a cellulase preparation to glucose. A thermotolerant yeast (Kluyveromyces marxianus IMB4) was added at the same time to ferment glucose to ethanol in a process known as simultaneous saccharification and fermentation (SSF). The temperature for SSF was 45 degrees C. In a subsequent experiment, K. marxianus IMB4 was evaluated at 37, 41 and 45 degrees C and compared to a control of Saccharomyces cerevisiae D5A at 37 degrees C. Switchgrass was prepared by hydrothermolysis at 200 degrees C and 10 minutes. SSF was initially conducted at pH 4.8. Due to a premature end to fermentation at pH 4.8 and 45 degrees C, an experiment was also done at initial pH 5.5 using K. marxianus IMB4 at 45 degrees C. K. marxianus IMB4 is of great interest because it can ferment sugars at 45 degrees C, which is an ideal temperature for cellulase hydrolysis. Other yeasts cannot survive at this temperature, so lower temperatures must be used during SSF. This results in a slower hydrolysis and fermentation. Results have been disseminated through a conference presentation and paper to the American Association of Agricultural and Biological Engineers Meeting and through a poster at the Oklahoma GROW Conference. PARTICIPANTS: Collaborators: Danielle D. Bellmer, OSU, Raymond L. Huhnke, OSU, Andrew Mort, OSU, Niels O. Maness, OSU and Ibrahim M. Banat, University of Ulster, United Kingdom. TARGET AUDIENCES: The target audiences are ethanol producers, government officials involved in bioenergy policy, enzyme producers, farmers and ranchers that may be involved in biomass production, undergraduate and graduate students interested in biofuels, and other biofuels researchers. Educational efforts to inform target audiences of the outputs of this project included a poster at the Oklahoma GROW Conference, a gathering of biofuels industry officials, Oklahoma farmers and ranchers, government officials, and academic researchers interested in biofuels. Also, a new course has been implemented at Oklahoma State University on renewable energy with several lectures focusing on activities and subjects related to this project.

IMPACT: 2006/10 TO 2007/09
Hydrothermolysis was determined to be a suitable pretreatment for hydrolysis of cellulose in switchgrass to glucose by cellulase. As temperature and hold time increased, more glucose was released through hydrolysis and more ethanol was produced. The conditions of 200 degrees C and 10 minutes were determined to be the best conditions for ethanol production. Also, K. marxianus IMB4 was found to be a promising candidate for use in SSF of biomass. K. marxianus IMB4 at 41 and 45 degrees C produced more ethanol than the control at 72 hours, but they ceased fermentation after 96 and 72 hours, respectively, while the control continued fermentation for 168 hours. Increasing pH to 5.5 resulted in greater ethanol productivity and fermentation continued for 96 h as opposed to 72 h at pH 4.8. SSF using K. marxianus IMB4 at 45 degrees C resulted in faster cellulose hydrolysis and faster ethanol production as compared to the most commonly used ethanol-producing yeast, S. cerevisiae D5A. The implementation of numerous standard procedures developed for analyzing biomass composition, enzymatic hydrolyses, and fermentations have been implemented as a result of this project. Three graduate students and the primary investigator have been trained in these methods, which has greatly expanded the capability of the biofuels program at Oklahoma State University (OSU). These procedures are commonly used in the biofuels industry and will make students trained during this project more marketable to employers. Also, more experienced graduate students have passed on their knowledge regarding enzymatic hydrolysis techniques and analysis procedures to new students, ensuring continuity of procedures and the ability to compare results over the course of the project.

PUBLICATIONS (not previously reported): 2006/10 TO 2007/09
Suryawati, L., M.R. Wilkins, D.D. Bellmer, R.L. Huhnke, N.O. Maness, and I.M. Banat. 2007. Effect of hydrothermolysis on ethanol yield from Alamo switchgrass using a thermotolerant yeast. Paper No. 077071. 2007 ASABE Annual Meeting. June 17-20, 2007.

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Item No. 47 of 59
ACCESSION NO: 0409037 SUBFILE: CRIS
PROJ NO: 1935-41000-072-00D AGENCY: ARS 1935
PROJ TYPE: USDA INHOUSE PROJ STATUS: NEW
START: 29 OCT 2004 TERM: 28 OCT 2009 FY: 2006

INVESTIGATOR: HICKS K B; TAYLOR F; BOATENG A A; MOREAU R A; MCALOON A J; FISHMAN M L

PERFORMING INSTITUTION:
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ECONOMIC COMPETITIVENESS OF RENEWABLE FUELS DERIVED FROM GRAINS AND RELATED BIOMASS

OBJECTIVES: Lower the cost of fuel ethanol production from corn and barley through improved dry grind and dry milling fractionation techniques, including a new 'ammoniation' process. Develop more efficient processes for converting hulled and hulless barley to fuel ethanol and improved, beta-glucan-free, feed coproducts. Through research assist in creation of a new hulless barley-to-ethanol industry in corn deficient regions, particularly the Mid Atlantic States and the North Western U.S. Use the low-starch ('low carb') and high-fiber, high-oil, and high-protein fractions recovered from corn and barley prior to ethanol fermentation to produce health-promoting food ingredients, functional foods, and extruded snacks. Develop improved processes to convert low valued crop-related biomass, byproducts and energy crops being researched in the ARS energy crop program into renewable hydrogen or liquid fuels and conduct economic feasibility studies for integrating this technology into co-located dry grind ethanol plants. Develop small-scale thermo-chemical technologies that economically, efficiently, and sustainably produce hydrogen and coproducts from agricultural materials.

APPROACH: Develop a continuous corn ammoniator for improving the conversion of corn to fuel ethanol. Conduct research to develop new dry de-germinators, roller mills, and associated fractionation devices with and without 'ammoniation' as a 'front end' to the traditional dry grind ethanol process. Use these techniques on corn and hulled barley to produce high-starch fractions for more efficient fermentations, and low-starch fractions that can be used as value added health-promoting 'low-carb' food ingredients, healthy edible oils and nutraceuticals. Use newly developed hulless barley cultivars and develop new beta-glucan-degrading enzyme technology to reduce fermentation viscosity and improve the production of ethanol from barley. Prepare hulless barley DDGS from beta-glucanase treated fermentations and examine as high-valued feeds for non-ruminants and aquaculture. Low-valued barley hulls and corn bran from corn and barley ethanol processing and energy crops and residues like switch grass, Eastern gama grass, reed canary grass, alfalfa, and corn stover will be studied as substrates for conversion to hydrogen and related liquid and gaseous fuels by thermochemical processes in a pyrolyzer (pyroprobe) coupled to a gas chromatograph/mass-spectrometer (PY-GC/MS) to analyze gasification products. Promising substrates will be converted in a 2-inch bench-top fluidized-bed reactor to test selected feedstock for yields of H2, syngas, char and pyrolytic oil. Process modeling and economic analysis will be conducted on all the technologies studied, to help direct research toward the most fruitful and commercially feasible areas.

PROGRESS: 2005/10 TO 2006/09
Progress Report 1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter? Agriculturally-derived renewable fuels have the potential to provide three major benefits to the United States: Energy independence; Economic opportunities for U.S. farmers and rural businesses; and Environmental improvements by cleaning the air and reducing greenhouse gas emissions. The demand for fuel ethanol in the U.S. is expected to at least double over the next several years due to federal mandates and the phase-out

