

REFINING THE BIOREFINING PROCESS

Promising Action on the Extraction Front

Finding the most efficient means to detoxify biomass hydrolysates may seem like a relatively minor, esoteric undertaking to people outside the renewable fuels arena. But it's the type of issue that researchers such as Colorado State University's (CSU) Ranil Wickramasinghe, PhD, PE, must help resolve if the U.S. government is to meet its goal of replacing 30% of America's petroleum-based transportation fuel consumption with biofuels by 2030.

Wickramasinghe, a professor in the Department of Chemical and Biological Engineering at CSU, is leading a team of researchers in a quest to find more efficient and cost-effective methods of detoxifying corn stover hydrolysates maximize conversion of sugars to ethanol. That in turn could lead to significant increases in bioethanol yields, thus bolstering the overall economic viability of the biorefining process. His team is performing its work under a subcontract with the U.S. Department of Energy's National Renewable Energy Laboratory (NREL) in Golden.

Instead of relying on conventional liquid extraction methods for removing toxics from a corn stover feedstock after it has been pretreated, Wickramasinghe and his team are exploring a membrane extraction method. Toxic compounds — acetic acid is the focus of their current work — are removed with a microporous hollow fiber membrane before the hydrolysate (the biomass) enters the fermentation

stage of the biofuel production process.

How do such seemingly obscure materials, methods and processes figure into the mainstream push to develop and commercialize renewable alternatives to petroleum-based fuels? According to Wickramasinghe, it comes down to bottom-line economics; minimizing the amounts of toxic compounds in the hydrolysate is critical not only to maximizing the overall yield from the fermentation process, but also to making bioethanol production facilities commercially feasible over the long run in terms of cost and scalability.

Wickramasinghe's team at CSU is about halfway through its work under the NREL subcontract. "We would like to show that the hollow fiber membrane, is a very flexible detoxification process that can be used for many different types of biomass," he explains. "We also want to see if we can use it to extract and concentrate a variety of toxic compounds, not only acetic acid."

Toxic compounds such as acetic acid are produced during the process of pretreating the corn stover, a lignocellulosic biomass. Because of its broad availability, this feedstock is widely seen as a practical choice for biofuel production, at least for the near term. The challenge lies in removing toxic compounds after pretreatment to improve enzymatic cellulose hydrolysis and co-fermentation of the sugars. "The sugars, are vital to producing bioethanol, but ethanol yields are lowered by the presence of toxic compounds that are released during pretreatment," explains Wickramasinghe.

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C2B2 REU — Year 2 in Review

The 2009 C2B2 Research Experience for Undergraduates Program (C2B2-REU) was record breaking! With more than 130 applicants from nearly 70 different universities, C2B2 selected 19 outstanding students to participate in cutting-edge bioenergy research this summer. Undergraduates were matched with a C2B2 principal investigator and mentor at one of the four C2B2 sites and worked in Colorado from June 1 to August 8, 2009. In addition to attending C2B2 Site Visits at CU, CSU, CSM and NREL, weekend hikes and rafting trips, students also took part in a new weekly seminar series and cultural experiences. Students learned about Boulder's sister city in Tajikistan at the Dushanbe Tea House, attended



performances by the Colorado Shakespeare Festival, and even presented their research to peers at the F/Stop Café and art gallery in Fort Collins. Three winners of the 2009 C2B2-REU Posters Session will present their C2B2 research later this year at related academic conferences.

C2B2-REU Students Pictured at Orientation Day 2009:

Front Row (left to right): Alejandra Vargas (Worcester Polytech), Jackie Johnson (UNC), Amy Oberlin (Michigan), Emilia Vesper (New Mexico IMT), and Kendall Fitzgerald (NC State).

Middle Row (left to right): Ray Smith (Clemson), Erica Jorgenson (Colorado), Andrew Mach, and Clay Beevers (New Mexico IMT).

Back Row (left to right): Brian Evanko (Colorado), Maclyn O'Donnell (Pennsylvania), Brian Ho (Florida), Michael Hoerner (Rose Hulman IT), Charlene Vela (Texas-Austin), Sasha Broadstone (Purdue), Marty Witt (Michigan), Jen Lewis (NC State), and Galo Atiaga (NC State).

Not pictured: Travis Lau (Northwestern).

C2B2 2009 SEED GRANT PROPOSALS

C2B2 researchers are constantly engaged in efforts to further fundamental scientific understanding and to develop new technologies for application to commercial processes in the biorefining and biofuels industries. The following information provides a snapshot of the proposals submitted by researchers to the 2009 C2B2 Seed Grant Program. Proposals were reviewed and selected for funding by C2B2 Sponsoring Members.