of Methyl tert-Butyl Ether (MTBE). Technological advances are still needed, however, to enhance the economic competitiveness of domestically produced renewable fuels, such as fuel ethanol, so they can compete in the marketplace with lower-priced fossil fuels. Especially needed are new and improved dry grind ethanol processes for corn that can produce cheaper ethanol while producing revenue-generating coproducts more valuable than traditional Distillers Dried Grains with Solubles (DDGS). Research in this area is critically needed to continue to decrease the cost and the energy required for producing fuel ethanol. There is now a major need for ethanol plants on the East and West Coasts, to satisfy growing local ethanol markets, especially in the North East and in California. Since most of these states are corn deficit, ethanol plants located there would need to either import higher-priced corn from the Midwest or develop an alternative grain more easily grown locally. Groups of East Coast, Pacific Northwest, and Upper Midwest farmers, farmer groups, trade associations, and University small grain breeders have identified barley, and specifically, hulless barley as such a crop. Similar interest in barley is being seen in other corn deficit states around the U.S. Research is needed however, to assist barley breeders in selecting varieties that are optimal for ethanol and coproduct production. Research is also needed to solve technical problems related to milling and fermentation of barley as well as expanding the feed applications for barley DDGS. Finally, major Federal initiatives are in place to create a future Hydrogen Economy. For this effort to be successful, hydrogen should be produced locally from renewable resources through improved gasification processes. The ARS has a growing energy crop program to produce energy crops for various regions of the country. The initial goal was to develop processes to convert these crops to ethanol, through hydrolysis and fermentation. No program on gasification of these crops to form hydrogen, syngas, and liquid fuels is available in ARS. The present research project will fill this void by conducting gasification research on these specific energy crops as well as crop residues and fibrous ethanol plant byproducts. Research leading to cost-effective, integrated fuel ethanol/ biomass hydrogen facilities using grain and energy crops plus crop-derived biomass would greatly benefit the hydrogen initiative. Development of new technology that integrates feedstock pretreatment, biological conversion and product recovery processes, as well as fundamental knowledge regarding fermentation, milling and separations resulting in additional income and reductions in capital and processing costs associated with biofuel production contributes to ARS National Program goals. This project responds directly to the "Ethanol" problem area of NP 307 Action Plan's Component I, Ethanol specifically by developing new Process Efficiencies (Ammoniation process, continuous fermentation, dry mill pre-treatments to remove non-fermentable kernel components, increasing the fermentability and ethanol yields from barley and hulless barley) and by conducting new CoProduct Development (hydrogen, premium high-protein feeds, high-protein, low carbohydrate food ingredients). The project also responds to the Action Plan's Component IV, Energy Crops in which conversion methods need to be further improved to reduce the cost of deriving fuels from biomass (Hydrogen from fibrous grain milling fractions and energy crops) and Crop residues such as corn stalks and wheat [barley] straw for biomass fuel production. Whereas there is no specific mention of hydrogen in the NP 307 Action Plan, National Program Staff gave approval for including hydrogen in NP 307 Research Goals in January 2004. The project also responds directly to NP306 action plan's Problem Area 2b - New Uses for Agricultural By-products and 2c - New and Improved Processes and Products). 2. List by year the currently approved milestones (indicators of research progress) FY 2005 1(a) To begin development of upstream dry grind and dry milling processes with potential to decrease the cost of fuel ethanol production from corn. 1(b) Begin to develop a continuous process to evenly ammoniate corn with potential to lower cost of fuel ethanol production. 1(c) To begin evaluation of the ammoniation of corn as a pretreatment of dry milling processes with potential to decrease the cost of fuel ethanol production from corn. 2(a) To begin development of upstream dry fractionation processes with potential to decrease the cost of fuel ethanol production from barley. 2(b) Begin development of b-glucanase and high-solids fermentation technology as a potential process to lower cost of fuel ethanol from barley 3(b)Begin to characterize phytonutrients in high-oil fractions such as those obtained by scarification with the goal of identifying new valuable coproducts for functional foods and ingredients. 4(a)To begin conducting Pyrolysis-GC/MS Studies to establish the thermo- chemical energy potential of grain milling byproducts and energy crops being developed and studied in the ARS bioenergy program FY 2006 1(a) To continue development and testing of upstream dry grind and dry milling processes with potential to decrease the cost of fuel ethanol production from corn. 1(b) Continue to develop a continuous process to evenly ammoniate corn with potential to lower cost of fuel ethanol production. 1(c) To conclude the evaluation of ammoniation of corn as a pretreatment of dry milling processes with potential to decrease the cost of fuel ethanol production from corn. 1(d) Begin to determine the effects of fractionation and ammoniation on fermentation and coproducts. 1(e) Begin process modeling and economic analysis of fractionation and ammoniation to determine their economic benefit in fuel ethanol production. 2(a) Continue development of upstream dry fractionation processes with potential to decrease the cost of fuel ethanol production from barley. 2(b) Continue development of b-glucanase and high-solids fermentation technology as a potential process to lower cost of fuel ethanol from barley 2(c) Begin process and economic analysis for new barley process: develop base case barley to ethanol model. 3(a) Begin characterization of fractionated streams, from corn and barley, resulting from upstream ethanol processes and combine them to make potential low carb flour replacements and provide to collaborators for evaluations. 3(b) Continue to characterize phytonutrients in high-oil fractions such as those obtained by scarification with the goal of identifying new valuable coproducts for functional foods and ingredients. Identify and provide high-oil samples to potential industry partners. 3(c) To begin evaluation of low carb extruded snacks and related products using low starch corn and barley fractions resulting from "upstream" ethanol processes 3(d) Begin economic analysis for feasibility of products in this objective. 4(a) Continue conducting Pyrolysis -GC/MS Studies to establish the thermo-chemical energy potential of grain milling byproducts and energy crops being developed and studied in the ARS bioenergy program. 4(b) To begin construction of a bench-top gasifier and conduct studies to determine the potential of grain byproducts and energy crops as feedstock for production of syn gas. 4(c) Begin initial process/cost simulation modeling for co-located fuel ethanol and thermochemical conversion plants. FY 2007 1(a) To complete development of "upstream" dry grind and dry milling processes with potential to decrease the cost of fuel ethanol production from corn. 1(b) Complete the development of a continuous process to evenly ammoniate corn with potential to lower cost of fuel ethanol production. 1(d) Continue to determine the effects of fractionation and ammoniation on fermentation and coproducts. 1(e) Continue process modeling and economic analysis of fractionation and ammoniation to determine their economic benefit in fuel ethanol production. 2(a) Complete development of upstream dry fractionation processes with potential to decrease the cost of fuel ethanol production from barley. 2(b) Complete development of b-glucanase and high-solids fermentation technology as a potential process to lower cost of fuel ethanol from barley 2(c) Continue process and economic analysis for barley processes: develop hulless barley to ethanol model. 2(d) Begin Scale up of barley dry fractionation and fermentation processes to prepare quantities for feed studies (if feasibility studies warrant). 3(a) Continue characterization of fractionated streams, from corn and barley, resulting from upstream ethanol processes and combine to make potential low carb flour replacements and provide to collaborators for evaluations. 3b) Complete the characterization of phytonutrients in high-oil fractions such as those obtained by scarification and determine their potential value as coproducts with functional food applications. 3(c) To begin development of potential low carb extruded snacks and related products using low starch corn and barley fractions resulting from upstream ethanol processes. 3(d) Continue economic analysis for feasibility of low carb extruded snacks. 4(a) To complete Pyrolysis-GC/MS studies and establish the thermo- chemical energy potential of grain milling byproducts and energy crops being developed and studied in the ARS bioenergy program. 4(b) To complete construction and testing of the gasifier and begin conducting studies to determine the potential of grain byproducts and energy crops as feedstock for production of syn gas. 4(c) To conduct process/cost simulation modeling for co-located fuel ethanol and thermochemical conversion plants to determine the economic feasibility of direct firing/co-firing of grain byproducts and energy crop biomass in such plants. FY 2008 1(d) Complete determination of the effects of fractionation and ammoniation on fermentation and coproducts. 1(e) Continue process modeling and economic analysis of fractionation and ammoniation to determine their economic benefit in fuel ethanol production 2(c) Continue process and economic analysis for fuel ethanol from barley processes: develop barley to ethanol model modified for feedstock from barley fractionation. 2(d) Continue scale-up barley dry fractionation and fermentation processes to prepare quantities for feed studies (if feasibility studies warrant). 3(a) Conclude low carb flour replacements studies with collaborators and determine their potential as value added coproducts from fuel ethanol plants/processes. 3(c) To continue development of potential low carb extruded snacks and related products using low starch corn and barley fractions resulting from upstream ethanol processes. 3(d) Continue economic analysis for feasibility of low carb extruded snacks. 4(b) To continue to conduct gasification studies to determine the potential of grain byproducts and energy crops as feedstock for production of syn gas. 4(c) To conduct process/cost simulation modeling for co-located fuel ethanol and thermochemical conversion plants to determine the economic feasibility of firing biomass-derived syngas in such plants. FY 2009 1(e) Complete process modeling and economic analysis of fractionation and ammoniation to determine economic benefit for lowering cost of fuel ethanol production. 2(c) Complete process and economic analysis for new barley process: Refine models using raw starch hydrolyzing enzymes. 2(d) Complete feed studies with collaborators and determine the potential for barley fractions and DDGS in animal feeds 3(c) To conclude development of potential low carb extruded snacks and related products using low starch corn and barley fractions resulting from upstream ethanol processes. 3(d) Complete economic analysis for feasibility of low carb extruded snacks. 4(b) To complete gasification studies to determine the potential of grain byproducts and energy crops as feedstock for production of syn gas. 4(c) To complete process/cost simulation modeling for co-located fuel ethanol and thermochemical conversion plants with the determination of the economic feasibility of firing biomass-derived bio-oil in such plants. 4a List the single most significant research accomplishment during FY 2006. Discovery of Key Enzymes to Boost Fuel Ethanol Production from Barley. New feedstocks for fuel ethanol production are needed. Currently 95% of fuel ethanol is made from corn and continued growth of the ethanol market could create shortages of corn for food and feed uses. Barley could be a potential alternative feedstock if problems with its high viscosity and low ethanol yields could be solved. Our research has found a new combination of enzymes (beta-glucanases and beta-glucosidases) that has the potential to solve both problems of viscosity and yield. The key discovery was the use of beta-glucosidase enzymes that are currently missing from commercial enzymes for barley conversion to ethanol. Development of new commercial enzyme preparations using this new knowledge will greatly benefit the small, but growing barley-to-fuel ethanol industry, which will allow building of ethanol plants outside the corn belt, in the barley belts of the East Coast, Northwest, and Upper Midwest and produce an additional 1-2 billion gallons of fuel ethanol for the US market. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol. 4b List other significant research accomplishment(s), if any. Tocotrienols in Barley Kernel Oil and Barley Fines Oil. The outer layers of the barley kernel are low in starch and high in oil. Experiments were conducted using a commercial scarifier to abrade the outer layers of barley cultivars and evaluate the yields and composition of the oil in these layers. Abrasion for one minute removed 12 to 15% of the mass from the four barley cultivars evaluated (two common hulled cultivars and two new hulless cultivars). The amount of oil in the abraded "fines" fractions was 3-5% in the two hulled barely cultivars and 7-9% in the two hulless barley cultivars. The levels of total phytosterols (natural cholesterol lowering substances) in barley oils obtained by extracting these fines fractions were about 6-9% for the hulled cultivars and 2-5% for the hulless cultivars. Previously we reported that the levels of vitamin E components (tocopherols and tocotrienols) were high in all of the oils. During the last year we have discovered that the levels of tocotrienols in the barley oils are the highest ever reported for any natural plant oil. Previously palm oil and rice bran oil were considered to have the highest levels of tocotrienols, but barley kernel oil and barley fines oil now appear to contain higher levels of tocotrienols. This information may lead to the development of new nutraceutical oil products from barley. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol Coproduct Development. Barley Hulls as a Feedstock for "Cellulosic" Ethanol. Most barley contains a hard and abrasive hull that makes up 10-15% of the weight of the kernel. When hulled barley is used to make fuel ethanol, the non- fermentable hull takes up valuable space in the fermentor, making the process inefficient. We developed a process to remove the hull from the barley, prior to grinding and producing fuel ethanol from the starchy portion of the kernel. The hull, which is rich in hemicellulose and cellulose, was then used to make "cellulosic ethanol" by pre-treatment with aqueous ammonia and then digestion with cellulases, followed by fermentation with a hexose and pentose-fermenting microorganism. The results of these studies showed that a 40 million gallon per year barley ethanol plant could increase ethanol production by 10-15% (4-6 million gallons) by converting the hull polysaccharides to additional "cellulosic" ethanol. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol Process Efficiencies. Combustion of Barley Hulls to Generate Process Steam for a Barley Ethanol Plant. In most barley ethanol plants, the hulls wind up being part of the ethanol coproduct, Distillers Dried Grains with Solubles (DDGS) that sells for less than $70/ton as ruminant animal feed ingredients. With the rising price of natural gas that is used to produce process steam in most ethanol plants, new sources of fuel are required. In this study, barley hulls were pyrolyzed in a special pyrolysis unit and the condensable and non-condensable gases and char produced were measured under different treatment conditions. Further thermochemical analyses of the hulls were carried out that determined hulls have an average calorific value of about 19MJ/kg (similar to tree bark). The information was placed into ERRC's SuperPro Process and Cost Simulator for a 40 MGY barley ethanol plant, and it was determined that use of barley hulls in a fluidized bed combustor could replace about 40% of the natural gas usage. This information will be of value to ethanol plant designers and builders as it will provide them a design option that would result in use of less fossil- and more renewable-fuels to provide plant energy. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol Process Efficiencies. New Shake-Flask Method Helps to Study Production of Fuel Ethanol from Grains. Studying new fermentation methods to increase process efficiency can be a long and tedious process if fermentation experiments are conducted one at a time, using a traditional 72-hour fermentation in a mid-sized lab fermentor. We developed a shake-flask method to evaluate the fermentation characteristics of corn. The method permits an accurate determination of ethanol yield after 50 hours of fermentation time, while using only 50 g of corn mash for each flask. Scores of shake-flasks can be incubated simultaneously, allowing rapid throughput of samples and faster and more accurate results. This method will be used by our researchers to save time and money during the early stages of ethanol fermentation research projects. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol Process Efficiencies. 4d Progress report. "Scarification" of Barley for Improved Fuel Ethanol and CoProduct Production. Scarification is a process that sequentially grinds off the outer surface (bran) of grain kernels as the kernels are tumbled on an abrasive surface. Based on preliminary results obtained from a limited scarification study initiated in 2004, a complete experiment was conducted to use scarification to produce fiber/oil rich and starch-rich fractions from hulless and hulled barley using three abrasive surfaces. Four barleys varieties, two hulled (Nomini and Thoroughbred) and two hulless (Doyce and Merlin) were scarified using three abrasive surfaces. All the fractions obtained at different degrees of abrasion are being evaluated to determine the chemical and physical composition of the fractions. The oil/fiber rich fractions are being characterized for potential nutraceutical coproduct applications while the starch-rich fractions will be considered for high-yielding fuel ethanol feedstock. The results of these studies will determine if scarification is a more profitable process that should be considered for future fuel ethanol plants using barley feedstock. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol Process Efficiencies and Coproducts. Roller Mill Flow Designed for Dehulling Barley May Produce Ethanol Feedstock, Value-Added Food Ingredients, and Energy to Run Ethanol Plants. The presence of a very hard and abrasive hull on most barley varieties is a major problem for using this grain in fuel ethanol production. A roller mill flow consisting of different roll passes followed by fractionation based on particle size and particle density was designed as a way to remove the barley hull and produce useful fractions. This flow is capable of separating the hulls of the hulled barley from the fractions rich in pericarp (fiber) and endosperm (starch). The fractions rich in endosperm will be used for fermentation in ethanol production and the ones rich in pericarp will be used in future studies for "whole grain" food product development. The hull fractions are used in gasification studies. If this process is successful, a roller milling process could lower the cost of fuel ethanol from barley by providing high-starch feedstock needed for high yields of ethanol, fiber and nutrient rich coproducts for nutritious foods, and hulls that could be used in a co-located fluidized bed gasifier or combustor, that could produce much of the process steam needed to run the ethanol plant. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol Process Efficiencies and Coproducts. Pyrolysis and Gasification of Energy Crops Yield Liquid and Gaseous Fuels. Pyrolysis studies were conducted on three herbaceous grasses and alfalfa stems all classified as energy crops in the national biomass initiative program. These experiments were carried out to establish their thermochemical conversion potential as a viable alternative to fermentation of energy crops to produce ethanol. While fermentation remains a popular biomass-to-biofuels conversion method, the thermochemical conversion e.g., pyrolysis to bio-oils, gasification, to syngas (CO, Hydrogen, Methane, etc.) and co-products may present a nearer term economic viability, if this can be experimentally verified. For this reason, the first ARS program on thermochemical conversion of biomass was initiated at the ERRC. Through this, pyrolysis studies employing a Pyroprobe-GC/MS setup were conducted on lowland ecotype Cave-in-Rock switchgrass (Panicum virgatum), reed canarygrass (Phalaris arundinacea), herbaceous C3 cool-season grass, eastern gamagrass (Tripsacum dactyloides) a warm-season herbaceous grass and alfalfa stems (Medicago sativa L.) All herbaceous grasses and alfalfa produced useful syngas during pyrolysis and their energy potentials were correlated with their maturity at harvest. In related research, ERRC is conducting collaborative studies with the USDA-ARS National Forage Seed Production Center in Corvallis, OR and the Western Research Institute to study a new dual-stage gasification concept to convert grass straw and other agricultural residues into syngas and other value-added energy products on a scale suitable for on- farm use. The results of these studies will be used to determine the economic viability of using pyrolysis and gasification processes to produce gaseous and liquid fuels for transportation and on-farm needs. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol Process Efficiencies and Coproducts. The following section serves to document research conducted under a CRADA between ARS and Archer Daniels Midland (ADM) Corporation. Additional details of research can be found in the report for the subordinate CRIS 1935-41000-072-O2T, "Biomass Research and Development for the Production of Fuels, Chemicals and Improved Cattle Feeds". The CRADA funding originally derived from a 2003 USDA-DOE Biomass R&D Grant that was co-written by ADM and ERRC. ADM was the submitting organization. The project builds on corn ammoniation and continuous fermentation and ethanol stripping technology that was developed under the parent CRIS. This technology will be combined with other new technologies to enhance the profitability of ethanol production by the dry grind process. Progress to date at ERRC includes: 1. Continuation of the research plan for ERRC that includes development of a continuous corn ammoniator, a new analytical method to measure ammonia levels in treated corn, new dry milling technologies for processing ammoniated and non-ammoniated corn for ethanol production, evaluating fermentability for ammoniated and non-ammoniated corn, developing more efficient fermentation processes, and conducting process and cost analysis to quantify the benefits (if any) for using ammoniation and new fermentation techniques in the corn to ethanol process. 2. Held regularly scheduled conference calls to coordinate research between two ADM locations and ERRC. Will hold quarterly meeting in fall of 2006 in Urbana-Champaign, Illinois. 3. Used the ERRC continuous ammoniator to produce 400 lb (7 bushel) and 800 lb (14 bushel) quantities of corn at two different ammoniation levels, 400 ppm and 800 ppm for evaluation of efficiency and consistency of treatment and fine-tuning its operation. 4. Updated the ARS 40 million gallon/year dry grind ethanol process and cost computer simulation model and provided this research tool to the ADM cooperators. Also provided models for dry pre-fractionation of corn, and for continuous fermentation with stripping, derived from the dry-grind base case. 5. Experiments were conducted and completed to show the effect of soaking in aqueous ammonia (SAA) on the digestibility of cellulose and hemicellulose in barley hulls. 6. Experiments were conducted to determine the effect of ammoniation on dry separations of dry-milled corn. 7. Developed a shake-flask method to evaluate the fermentation characteristics of ammoniated and non-ammoniated corn. The method permits an accurate determination of ethanol yield after 50 hours of fermentation time, while using only 50 g of corn mash for each flask 5. Describe the major accomplishments to date and their predicted or actual impact. Researchers Built First Continuous, Pilot Plant-Scale Corn Kernel Ammoniation Device. Exposing whole corn to anhydrous (gaseous) ammonia may loosen the hull and help to separate it from the kernel prior to fuel ethanol production, a process that could lead to cheaper fuel ethanol production. To test this hypothesis, a method was needed to safely, uniformly and reproducibly treat whole corn with ammonia. It was necessary to control the amount of ammonia and time of exposure. Batch methods were not suitable, because hot spots form and the treatment is uneven. To solve this problem, a continuous flow ammoniator was designed, built and tested. The device was shown to reproducibly and safely distribute up to 1000 ppm of anhydrous ammonia into 2.5 kg/min of dry corn. Distribution of ammonia in corn was measured by assaying the ammonia content of individual corn kernels. Distribution was found to be much more uniform than in a bench-scale batch ammoniator. The idea of using ammonia to help remove the hull from corn was previously patented by ARS. The continuous ammoniator will enable research both here at ERRC and with A CRADA partner to determine the practical utility of the patent and of the technology. If the idea is found to be practical, the device may serve as a prototype, leading to industrial application of the technology to lower the cost of fuel ethanol from corn. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol Coproduct Development and Process Efficiencies. Development of the ERRC Grain Processing Research Pilot Plant. One way to lower the cost of fuel ethanol from grain is to remove non-fermentable components from corn kernels prior to fermentation to ethanol. There was a major need for a grain milling research facility that contained bench and pilot scale grain fractionation equipment to test this hypothesis. In 2004, a new Grain Processing Research Pilot Plant, unique to ARS was opened at ERRC. To expand the capability of this facility, new particle reduction and particle separation equipment was added in FY 2005. This new equipment includes four new roller mill stands and two additional sets of rolls that provide a wide range of applications in dry milling of barley and corn. Particle separation equipment includes a micron air jet separator, rotary sifter and air aspiration units. In addition, a whitener unit and a color analyses unit complete the equipment necessary to conduct fractionation studies and evaluation of milling alternatives for new barley varieties. This new facility provides unique resources that will enable ARS researchers to lower the cost of fuel ethanol through grain fractionation and fermentation research. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol Coproduct Development and Process Efficiencies. Experimental Milling Techniques May Improve Barley as a Fuel Ethanol Feedstock. In research designed to facilitate the use of barley as a feedstock for fuel ethanol production in "corn deficit states", four different experimental milling processing units designed for wheat flour production were tested with three barley varieties. This evaluation was used to determine the performance of the experimental mills and the barley varieties in the "upstream" (prior to fermentation) fractionation of barley for ethanol production. The milled streams were fermented and preliminary ethanol production characteristics determined. The chemical composition and particle size distribution of the streams produced had a major effect on fermentation rates, with some fractions fermenting 100% faster than others. The experimental milling results were used to select the most appropriate grinding equipment purchased for the ERRC Grain Processing Plant and for planning additional experiments on the small and pilot scale to fully understand the full potential of these techniques to improve fuel ethanol production from barley. This work supports the ARS National Program 307, Bioenergy and Energy Alternatives, Component 1, Ethanol Coproduct

Development and Process Efficiencies. 6. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end- user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products? Unit scientists and engineers held a meeting with Novozymes to transfer new information about barley fermentation. Novozymes has provided enzymes to ARS for this purpose. December 2005. A sophisticated computer simulation and cost model for 40 million gallons per year fuel ethanol from dry-grind corn that was developed in our Unit, was provided to approximately 100 requestors during this reporting period. These included academic researchers, private consultants and small and large companies involved in or seeking to become involved in the fuel ethanol industry including the USDA office of the Chief Economist, US Environmental Protection Agency, the National Corn to Ethanol Research Center, the Nebraska Ethanol Board, ADM, Dupont, Batelle Institute, Monsanto, Genencor , Novozymes, Booze Allen & Hamilton, Chevron, Cornel University, Lafayette College, Monsanto, Purdue University, Southern Illinois University, the Wharton School at the University of Pennsylvania, the University of Minnesota, the University of Illinois, Washington University and the University of Nebraska. Access to this model has helped the recipients with understanding the costs, energy, inputs and outputs of the ethanol process. They have used it for modeling, design and business decisions, and thus helped to improve the cost-effectiveness of fuel ethanol production from corn. The model was used by researchers in the USDA's office of the Chief Economist and many other groups across the US to study the net energy balance for ethanol. January 2006 to present. A rigorous computer simulation model for a corn wet milling plant was developed in our Unit and it represents the only one of its kind in the public domain. Nineteen copies of the corn wet milling model have been requested and distributed to various organizations and individuals. These organizations included other ARS research centers, the US Environmental Protection Agency, Booze Allen & Hamilton, Corn Products, Lafayette University, the University of California, Purdue University the Wharton School at the University of Pennsylvania. Requestors used this model to understand the corn wet milling process, to understand the inputs and outputs, capital and operating costs and construction costs. January 2006- present A Unit researcher worked through a specific cooperative agreement to transfer engineering information and assist in the design and construction of a farm-scale switchgrass gasifier in Ligonier PA. The unit was constructed and was tested. It purpose is to gasify switchgrass and generate electricity using the gas produced. It is now available for research and development by the owner and other interested parties. January to May 2006. Unit scientists and engineers held a meeting with Genencor International to begin a new CRADA on fuel ethanol production from barley. Unit scientist and engineers transferred new computer cost and process models on the fuel ethanol process and new information on unique enzymes that have potential to increase the yield of fuel ethanol from barley, an alternative to corn that will play a major role in fuel ethanol production as corn feedstocks begin to tighten around the country due to unprecedented growth in ethanol production. Genencor will provide new enzymes and processing technologies. During the term of the CRADA new technology for barley fermentation will be developed and transferred to the ethanol industry by ARS and Genencor. June 2006. Unit scientists and engineers met with approximately 25 different groups during the year to transfer information on fuel ethanol production technologies, economics, energy balance, feedstocks, and other technical matters related to ethanol production from grains and cellulosic feedstocks. January 2006 present 7. List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below). Hicks, K.B., Stewardship of the Earth Through Renewable Fuels. Union League of Philadelphia, November, 2005. Hicks, K.B., Flores, R.A, Kurantz, M. Taylor, F., Johnston, D., Moreau, R., Wilson, J., Kohout, K., and McAloon, A.J. 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Ramirez, E., and Singh, V. Corn Wet Fractionation Prior to Fermentation for Recovery of Valuable Co-Products. Fifth Corn Utilization and Technology Conference, Dallas Texas, June 2006. McAloon, Andrew, Modeling the Wet Milling Process. Fifth Corn Utilization and Technology Conference, Dallas Texas, June 2006 Burkdoll, S., Researcher shares ways to improve barley feasibility in ethanol production. The Prairie Star. Wednesday, July 5, 2006. http://www. theprairiestar.com/articles/2006/07/05/ag news/farm and field/farm12.txt

PUBLICATIONS (not previously reported): 2005/10 TO 2006/09
No publications reported this period.

Item No. 48 of 59
ACCESSION NO: 0407996 SUBFILE: CRIS
PROJ NO: 1935-41000-072-02T AGENCY: ARS 1935
PROJ TYPE: USDA INHOUSE PROJ STATUS: NEW
START: 01 MAY 2004 TERM: 30 APR 2007

INVESTIGATOR: TAYLOR F

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BIOMASS RESEARCH AND DEVELOPMENT FOR THE PRODUCTION OF FUELS, CHEMICALS, AND IMPROVED CATTLE FEED

OBJECTIVES: Lower the cost of fuel alcohol through innovative engineering process development.

APPROACH: Treat whole corn kernels with anhydrous ammonia in a special continuous reactor to improve processing and conversion of the grain to fuel ethanol and valuable coproducts.

PROGRESS: 2005/10 TO 2006/09
Progress Report 4d Progress report. This report serves to document research conducted under a CRADA between ARS and the Archer Daniels Midland Corporation. Additional details of research can be found in the report for the parent CRIS 1935-41000-072- OOT, "Biomass Research and Development for the Production of Fuels, Chemicals and Improved Cattle Feeds". The CRADA funding originally derived from a 2003 USDA-DOE Biomass R&D Grant that was co-written by ADM and ERRC. ADM was the submitting organization. The project builds on corn ammoniation and continuous fermentation and ethanol stripping technology that was developed under the parent CRIS. This technology will be combined with other new technologies to enhance the profitability of ethanol production by the dry grind process. Progress to date at ERRC includes: 1. Continuation of the research plan for ERRC that includes development of a continuous corn ammoniator, a new analytical method to measure ammonia levels in treated corn, new dry milling technologies for processing ammoniated and non-ammoniated corn for ethanol production, evaluating fermentability for ammoniated and non-ammoniated corn, developing more efficient fermentation processes, and conducting process and cost analysis to quantify the benefits (if any) for using ammoniation and new fermentation techniques in the corn to ethanol process. 2. Held regularly scheduled conference calls to coordinate research between two ADM locations and ERRC. Will hold quarterly meeting in fall of 2006 in Urbana-Champaign, Illinois. 3. Used the ERRC continuous ammoniator to produce 400 lb (7 bushel) and 800 lb (14 bushel) quantities of corn at two different ammoniation levels, 400 ppm and 800 ppm for evaluation of efficiency and consistency of treatment and fine-tuning its operation. 4. Updated the ARS 40 million gallon/year dry grind ethanol process and cost computer simulation model and provided this research tool to the ADM cooperators. Also provided models for dry pre-fractionation of corn, and for continuous fermentation with stripping, derived from the dry-grind base case. 5. Experiments were conducted and completed to show the effect of soaking in aqueous ammonia (SAA) on the digestibility of cellulose and hemicellulose in barley hulls. At optimum conditions, pretreatment by SAA resulted in 83% digestibility of cellulose, and 63% digestibility of xylan. 6. Developed a shake-flask method to evaluate the fermentation characteristics of ammoniated and non-ammoniated corn. The method permits an accurate determination of ethanol yield after 50 hours of fermentation time, while using only 50 g of corn mash for each flask.

PUBLICATIONS (not previously reported): 2005/10 TO 2006/09
No publications reported this period.

Item No. 49 of 59
ACCESSION NO: 0200354 SUBFILE: CRIS
PROJ NO: SD00134-G AGENCY: CSREES SD.
PROJ TYPE: SPECIAL GRANT PROJ STATUS: EXTENDED
CONTRACT/GRANT/AGREEMENT NO: 2004-34474-15053 PROPOSAL NO: 2004-06070
START: 01 SEP 2004 TERM: 30 APR 2007 FY: 2006 GRANT YR: 2004
GRANT AMT: $626,826

INVESTIGATOR: Kephart, K. D.

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PLANNING THE SUN GRANT INITIATIVE

NON-TECHNICAL SUMMARY: The Sun Grant Initiative is a plan to implement land grant research, Extension, and teaching programs in renewable energy and biobased products. This project will focus on developing an administrative structure for the Sun Grant Initiative. The participating universities will organize planning conferences and summarize input to develop a planning report.

OBJECTIVES: The objective of this project will be to define priorities and conduct public education in regard to the Sun Grant Initiative. The mission of the Sun Grant Initiative will be to `(1) to enhance national energy security through the development, distribution, and implementation of biobased energy technologies; (2) to promote diversification in, and the environmental sustainability of, agricultural production in the United States through biobased energy and product technologies; (3) to promote economic diversification in rural areas of the United States through biobased energy and product technologies; and (4) to enhance the efficiency of bioenergy and biomass research and development programs through improved coordination and collaboration between the Department of Agriculture, the Department of Energy, and the land-grant colleges and universities.

APPROACH: There are three primary goals of this planning period. The first is to activate land grant faculty for roadmapping and for analysis of other domestic and international efforts. Land grant faculty, administrators, and stakeholders will assess previous roadmapping efforts that have been conducted by various federal agencies, the Experiment Station Committee on Organization and Policy (ESCOP), and others. The regional roadmapping committees will determine how activities supported by the Sun Grant Initiative complement these roadmaps and how the Sun Grant Initiative can initiate appropriate activities. Sun Grant Initiative centers will initiate national and regional policy analysis efforts. The policy topics will include: Assist USDA in certification of biobased products and Federal procurement (Economic diversification); Pick up feedstock development questions left unanswered by previous DOE effort (National energy security); Interaction with Federal price supports (Economic diversification); Determine the net positive and negative externalities, assign dollar values to energy security, climate impacts, offset of imported petroleum (Economic diversification and Sustainability); Assess the status of biobased technology development and implementation (Systems and collaboration). The second goal is to develop a broad educational program on the Sun Grant Initiative. The planning activities held during 2002 and 2003 brought widespread attention to the Sun Grant Initiative within the land grant community. A common conclusion from the regions was that outreach to stakeholders that are outside of the routine land-grant community is needed. Examples of these stakeholders groups are urban energy consumers, national and state decision makers, and the energy industry. The third goal is to activate regional Sun Grant advisory boards. During 2002, every region recognized the need to have multistate involvement in directing Sun Grant activities. These boards will provide guidance and feedback for roadmapping and public education efforts. Each of the five center institutions will be responsible for establishing their advisory boards. The regions will conduct their own roadmapping efforts; however, there will also be an effort to coalesce these activities into a single vision. Public education activities will have a national component and regional components.

PROGRESS: 2006/01 TO 2006/12
The regional Sun Grant Centers continued to engage the Land-Grant Universities in their respective regions by providing update reports at various regional and national meetings which included the 2006 Pacific Rim Summit on Industrial Biotechnology and Bioenergy. Each has also developed and maintained a Regional Sun Grant web page. All of the centers' web pages can be accessed through a central portal at www.sungrant.org . The five Centers have kept in close communication via teleconferences and semi-annual meeting in Washington DC. The South Eastern and North Central regions collaborated with the US Department of Energy, US Department of Agriculture, and respective governor's associations to host regional biomass biofuel feedstock workshops. The overall goal of these workshops was to help resolve the potential contribution each region could make to provide the 1 billion ton supply of cellulosic material necessary to achieve the nation's 30 X 30 renewable fuels goal outline in President Bush's 2006 State of the Union Address. The outcomes of these workshops is available at http://www.feedstockpartnership.biomass.govtools.us/. The other three Sun Grant Regions are planning their respective biomass biofuel workshops for late summer/fall 2007.

IMPACT: 2006/01 TO 2006/12
The Sun Grant Initative is linking Land Grant Institutions across the nation to develop teaching, research, and outreach programs in biofuels and bioproducts to help America decrease its dependency on imported petroleum.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
No publications reported this period

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Item No. 50 of 59
ACCESSION NO: 0194763 SUBFILE: CRIS
PROJ NO: SD00392-R AGENCY: CSREES SD.
PROJ TYPE: HATCH PROJ STATUS: NEW MULTISTATE PROJ NO: S-1007
START: 01 OCT 2002 TERM: 30 SEP 2007

INVESTIGATOR: Gibbons, W. R.; Julson, J. L.

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THE SCIENCE AND ENGINEERING FOR A BIOBASED INDUSTRY AND ECONOMY

NON-TECHNICAL SUMMARY: Agriculture and many other facets of the U.S. economy are highly dependent on a low cost, abundant source of fossil fuels. This has placed our economy in a very vulnerable situation. This project will utilize the Land Grant University System to develop technologies that can "wean" us from use of fossil fuels. This will involve technology development, outreach to the private sector, and education/training of students to develop the manpower needed to implement this technology.

OBJECTIVES: 3. Develop, evaluate, and optimize integrated processes to convert biomass resources into biomaterials with commercial applications.

APPROACH: SD scientists will evaluate the mechanical properties (strength, elasticity, flexibility, etc) of microbial exopolysaccharides (gellan, scleroglucan, and polyhydroxyalkonoate) produced by fermentation of corn byproducts. SD scientists will also evaluate the use of C-4 warm season native grasses (big buestem, switchgrass, etc) soybean hulls, and other natural fibers as reinforcing fiber sources for use in blended plastic composites.

PROGRESS: 2006/01 TO 2006/12
The 2006 annual meeting of S1007 was held in Minneapolis, MN (Sept). Kasi Muthukumarappan reported on research being conducted at SDSU to convert corn ethanol byproducts into valuable chemicals. We studied the effect of conventional extrusion processing parameters (temperatures of 25, 100 C and screw speeds of 200 and 400 rpm) on DDGS digestibility. Moisture content adjusted DDGS samples (15, 20, 25, 30, 40% wet basis) were extruded using a Twin-Screw conventional extruder with a barrel length to diameter ratio of 30:1 and compression ratio of 3:1. The amount of glucose in the extruded DDGS was measured by HPLC. We found no significant difference in the glucose recovery of DDGS when the moisture content was increased from 15 to 40% wb. However, we observed higher glucose recovery of DDGS samples when extruded at 200 rpm compared to 400 rpm, primarily due to higher reaction time at 200 rpm. Moreover, we were able to achieve about 33% glucose recovery when the fine ground DDGS were extruded compared to coarse ground (16%) DDGS. In general, we observed higher glucose recovery when the samples were extruded at 100 C compared to 25 C. Between single-screw extruder and twin-screw extruder studies we didn't observe any significant differences in glucose recovery of DDGS. Research on production of citric acid from Aspergillus niger and fumaric acid from Rhizopus oryzae used untreated and acid-pretreated DDGS as substrates. Fungal strains were grown in shake flask cultures via solid-state fermentation at 25oC for 10 days. A. niger ATCC strains 9029, 9142, 10577, 11414, 12846, 26550 and 201122 were investigated for citric acid production. Strain ATCC 9142 produced the highest citric acid level (10.6 g/Kg) on untreated wet corn distillers' grains after 10 days of growth, while ATCC 11414 and ATCC 12846 produced slightly lower citric acid levels (7 g/Kg). We found found that R. oryzae ATCC 20344 was capable of producing 3 g/Kg fumaric acid on the untreated or treated grains after 10 days of growth. Another research project aims to produce short chain length polyhydroxyalkanoates (scl-PHA) from cellulosic feedstocks. We determined that the best growth medium for R. eutropha consists of 240 g/l CCS, supplemented with nitrogen to a 50:1, carbon to nitrogen ratio, with continual pH adjustment. This resulted in a growth rate of 0.25h-1 and a generation time of 2.77 h. Trials to evaluate how volatile fatty acids normally present in CCS (succinic, propionic, acetic, and lactic) were utilized by R. eutropha showed complete utilization. Even though it was present in the highest amount (1.5-2.5 g/L), lactic acid was utilized at the fastest rate (consumed by 12-18 h). Succinic acid was initially present at 0.7 g/L and was also consumed by 12-18 h. Acetic acid took slightly longer for complete metabolism (18-20 h), while propionic acid took up to 48-54 h for utilization. This data suggested the preference order for VFAs was lactic, succinic, acetic, and propionic.

IMPACT: 2006/01 TO 2006/12
Research to develop a biobased economy will require collaboration across disciplines and institutions. S1007 is a multi-institutional organization that is using a multidiciplinary approach to develop fuels, chemicals, and biomaterials from renewable resources.

PUBLICATIONS (not previously reported): 2006/01 TO 2006/12
1. Xie, G. and T. P. West. 2006. Citric acid production by Aspergillus niger on wet corn distillers grains. Lett. Appl. Microbiol. 43:269-273.
2. Xie, G. and T. P. West. 2006. Comparison of citric acid production by Aspergillus niger ATCC 9029 and ATCC 12846 on corn distillers grains with solubles. Res. J. Microbiol. 1:540-545.
3. West, T. P., and B. V. Reed. 2006. Effect of treatment of corn distillers grains with solubles on fungal fumaric acid production. Abstracts of the 2006 Society for Industrial Microbiology Annual Meeting, Abstract P76A, page 182.

PROJECT CONTACT:

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Item No. 51 of 59
ACCESSION NO: 0212915 SUBFILE: CRIS
PROJ NO: SD00H238-07IHG AGENCY: CSREES SD.
PROJ TYPE: HATCH PROJ STATUS: PENDING NEW
START: 01 OCT 2007 TERM: 30 SEP 2008

INVESTIGATOR: Rupp, S. P.; Jensen, K. C.; Boe, A.; VanDerSluis, E.

PERFORMING INSTITUTION:
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DEVELOPING SUSTAINABLE HARVEST STRATEGIES FOR CELLULOSE-BASED BIOFUELS: THE EFFECT OF INTENSITY AND SEASON OF HARVEST ON WILDLIFE AND BIOMA

NON-TECHNICAL SUMMARY: Use of native grasses for biofuel production may negatively affect wildlife populations. Season and intensity of harvest need to be considered. This project examines how harvest intensity and timing affect both wildlife and biomass production. An full economic analysis will also be completed.

OBJECTIVES: In his 2006 State of the Union Address, President Bush espoused the goal of making cellulosic ethanol practical and competitive within six years. Government incentive programs, such as the Conservation Reserve Program (CRP), were initially established to diminish soil erosion and improve water quality on highly erodible lands, but have also been considered for use as cellulose-based biofuels. Because 23% of CRP is in the Northern Great Plains, even small, incremental reductions in wildlife benefits due to misdirected policies may have wide-ranging impacts on wildlife populations. As a result, developing appropriate harvesting strategies which consider maximizing both yield and wildlife use of bioenergy stands has been identified as a research priority if native warm-season perennial grasses are to significantly expand as energy crops. Therefore, the objectives of this research are to 1) determine which combination(s) of stubble height and season of harvest are most beneficial for maximizing biomass production/quality in mixed-grass native grasslands, 2) determine which combination(s) of stubble height and season of harvest are most beneficial for maximizing nesting success/diversity of economically important bird species in eastern South Dakota, and 3) provide an economic analysis of the trade-offs associated with various combinations of biomass and wildlife production based on results obtained.

APPROACH: A series of study sites on South Dakota Game Production Areas, Waterfowl Production Areas, and/or private lands in eastern South Dakota will be identified. Data will be analyzed either with (a) a two-factor factorial randomized complete block design, (b) a completely randomized design or a randomized block design with a split plot arrangement, or (c) a completely randomized design with a factorial arrangement of stubble height and season of growth, to determine the effects of treatment application on bird species diversity and nesting success of pheasants and waterfowl over 6 replicates. Harvest intensity will be assessed at 3 levels (10-cm stubble, 30-cm stubble, and a control) and season at two different times of the year (fall and spring) over the course of two years. Tiller density, biomass production, species composition, aerial partitioning of the biomass, and effect of aerial position on chemical composition of the biomass will be analyzed. Mineral, lignin, and carbohydrate analyses will be completed at South Dakota State University. Potential ethanol yield will be calculated using U.S. Department of Energy protocols. Study sites will be searched for pheasants and ducks by chain-dragging. Data recorded will include species, clutch size, age of clutch, dominant plant species, and vegetation height/density. Microclimate measurements will be taken. Hatching date will be estimated by using the egg flotation method for pheasants or candling for all other species. Nests will be revisited at least once per week to determine fate between 23 June and 24 July. A generalized linear mixed model with a binomial link function will be used to analyze nest success. Diversity will be measured using Simpsons and

Shannon-Weiner indices. Yield and quality data will be analyzed using a mixed model analysis of variance. Variance assumptions will be tested using a Mauchly or Hartley test. If sphericity is violated, a univariate F-statistic with the Greenhouse and Geisser adjustment will be used. Pairwise comparisons will performed with an LSD test using error terms specific to each contrast. Nest success data will be analyzed using Program MARK and compared among treatments utilizing Program CONTRAST. An analysis of the economic trade-offs associated with various intensities and timing of biomass harvest and utilization will be conducted. Besides analyzing the benefits associated with alternative biomass harvesting regimes and the costs of producing, collecting, storing, and transporting the biomass to energy production facilities, the economic analysis also includes providing estimates of the costs involved with foregone revenues due to the production and presence of wildlife. Additional elements included in the total economic costs involved with biomass harvest include estimating soil productivity losses due to nutrient and organic matter losses and as a result of soil compaction. The framework and projections of this research will provide a basis for policy makers to develop appropriate policies related to energy production from biomass.

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Item No. 52 of 59
ACCESSION NO: 0197017 SUBFILE: CRIS
PROJ NO: TEN02003-02985 AGENCY: CSREES TEN
PROJ TYPE: NRI COMPETITIVE GRANT PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2003-35400-13860 PROPOSAL NO: 2003-02985
START: 15 AUG 2003 TERM: 31 AUG 2006 FY: 2006 GRANT YR: 2003
GRANT AMT: $136,000

INVESTIGATOR: English, B. C.; De La Torre Ugarte, D. G.

PERFORMING INSTITUTION:
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ECONOMIC IMPACTS FROM INCREASED COMPETING DEMANDS FOR AGRICULTURAL FEEDSTOCKS TO PRODUCE BIOENERGY & BIOPRODUCTS

NON-TECHNICAL SUMMARY: We propose to develop expansion curves for the growth of the bioenergy and bioproducts industries by estimating a national bioenergy and bioproducts demand for agricultural feedstock, the agricultural resources demanded and the price and income impacts on the agricultural sector.

OBJECTIVES: The overall objective of this proposed project is to develop a national bioenergy and bioproduct expansion curve. As bioenergy and bioproduct production increases, demand for, and price of agricultural products will increase. This analysis will quantify these expected increases considering various demand quantities of bioenergy and bioproducts.

APPROACH: Develop national ethanol, biodiesel, electricity, and bioproduct demand quantities and incorporate them into a dynamic agricultural sector model (POLYSYS). Estimate regional feedstock supply curves necessary to meet national bioenergy and bioproduct demand quantities by modifying a dynamic agricultural sector model (POLYSYS) to include cellulose feedstock in addition to existing agricultural grain and oilseed crops. Develop regional bioenergy and bioproduct supply curves using regional feedstock supply curves, representative transportation costs, and representative technical and economic cost estimates for each feedstock-technology-product combination considered. Construct a national expansion curve for the bioenergy and bioproduct industry. Evaluate key indicators of agricultural sector performance including net farm income, agricultural prices, government cost, etc. in meeting national bioenergy and bioproduct demand quantities.

PROGRESS: 2003/08 TO 2006/08
Supply and demand for bioenergy and bioproducts have been estimated. Expansion curves reflect growth of bioenergy and bioproduct industries. The impact that the growth of these industries has on the agricultural sector has been estimated including price, income, and government payment impacts. Feedstocks included in the expansion path are crop residues (corn stover and wheat straw), traditional crops (corn and soybeans), and dedicated crops such as switchgrass. Each of these feedstocks can be converted to bioenergy, bioproducts, or both. These feedstocks compete for land and other resources based on net returns. As resources become limited, price of feedstock increases. End product demands modeled include transportation fuels, ethanol and biodiesel, and electricity as well as bioproduct building blocks of Lactic, Levulinic, and Succinic Acids, and 1,3-Propanediol. According to the model, demand for biopower increases from 88 bil kwh in 2005 to 160 bil kwh in 2014. Projected demand for ethanol increases from 2.3 bil gal in 2005 to 18.4 bil gal in 2014 and demand for biodiesel increases from .2 to .6 bil gal in 2005 and 2014, respectively. Demands for biochemicals are also placed on the system. While demand from Levulinic (175 mil lbs.) and succinic (33 mil lbs.) acid do not change over time, demand for Lactic acid increases from 400 to 1700 mil lbs. and demands for 1,3 Propanediol increase from .2 to 320 mil lbs. over the 2005 to 2014 period. Little change occurs in agricultural sector commodity prices through 2010. However, as demands build, and conversion of pasture and hay opportunities become limited, prices increase. In 2010, corn prices increase from $2.45/bu to $3.40, and soybeans increase from $5.55/bu to $6.37. Other nonfeedstock prices also increase. Wheat goes from $3.40/bu to $3.81. Cotton and rice prices increase in 2010 only $0.02/lb and $0.77/cwt, respectively. By 2014, price gains would be very significant. Corn rose from a baseline of $2.45/bu to $4.16, and soybeans increased from $5.70/bu to $6.84. Wheat went from $3.60/bu to $4.04. Cost of cellulostic feedstock also increases. Starting at $31/dry ton, price of biomass jumps to $50/ton in 2014. Electricity and ethanol are the largest potential users of biological feedstocks. Simulation of potential bioproduct demands showed that a biomass price of $30/dry ton (dt) could fill all cellulosic demands until 2011. Ethanol demand, in excess of what could be provided by $30/dt biomass, can be met by use of corn grain as a feedstock at a total cost less than biomass until 2011. In 2011, stresses upon the corn grain sector raise corn price so that biomass becomes competitive in ethanol production. Beyond 2011, supply stresses push biomass prices up to $49.34/dt and corn grain price up to $4.16 by 2014. Nationally, pasture conversion allows 13.6 mil ac of additional land to come into production. MO, KY, and TN can gain more than 250,000 ac, with a majority of them being converted to switchgrass. By 2014, biofuels, biopower, and bioproduct demand increases total net farm income from $54.5 bil to $72.1 bil, a gain of $17.7 bil to the agricultural sector.

IMPACT: 2003/08 TO 2006/08
The project provides insight into the regional and national viability and impacts of different feedstock choices in an increasingly larger bio-based industry, and provides key information needed in the decision making process to meet research and commercialization goals. The project showed that crop residue collection can be quickly increased to fill biomass demand in the initial years while switchgrass production matures. As switchgrass matures, residue collection falls until stresses upon corn grain demand necessitate increased collection. The use of all three bioproduct feedstock sources (biomass, residues, and corn grain) allows the market to smoothly adjust to the rapid increase in demand. Without the use of all three, the ability of the agricultural sector to meet bio-product demands would be severely stressed. The conversion of lands currently in pasture into cropland also increases feedstock quantities.

PUBLICATIONS (not previously reported): 2003/08 TO 2006/08
No publications reported this period

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Item No. 53 of 59

ACCESSION NO: 0211192 SUBFILE: CRIS
PROJ NO: TEX09131 AGENCY: CSREES TEX
PROJ TYPE: HATCH PROJ STATUS: NEW
START: 25 JUN 2007 TERM: 24 MAY 2012

INVESTIGATOR: Capareda, S. C.

PERFORMING INSTITUTION:
BIOLOGICAL & AGRICULTURAL ENGINEERING
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ADVANCED BIO-ENERGY AND ENVIRONMENTAL QUALITY RESEARCH FOR SUSTAINABLE AGRICULTURE

NON-TECHNICAL SUMMARY: The goal of this research work is to focus on researchable aspects of bio-fuels that have the potential to play a larger role in the future economy of the US. The leading bio-fuel candidate in the US today is ethanol. The current US ethanol production is 4.8 billion gallons in year 2006 (RFA, 2006). Biodiesel is the second most popular bio-fuel whose current production is estimated at 75 million gallons per year (Urbanchuk, 2006). Cellulosic bio-fuels are of particular importance primarily because the feedstock is not used as food and fiber. However, bio-fuel production via cellulosic feedstock has a number of researchable gaps that need to be addressed very quickly in addition to identifying high cellulosic biomass candidates. Sorghum biomass is a promising biomass resource due to its having high yielding characteristics coupled with lower water requirements compared to corn (McCutchen, 2006). The research studies outlined in this proposal will build on a solid science-based approach in all aspects of bio-fuels production and environmental quality to address the needs of bio-refineries in the future. This project will evaluate effective ethanol and biodiesel production studies and improved gasification research using numerous biomass feedstocks. In addition, all environmental aspects associated with the establishment of biorefineries will be investigated.

OBJECTIVES: The following objectives outline an overarching research framework in which laboratory and pilot experiments will be planned to address interrelated key questions in energy and air quality. In the context of understanding energy and air quality interactions, research activities will address the following objectives: a. To investigate optimal ethanol production process from wood sugar, highly digestible sorghum strain, sweet sorghum, and sorghum biomass; b. To study engine emissions of biodiesel blends and emerging bio-fuels such as ethanol, synthesis gas and synthetic petroleum; c. To investigate the slagging and fouling problems of selected biomass (cotton gin trash, animal manure and sorghum biomass) through advanced gasification/pyrolysis systems; and d. To evaluate environmental issues associated with the production and use of emerging bio-fuels.

APPROACH: Objective 1. Optimal Ethanol Production Systems from Highly Digestible Grain Sorghum (HDGS), Wood Sugar (Temulose) and other Sorghum Feedstock. This study will investigate the production of ethanol from various biomass resources most abundant in the state of Texas that are potential candidates for high volume production. Objective 2. Engine Emissions Testing of Biofuels (Biodiesel and Blends, Ethanol and Producer Gas). This study will investigate the various barriers to improved production and how to reduce production costs. For every new oil feedstock that will be converted into biodiesel, an understanding of the unique properties of the oil needs to be investigated. This will ensure that gumming, wax formation, phosphates accumulation, rancidity, discoloration, among others are not encountered. Pre-treatment and other removal processes have to be developed for each new oil feedstock. Two potential areas of research include: enzymatic biodiesel production and solid state processes. Objective 3. Slagging and Fouling Problems of Biomass Through Thermal Conversion Systems. Bio-fuels can be readily produced from any biomass resource through thermal conversion processes. The high ash content of biomass makes it unsuitable for combustion system. Only gasification processes would work for high ash biomass. Possible processes include pyrolysis (absence of oxidant) and gasification (deficient O_2). TAMU has developed and patented a fluidized bed gasifier that is capable of producing synthesis gas (CO and H_2) that could be readily reformed to synthetic fuels such as gasoline, diesel, or aviation fuel (Parnell and LePori, 1988). Objective 4. Environmental Issues Associated with the Production and Use of Biofuels. This study involves the evaluation of all environmental and air quality issues associated with the production and use of bio-fuels.

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Item No. 54 of 59
ACCESSION NO: 0205429 SUBFILE: CRIS
PROJ NO: VA-135747 AGENCY: CSREES VA.
PROJ TYPE: HATCH PROJ STATUS: NEW
START: 01 OCT 2005 TERM: 30 SEP 2010 FY: 2006

INVESTIGATOR: Agblevor, F. A.

PERFORMING INSTITUTION:
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BIOFUELS PRODUCTION FROM COTTON GIN WASTE AND RECYCLED PAPER SLUDGE

NON-TECHNICAL SUMMARY: Renewable energy sources can provide the United States with a stable reliable domestic energy supply, contribute very little, if any, net carbon dioxide into the atmosphere, reduce the demand for fossil fuels and hence reduction in NOx, SOx, carbon monoxide, and hydrocarbon emissions into the atmosphere. It can revitalize rural agricultural economies and thus create new jobs. USDA estimated that a 100 million gallon bioethanol plant could create 2,250 local jobs (temporary and/or permanent) in the United States. Cotton gin waste (CGW) is an agro-industrial residue, which could be potentially used for ethanol production. Unlike other lignocellulosic feedstocks, this material is concentrated at the processing sites and therefore harvesting and transportation costs could be considerably less than those for agricultural and forestry residues and dedicated biomass feedstocks. Recycled paper sludge is a short fiber cellulosic feedstock, which could also be potentially used for ethanol production. Similar to the CGW, this material is concentrated at processing sites and therefore transportation cost could be considerably reduced. By combining these feedstocks, ethanol in high yields can be produced for fuel applications and waste disposal problems in these industries can be solved simultaneously. The purpose of this project is to develop an in situ detoxification process for the bioconversion of cotton gin waste and recycled paper sludge mixtures into ethanol in high yields.

OBJECTIVES: Our previous studies on cotton gin waste showed that we could produce ethanol at very good yields at one-liter scale level if the pretreated material is properly detoxified. However, the process was not been demonstrated for the cotton gin waste/recycled paper sludge combination. The overall goal of this project is to develop an in situ detoxification process for the bioconversion of cotton gin waste and recycled paper sludge mixtures into ethanol using steam explosion, enzyme hydrolysis, and fermentation technologies. The process has the potential of solving a waste disposal problem while simultaneously generating a new product stream for the cotton and paper and pulp industries. The specific objectives of this project are: 1. Collect and fractionate cotton gin waste from cotton gins in five states to access the impact of harvesting method on the quality of the feedstock. 2. Characterize recycled paper sludge and cotton gin waste from various states to assess impact of ginning on feedstock composition. 3. Investigate the in situ detoxification of cotton gin waste/recycled paper sludge during steam explosion pretreatment. 4. Investigate the bioconversion of steam exploded cotton gin waste/recycled paper sludge mixture to ethanol. 5. Analyze biomass-to-ethanol fermentation residue to assess the nature of the unhydrolyzed residual cellulose. This technology could be applied to other biomass feedstocks and thus eliminate the extra processing step required for the bioconversion of biomass to fuels and chemicals. This will obviously improve the biomass-to-ethanol process economics. The conversion of RPS and CGW to fuel ethanol has other potential advantages. It will solve waste disposal problem in two industries, it will create new jobs in rural America and further it will stimulate both paper recycling and increase processing of cotton. Increased processing of cotton will of course impact USA agriculture, which is one of the goals of the Hatch Program. The processing of cotton gin waste and recycled paper sludge to ethanol will reduce greenhouse gas emission, and replace petroleum products. It cannot be overemphasized that this proposal fits the objectives of this program because it will assist in implementing new ethanol production capacity and it has a potential for near-term applicability and replication in Southern United States.

APPROACH: Objective 1. Collect and fractionate cotton gin waste from cotton gins in five states to access the impact of harvesting method on the quality of the feedstock. a) Collection of cotton gin waste. Cotton gin waste samples will be collected from five cotton gins in Virginia, Mississippi, Texas, Georgia, and North Carolina, and the RPS will be collected from the International Paper recycling facility in Franklin, Virginia. Samples will be collected every year for three years during the project. b) Fractionation of cotton gin waste. About 1 kg dry CGW samples from the five gins will be shipped to the USDA-ARS Cotton Ginning Laboratory, (Stoneville, MS) to be fractionated into clean lint, hulls, sticks/stems, grass, seeds, small leaf, and pin trash. Additionally, some cotton gin waste fractions will be generated from the pilot scale ginning machine for the studies. Objective 2. Characterize recycled paper sludge and cotton gin waste from various states to assess impact of ginning on feedstock composition. The CGW, RPS, and CGW/RPS samples will be analyzed for their summative composition (cellulose, lignin, hemicellulose, ash, and extractives) using ASTM standard methodologies. In addition, the RPS will also be analyzed for elemental composition. Objective 3. In situ detoxification of cotton gin waste/recycled paper sludge during steam explosion pretreatment. a) Steam explosion of cotton gin waste and recycled paper sludge. We will steam explode CGW and RPS mixtures at various CGW to RPS ratios, moisture contents, and severity parameters in the batch steam explosion unit. The CGW and RPS will be steam exploded as received without any milling or presoaking. b) Enzyme hydrolysis of steam exploded material. Steam exploded CGW and RPS will be hydrolyzed independently to determine their hydrolysis rates and their fermentable sugar yields. The hydrolysis will be carried out for 72 h at 50 deg. C using commercial cellulase enzyme preparation such as Spezyme CP. We will investigate various CGW/RPS ratios and the influence of potential heavy metal content of the RPS on the sugar yield. Objective 4. Investigate the bioconversion of steam exploded cotton gin waste/recycled paper sludge mixture to ethanol. a) Fermentation of hydrolysates. We will use both Saccharomyces cerevisiae, and E. coli KO11 for the fermentation. We will investigate the effect of the following parameters on ethanol yield: severity of steam explosion, CGW/RPS ratio, degree of enzyme hydrolysis, composition of CGW, feedstock source, temperature, pH and additives content of RPS. b) Optimization of in situ detoxification process. Factorial designs and response surface methodology will be used to determine the best operating parameters for in situ detoxification and ethanol production. c) Scale-up of process to 1-liter scale. We will scale-up the process from shaker flask level to one-liter scale after the optimization of the process parameters.

PROGRESS: 2005/10 TO 2006/09
We have investigated steam explosion pretreatment of mixtures of cotton gin waste and recycled paper sludge for bioethanol production. The cotton gin waste was obtained from Emporia, VA, whereas the recycled paper sludge was obtained from Weyerhaeuser recycling paper mills in Cedar Rapids, IA and Marlboro, SC. We investigated the insitu detoxification of the cotton gin waste samples. In this process, no overliming of the steam exploded material is necessary before hydrolysis and fermentation to ethanol. The mixtures of cotton gin waste to recycled paper sludge in the ratios of 1:1, up to 4:1 were steam exploded at a severity of 3.8. We investigated enzyme hydrolysis of the steam exploded materials using proprietary enzymes from Novozymes Inc, and Genecor Int. Because of the insitu detoxification process, the steam exploded materials were not overlimed before the detoxification. We successfully hydrolyzed 90 percent of reducing usgars in the steam exploded material into monomeric sugars. These results showed a proof of concept that it is not necessary to overlime the samples if the insitu detoxification method is used. The steam explosion process was scale-up to 1000 kg/h and the recycled paper sludge and cotton gin waste were processed on that scale. Our results show that we could scaleup the process successfully.

IMPACT: 2005/10 TO 2006/09
Southside Virginia cotton industry is experiencing waste disposal problems because of potential particulate emissions if the cotton gin waste is combusted. The conversion of the cotton gin waste to bioethanol will simultaneously solve the waste disposal problem and generate new product streams for both industries. Thus, we expect this project to create new jobs in the area when completed. Furthermore, Xethanol Inc,

which has acquired rights to the process, plans to build small footprint plants in Southside Virginia and thus provide new jobs in rural Virginia.

PUBLICATIONS (not previously reported): 2005/10 TO 2006/09
No publications reported this period

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Item No. 55 of 59
ACCESSION NO: 0210227 SUBFILE: CRIS
PROJ NO: VAK-2007-00181 AGENCY: CSREES VA.K
PROJ TYPE: SMALL BUSINESS GRANT PROJ STATUS: NEW
CONTRACT/GRANT/AGREEMENT NO: 2007-33610-18017 PROPOSAL NO: 2007-00181
START: 01 JUN 2007 TERM: 31 JAN 2008 GRANT YR: 2007
GRANT AMT: $80,000

INVESTIGATOR: Blaylock, M. J.

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PLANT EXPRESSION OF CELLULASE FOR BIOMASS ETHANOL PRODUCTION

NON-TECHNICAL SUMMARY: Cellulosic ethanol production has the potential to provide a renewable, clean source of automobile fuel. Current production costs (including the costs of required enzymes) are prohibitively expensive. This project seeks to provide a significant cost savings for cellulosic ethanol production by incorporating the ability to produce stable enzymes in the plant tissue of select Energy Crops.

OBJECTIVES: Edenspace Systems Corporation seeks to achieve a substantial decrease in the production costs of cellulosic ethanol biofuel by engineering a promising biofuel crop, switchgrass (Panicum virgatum L.), with up to three different genes that produce cellulases. When activated by heat, these enzymes break down biomass cellulose into simple sugars that can serve as biofuel feedstocks. Microbially-produced cellulases currently used to produce ethanol from corn and other biomass cost more than $0.45 per gallon of ethanol produced. With successful completion of this project to produce the cellulases in switchgrass biomass, this cost can be drastically reduced to the current goal of $0.10 per gallon or perhaps eliminated, with further cost savings from simplified ethanol production operations. Switchgrass, a versatile, perennial, high-biomass grass, provides significant environmental benefits with respect to maintaining soil quality and sequestering carbon while providing feedstocks for biomass based fuels. In Phase I, an existing accession of switchgrass transformed with the Acidothermus cellulolyticus E1 endoglucanase gene will be tested for biomass production and composition, cellulase activity, and enzymatic stability with respect to ethanol production from plant biomass. Switchgrass will be engineered with additional cellulase genes for testing in Phase II. Successful completion of this project will provide substantial cost benefits for the production of ethanol and other biomass feedstocks. In addition, growing enhanced switchgrass for biofuels could provide new income potential for farmers, particularly those participating in the Conservation Reserve Program.

APPROACH: The Phase I study has three major objectives: 1. Demonstrate that E1 switchgrass shows comparable growth and biomass characteristics to nontransformed switchgrass. 2. Show that cellulase activity and conversion of cellulose to fermentable sugars in E1 switchgrass biomass takes place at rates comparable to levels obtained in nontransformed switchgrass using external cellulases. 3. Prepare and constructs for additional cellulases (hemicellulase, and exoglucanase) and begin swtihcgrass transformation for testing in Phase II. This project will utilize transgenic E1 switchgrass lines being developed under a CRADA between Edenspace and the USDA-ARS Western Regional Research Center (WRRL) in Albany, CA. Over an 8-month demonstration period, the team will seek to achieve the SBIR objectives by completing the following tasks: Task 1.0. Demonstrate that E1 switchgrass shows comparable growth and biomass characteristics to nontransformed switchgrass. The growth characteristics and biomass yield of transformed switchgrass (T0) will be compared with existing high biomass lines (i.e. Alamo). Seeds of transformed and Alamo switchgrass will be planted in commercial potting mix (e.g., Promix)in the growth chamber using twelve replicates of each genotype. Leaf tissue samples from each plant genotype will be collected at four week intervals beginning one month after emergence. The tissue samples will be analyzed for cellulose, hemicellulose and lignin content to assess differences in composition. Soluble sugars in the plant tissue will also be analyzed at monthly intervals. Task 2.0. Show that cellulase activity and conversion of cellulose to fermentable sugars in E1 switchgrass biomass takes place at rates comparable to levels obtained in nontransformed switchgrass using external cellulases. The T0 generations (i.e., those plants formed through the transformation procedure) of transformed switchgrass generated by WRRL will be grown for six months in the growth chamber at Edenspace using a 16:8 hour light:dark regime. Seeds will be planted in containers with 1 kg of a silt loam soil limed to pH 6 or above and fertilized according to soil fertility recommendations to provide a minimum of 50 mg/kg of N, P and K. The growth will be monitored and documented through weekly digital images. Cellulase activity will be evaluated in those lines through several methods: 1. Analysis of purified extracts. 2. Cellulose hydrolysis in biomass after pretreatment to remove hemicellulose and lignin. 3. Enzymatic Stability. Task 3 Prepare constructs for additional cellulases (hemicellulase and exoglucanase) and begin switchgrass transformations for testing in Phase II. Gene constructs for additional glycoside hydrolases with reported endo, exo, and hemicellulase activities will be synthesized for use in plant transformations. Stable switchgrass calli will be generated and propagated and used for transformation.

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Item No. 56 of 59
ACCESSION NO: 0194359 SUBFILE: CRIS
PROJ NO: WVA00428 AGENCY: CSREES WVA
PROJ TYPE: HATCH PROJ STATUS: TERMINATED MULTISTATE PROJ NO: S-1007
START: 01 OCT 2002 TERM: 30 SEP 2007 FY: 2005

INVESTIGATOR: Russell, R. W.

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THE SCIENCE AND ENGINEERING FOR A BIOBASED INDUSTRY AND ECONOMY

NON-TECHNICAL SUMMARY: The availability of quantities of oil necessary to power our economy is becoming less certain. We are challenged with harnessing solar energy in quantities to meet our food and fuel needs of the present and future. The purpose of the project is to organize the expertice and activities of the land-grant university system to form the science and engineering base necessary for developing a biobased economy and associated biobased industries.

OBJECTIVES: Objective 2. Expand the scientific knowledge leading to significant economic improvements in biofuel production processes.

APPROACH: Development of improved thermochemical processes for biofuel production. Three basic thermochemical conversion approaches to be evaluated include direct combustion (CA, NE), gasification (CA, OK, NE), and direct conversion to liquid fuel (WV). Problems of fouling, slagging, and boiler efficiency associated with co-firing biomass with coal will be studied (CA). A close-coupled fluidized bed biomass gasification and direct combustion system will be investigated for reduction of NOx emmissions through reburning (CA). Other gasification studies will optimize production of syngas for use as a substrate for fermentation to fuel alcohol (OK). The reaction of biomass with water under conditions of high temperature and high pressure will be optimized with respect to reaction time, temperature, and water content for different types of biomass.

PROGRESS: 2002/10 TO 2007/09
This project has demonstrated the process of using heat and pressure for effective conversion of agriculture and forestry biomass/waste to a diesel fuel additive at the experimental level. Demonstrations through the national Farm Bureau organization have been conducted to demonstrate that the end product of this conversion process can successfully run a diesel generator. The patent disclosure has been filed for the process. This project has supported the faculty structure within the University to garner effective input from the College of Engineering with the collaboration of two faculty members. The Davis College has been a participant in the regional project for the development of alternative fuel systems.

IMPACT: 2002/10 TO 2007/09
The purpose of the project is to organize the expertice and activities of the land-grant university system to form the science and engineering base necessary for developing a biobased economy and associated biobased industries.

PUBLICATIONS (not previously reported): 2002/10 TO 2007/09
No publications reported this period

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Item No. 57 of 59
ACCESSION NO: 0212875 SUBFILE: CRIS
PROJ NO: WIS01302 AGENCY: SAES WIS
PROJ TYPE: STATE PROJ STATUS: NEW
START: 01 OCT 2007 TERM: 30 SEP 2012

INVESTIGATOR: Donohue, T.

PERFORMING INSTITUTION:
GREAT LAKES BIOENERGY RESEARCH CENTER
UNIV OF WISCONSIN
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BIOENERGY RESEARCH

NON-TECHNICAL SUMMARY: The potential for biofuels to address a looming energy crisis is significant, yet their contribution to the US energy portfolio is small. A major reason for their relatively small contribution is that various factors limit transformation of sunlight or plant biomass into biofuels. To apply the full power of contemporary science to this looming crisis, we will create the Great Lakes Bioenergy Research Center (GLBRC). The GLBRC will conduct basic, genomics-based research to design the microbial and plant systems needed to realize the potential of biofuels.

OBJECTIVES: To increase the contribution of biofuels to the US energy portfolio, the Great Lakes Bioenergy Research Center (GLBRC) will conduct fundamental, genomics-based research to remove bottlenecks in the biofuel pipeline. The Principal Investigator and Scientific Director of the GLBRC will be Professor Timothy Donohue. The GLBRC will be housed at the University of Wisconsin-Madison, with Michigan State University as a major partner. Additional scientific partners will be based at DOE National Laboratories (Pacific Northwest and Oak Ridge National Labs), other universities (University of Florida, Illinois State University, Iowa State University), and biotechnology companies (Lucigen Corporation). To function as a worldwide center of excellence, the GLBRC will develop programs that bring bioenergy breakthroughs and technology to stakeholders, including other centers with complementary missions, both here and abroad; academic and research hubs; regional, national or international members of the agricultural and private sector; educators in the scientific, business, or academic community; and the general population.

APPROACH: To achieve its vision, the GLBRC will develop a set of vertically integrated research programs aimed at removing existing bottlenecks in the bioenergy pipeline, developing economically- and environmentally-sustainable bioenergy practices, and educating society scientists and biomass producers or consumers about bioenergy issues. To support its programs, the GLBRC will assemble an interactive management team that has regular feedback from a scientific advisory board, pilot plant partners, and a collaborative network to align GLBRC products with our needs for energy, thereby increasing the contribution of biofuels to the US energy portfolio. To accomplish its short-, intermediate-, and long-term goals, the GLBRC will have the following major research thrusts: (1) Improve the characteristics of biomass plants. Difficulties in using biomass for bioenergy production include degradation of the major plant cell wall constituents (cellulose, hemicellulose, and lignin) and the inability of many plants to store carbon in energy-rich hydrocarbons. Long-term goals of the GLBRC for improving plant biomass are to increase the yields of easily degraded polysaccharides within cell walls and to increase the yields of hydrocarbons in biomass tissues. (2) Improve the processing of plant biomass. Processing of plant biomass to sugars is a major problem in economical production of cellulosic ethanol or other bioenergy products. To remove this bottleneck, new treatments are needed for processing the feedstocks (corn stover, switchgrass, or wood chips) envisioned for the bioenergy pipeline. In this area, the long-term goal of the GLBRC will be to develop new physical and biological ways to process existing and improved plant biomass. (3) Improve how we convert biomass into energy products. To accomplish this, the GLBRC will overcome difficulties encountered during conversion of plant-derived chemicals to bioenergy compounds. The long-term biomass conversion goals of the GLBRC are to improve methods for conversion of cellulosic biomass into ethanol and to develop novel biological or chemical ways to convert plant material into H2, electricity, or other chemical feedstocks that can replace fossil fuels. (4) Evaluate the economic and environmental sustainability of the biomass to biofuel pipeline. For a bioenergy economy to positively impact the US energy sector, we must address complex issues in agricultural, industrial, and behavioral systems. Another long term goal of the GLBRC is to develop economically viable and environmentally responsive practices for the biomass-to-bioenergy pipeline. (5) Integrate enabling technologies into bioenergy research. To achieve its short- and long-term goals, the GLBRC will deploy high-throughput technologies; integrate information from computational, physical, and genomic approaches; and develop predictive models for relevant enzymes, pathways, or networks. Success of the GLBRC will hinge on having biological, physical and computational approaches to enable biomass production, processing, conversion, and sustainability.

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Item No. 58 of 59
ACCESSION NO: 0189207 SUBFILE: CRIS
PROJ NO: WIS04517 AGENCY: CSREES WIS
PROJ TYPE: NRI COMPETITIVE GRANT PROJ STATUS: TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2001-35504-10695 PROPOSAL NO: 2001-01418
START: 15 SEP 2001 TERM: 30 SEP 2004 FY: 2004 GRANT YR: 2001
GRANT AMT: $200,000

INVESTIGATOR: Jeffries, T. W.

PERFORMING INSTITUTION:
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QUANTITATIVE ANALYSIS OF GENE EXPRESSION DURING XYLOSE FERMENTATION BY YEASTS

NON-TECHNICAL SUMMARY: Agricultural residues could be used to produce renewable fuels and chemicals if ways can be found to efficiently ferment the hemicellulosic sugars. The resulting fermentation industry would create new income sources and provide rural jobs while reducing global change. This project will elucidate the regulatory patterns and genes necessary for efficient fermentation of xylose by yeasts. It will use contemporary techniques to track and quantify the large-scale expression of genes in yeasts.

OBJECTIVES: The overall goal of this research is to understand the principal genes and physiological conditions that govern metabolism of xylose in yeasts so we can manipulate their expression to enhance ethanol production from lignocellulosic residues. Ultimately this research will be used to establish an agro-based industry for the production of clean burning renewable fuels from agricultural residues. Implementation of this technology will enable a sustainable economic environment for rural communities while helping to stem global warming. Objectives supporting this goal are the identification of genes essential for xylose metabolism, understanding their patterns of induction and integrating that information into a model that will enable us to interpret the overall flow of metabolites. This research addresses two basic questions: First, how does one maintain and maximize a flow of metabolites (a metabolic flux) from xylose to ethanol under respiro-fermentative conditions in P. stipitis? Second, how would one go about altering the flow of metabolites in central pathways of other organisms? This research is relevant to general metabolic engineering in eukarya and more specifically to the enhancement of xylose fermentations for value added products from agricultural residues.

APPROACH: We will transform S. cerevisiae with genes for xylose metabolism and cultivate the cells on glucose and xylose to determine which sets of genes are induced on xylose. The patterns of expression (or lack thereof) should indicate which regulatory cascades are essential for fermentation. Large data sets already exist that describe induction patterns for S. cerevisiae cultivated under aerobic and oxygen limited or fermentative conditions, and the regulatory proteins controlling these patterns are under active investigation. These prior and contemporaneous studies will serve as reference standards for induction (or lack thereof) for cells cultivated on D-xylose. Depending on the results from the initial carbon source studies, we might also examine the effect of aeration levels on mRNA induction in cells transformed with the xylose assimilation genes. The gene chip experiments should identify sets of enzymes that are regulated in response to oxygen, xylose and the presence or over expression of xylose metabolizing genes in the Saccharomyces genome. These direct studies will not, however, reveal what additional proteins are necessary for high fermentation levels, so we must examine P. stipitis as well. We do not have a complete sequence of the P. stipitis genome, and the gene sequences that we do have for P. stipitis show that it has diverged far enough from S. cerevisiae such that cross hybridization with a S. cerevisiae gene chip would not render productive data. Therefore, it is essential to use sequential analysis of gene expression (SAGE) technology to identify the relevant genes in this organism. There is a strong correlation between the levels of mRNA detected by SAGE and gene chip technologies, so the results obtained by these two approaches should be comparable. In the case of P. stipitis, we will compare mRNA expression levels of wild-type cells grown on glucose and xylose under fully aerobic and oxygen limited conditions. This will tell us which enzymes are essential for xylose metabolism and fermentation in this organism. We will then perform comparative analyses of control cells and mutants that have site-specific deletions of alcohol dehydrogenase (PsADH1 and PsADH2), which blocks ethanol formation, and cytochrome c (PsCYC1), which blocks cytochrome respiration. These analyses should indicate the adaptive response that the cells take to blockages in their fermentation and respiration pathways, respectively. We will cultivate parental and adh1 cells on xylose under identical continuous oxygen limiting conditions, harvest the cells, recover the mRNA, convert the pools into SAGE libraries then compare the homeostatic adaptation of cells at the mRNA level to this targeted disruption. Incomplete fermentation of xylose to ethanol should reduce the internal ATP charge. The SAGE analysis should show us whether any other proteins are induced along with PsADH2.

PROGRESS: 2001/09 TO 2004/09
The overall objective of this research has been to understand how intermediary metabolism is regulated. We will apply this knowledge to increase the fermentation of xylose by engineered yeasts. This research has shown that Saccharomyces cerevisiae engineered for xylose metabolism through the overexpression of three genes, XYL1, XYL2 and XYL3, requires further genetic manipulation for efficient xylose fermentation. First, the overexpression of XYL3 inhibits the growth of some strains of S. cerevisiae when the transformed cells are cultivated on xylose. However, several mutational events can occur spontaneously in S. cerevisiae to relieve that inhibition. Moreover, the extent of inhibition depends on the strength of the promoter used for XYL3, the number of copies present in the cell and the carbon source used for growth. Because of these numerous variables, and the fact that inhibition resistant mutants arise spontaneously with high frequency, the literature on this subject is somewhat contradictory. Our research used two approaches to identify the mutational events. We transformed S. cerevisiae with a cassette that overexpressed XYL1, XYL2 and XYL3 on a multi-copy expression vector, and we then transformed the resulting strain (which would not grow on xylose) with a transposon-mutated library of the S. cerevisiae genome. These were targeted to create insertional mutations. We were able to recover and verify two mutational events that overcome the growth inhibition imposed by XYL3 overexpression. These studies have formed the basis for a patent disclosure. In a separate approach we transformed the cells carrying XYL123 with an overexpression library. This also revealed a number of genes that could overcome the XYL3 growth inhibition. Second, our research has shown that even when S. cerevisiae is transformed with XYL123 in a construct that allows an appropriate low level of expression and when mutational events are present in the genetic background that enable the cells to resist growth inhibition, they still do not recognize xylose as a fermentable carbon source. Rather, they induce the TCA cycle and respiration pathways. By creating respiration deficient mutants of these genetically engineered cells it has been possible to increase the fermentation rate and the ethanol yield. A metabolic model based on metabolic flux and redox balances predicted that these engineered cells would require oxygen for xylose assimilation. In keeping with this model, our respiration deficient mutants would not grow on xylose. A similar study in the xylose assimilating yeast, Kluyveromyces marxianus showed that disruption of CYC blocked growth on xylose with this organism as well. In separate studies, we showed that when D-xylulokinase is deleted in Pichia stipitis, the resulting cells tend to produce xylitol rather than ethanol. This is consistent with our studies of engineered S. cerevisiae and the finding confirms that in order to produce ethanol from xylose it is necessary to increase the flux of metabolites through the pathway.

IMPACT: 2001/09 TO 2004/09
The efficient fermentation of xylose is essential for the commercial conversion of renewable biomass to fuels and chemicals. This research identifes which changes in genes and regulatory pathways are necessary for the fermentation of xylose in Pichia stipitis. This information will be used to increase efficiency of xylose fermentation to ethanol by both P. stipitis and S. cerevisiae. This research will increase utilization of agricultural processing wastes such as corn, oat, wheat and rice hulls by enabling improved ethanol yields. It will also enable farmers to realize additional revenues through the sale of corn cobs, corn stover, and soybean residues. The ethanol and other renewable chemicals produced from lignocellulosic xylose will reduce dependence on imported petroleum and thereby increase domestic security while reducing the accumulation of greenhouse emissions.

PUBLICATIONS (not previously reported): 2001/09 TO 2004/09
1. Mu, Yi, Jose M. Laplaza, T.W. Jeffries. Cloning and disruption CYC in Kluyveromyces marxianus CBS 6556. 26th Symposium on Biotechnology for fuels and Chemicals. Chattanooga, TN, May 9-12, 2004
2. Laplaza, Jose M., Chenfeng Lu, and T.W. Jeffries. Screen of novel yeast strains for fermentation of xylose and glucose. 26th Symposium on Biotechnology for Fuels and Chemicals. Chattanooga, TN, May 9-12, 2004.
3. Jin, Yong-Su and T. W. Jeffries. 2004. Stoichiometric network constraints on xylose metabolism by recombinant Saccharomyces cerevisiae. Metabol. Engineer. 3:229-238
4. Jin,Yong-Su, Laplaza, J.M., and T.W. Jeffries. 2004 Saccharomyces cerevisiae engineered for xylose metabolism exhibits a respiratory response. Appl. Environ. Microbiol. 70:6816-6825.

5. Jin, Yong-Su, and TW Jeffries. Xylitol production by a Pichia stipitis D-xylulokinase mutant. 2005. Appl. Microbiol. Biotechnol. (in press)
6. Jeffries, T.W. and Jin, Y.S. Xylose Fermenting recombinant yeast strains. US application B0190030 Filed January 2003

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Item No. 59 of 59
ACCESSION NO: 0196241 SUBFILE: CRIS
PROJ NO: WIS04749 AGENCY: SAES WIS
PROJ TYPE: STATE PROJ STATUS: TERMINATED
START: 01 SEP 2002 TERM: 31 AUG 2011 FY: 2006

INVESTIGATOR: Weimer, P. J.

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COMPARATIVE INVESTIGATION OF PHYSIOLOGICAL FEATURES OF CELLULOSE

NON-TECHNICAL SUMMARY: Clostridium thermocellum is a cellulose-fermenting bacterium with potential for conversion of biomass resources into fuels and industrial chemicals, particularly ethanol. The purpose of this research is to determine how much of its energy and resources this organism invests in assembling the surface layer of the cell that is partially responsible for the process of cellulose fermentation.

OBJECTIVES: The purpose of this research is 1) to determine the composition and structure of the glycocalyx (extracellular polysaccaharide and associated protein) of the anaerobic bacterium Clostridium thermocellum; and 2) to identify potential genes involved in biosynthesis of the EPS component of the glycocalyx.

APPROACH: Clostridium thermocellum will be grown on cellulose and on cellobiose as energy sources, and cells will be treated to remove the glycocalyx. Chemical hydrolysis and derivatization, combined with gas chromatography/mass spectrometry, or liquid chromatography/mass spectrometry, will be used to identify and quantify the monosaccharides in the C. thermocellum glycocalyx, and to determine their linkage patterns. The protein content of the glycocalyx will be determined by standard chemical techniques, and mode of interaction (covalent or noncovalent) between protein and EPS will be determined. Mass balances of cells, fermentation products, and glycocalyx components will be determined to estimate the allocation of metabolic effort required for biosynthesis of the glycocalyx, and candidate genes involved in biosynthesis of glycocalyx precursors will be identified from known pathways of EPS biosynthesis and from the nearly completed and publicly available C. thermocellum genome in the TIGR Website for Unfinished Genomes.

PROGRESS: 2002/09 TO 2011/08
Expression of 17 genes involved in cellulose degradation and primary catabolism by the thermophilic, anaerobic cellulose-degrading bacterium Clostridium thermocellum ATCC 27045 were determined, using real-time PCR of mRNA recovered from cells grown in continuous culture on cellulose or on a soluble cellulose building block, cellobiose. Relative to growth on cellulose, expression of genes encoding the cellulosome scaffoldin (CipA) and main exoglucanase (CelS), as well as a mannanase (ManA) and one alcohol dehydrogenase was decreased when cells were grown on cellobiose at high growth rates, but not at low growth rate, suggesting that expression of these genes was regulated by growth rate, not (as previously

believed) by substrate type. Expression of genes encoding known catabolite regulatory proteins was not quantitatively affected by growth rate or by substrate type. Growth on cellulose, but not on cellobiose, was accompanied by production of a polysaccharide-rich sticky substance (called a glycocalyx) that allowed cells to adhere tightly to cellulose (a requirement for cellulose degradation by this species). The glycocalyx from C. thermocellum and from a related bacterium, Ruminococcus albus, had similar carbohydrate compositions, suggesting a similar function and a similar evolutionary origin. Variable pressure scanning electron microscopy (VP-SEM), a technique not previously applied to the study of biofilms, allowed accurate visualization of the R. albus cells without physical disruption of the biofilm. The biofilm consisted of a single layer of bacterial cells attached by the glycocalyx to one another and to the cellulose substrate. Compositional and linkage analysis of the polysaccharide component of the glycocalyx revealed chains of six-carbon sugars of up to 26 units in length, with substantial branching of the main polysaccharide chain, indicating that the glycocalyx is structurally different from other bacterial polysaccharides studied thus far. Analysis based on known biochemical pathways for microbial polymer assembly suggest that R. albus must invest only a small fraction (<4%) of the energy available from cellulose fermentation in order to assemble the polysaccharide component of the glycocalyx that it needs to attach to the cellulose.

IMPACT: 2002/09 TO 2011/08
This research indicates that important cellulolytic functions in C. thermocellum, a potentially useful organism for biomass conversion to ethanol, are not exclusively controlled by substrate type. This, in turn, suggests novel strategies for regulating biomass conversion by this organism. This research also identifies the unique structure of the polysaccharide component of this glycocalyx, provides both a means of estimating glycocalyx yield and energy demands, provides guidance for future studies to link composition and structure of the glycocalyx-containing fermentation residues to the functional behavior of the glycocalyx as a potentially adhesive for manufacture of plywood and other bonded wood products.

PUBLICATIONS (not previously reported): 2002/09 TO 2011/08
1. Weimer, P.J., N.P.J. Price, O. Kroukamp, L.M. Joubert, G.M. Wolfaardt, and W.H. Van Zyl. 2006. Studies of the extracellular glycocalyx of the anaerobic cellulolytic bacterium Ruminococcus albus. Appl. Environ. Microbiol. 72:7559-7566.
2. Lynd, L.R., P.J. Weimer, G. Wolfaardt, and Y.H.P. Zhang. 2006. Cellulose hydrolysis by Clostridium thermocellum: A microbial perspective. In: I.A. Kataeva (ed.). Cellulosome: Molecular Anatomy and Physiology of Proteinaceous Machines (Uversky V.N., Series Editor). Nova Science Publishers, Hauppauge, New York (In press).

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