Proposal titles below have been organized according to C2B2 Research Thrust and listed alphabetically by lead principal investigator. The six proposals selected for funding beginning in January 2010 are delineated in **BOLD**.

(Ali) *The primary goal of this project is to identify genes involved in lipid biosynthesis in algae. Ultrahigh-throughput gene expression analysis will be used for the RNA sequencing (RNA-Seq) of Chlamydomonas reinhardtii under normal and a variety of stress conditions to induce lipid accumulation.*

FEEDSTOCK ENGINEERING, PLANT BIOTECHNOLOGY & CROP SCIENCE

Ali, Gul - CSU • Functional Genomics of Lipid Biosynthesis in Algae

(Darzins) *This research will identify the best lipid producing algae in the C2B2 culture collection utilizing both established and novel analytical techniques to comprehensively and rapidly assess the biofuel feedstock potential of the most promising isolates. A diversity of media formulations will be screened to determine optimized media and growth conditions for the most productive strains using (a) high-throughput spectrophotometry and cell counts to assess algal growth, (b) fluorescence microscopy and flow cytometry to rapidly probe the non-polar lipid content, (c) high-throughput fatty acid fingerprinting and lipid characterization in the most productive strains using NIR and FTIR spectrophotometry, and (d) genetic techniques to isolate and clone 18S rRNA gene sequences from selected organisms for sequencing and species identification.*

Chisholm, Stephen - CSU • The CSU_Biofuel Information Resource (CSU_BIR): Building Bioinformatic Infrastructure for Biofuel Research in Colorado

Demmig-Adams, Barbara - CU • Identification of Regulators that Trigger Energy Carrier Accumulation and/or Secretion in Algae Without Stress

Darzins, AI - NREL • Characterization and Optimization of Lipid Accumulation in Oleaginous Algae from the C2B2 Culture Collection

(Pilon) *Project objective will be to analyze the role of the diverse family of laccase copper-enzymes in the process of lignification in Poplar, a fast growing model tree with a sequenced genome. Recently discovered micro-RNA mediated regulation which links laccase activity to Cu availability will be used. By uncoupling micro-RNA production from Cu availability we aim to produce trees in which the contribution of the majority of laccases to lignification and wood formation can be tested.*

McKnight, Dianne - CU • Optimization of Growth Algae for Biofuels Using Wastewater Effluent

Peebles, Christie A. M. - CSU • High Throughput Generation and Selection of Lipid-accumulating Cyanobacteria

Pilon, Marinus - CSU • Micro-nutrient Effects on Biomass Quality in Poplar: Effects of Copper Status on Laccase Activity and Lignification.

(Stephens) *Proposal seeks to elucidate the relationship between cell wall composition, biomass and the ultimate availability of cellulose for conversion to ethanol. Data will provide critical preliminary information for designing cell wall modifications in proposed biofuel feedstock grasses, that will improve access to plant cell wall sugars. Researchers will determine the type, quantity and distribution of different cell-wall components from a diverse set of rice lines and rice mutants altered in key wall-modifying enzymes. Data will provide critical preliminary information for designing cell wall modifications in biofuel grasses that will improve access to plant cell wall sugars.*

Qian, Yaling - CSU • Growing Switchgrass as an Energy Crop in Colorado: Plant Growth and Physiological Response under Different Management Regimes

Stephens, Jan - CSU • Towards Improving Carbohydrate Availability for Cellulosic Ethanol: Analysis of Cell Wall Composition in Rice Variants

(Gill) *This study will investigate the relation between energy metabolism and growth in selections by making gene deletions that alter E. coli's energy metabolism (ATP availability). Elementary Mode Analysis will be used to make quantitative estimations of these effects, which will be experimentally verified using glucose and xylose limited continuous cultures. This will allow for an accurate quantification of the energetic and metabolic (growth) effects of the deletions.*

BIOCHEMICAL ENGINEERING

Gill, Ryan - CU • Tools for Improving Strain Selection Through Engineering of Energy Metabolism

(Prasad) *A mathematical model of the lipid biosynthesis process will be developed in a well studied green algal species that includes the linkages between lipid biosynthesis and photodamage, and lipid biosynthesis and food metabolism. Flux analysis will be performed under different conditions on the network and will obtain rules for optimization of lipid production. Use will be simulated in silico experiments to help elucidate the physiological role of lipid accumulation. A mathematical model for lipid biosynthesis in a well studied cynaobacteria species will also be developed.*

Prasad, Ashok - CSU • Systems Biology Analysis of Lipid Biosynthesis pathways in algae and cyanobacteria

Weir, Tiffany - CSU • Discovery of Highly Efficient Lignin-degrading Enzymes and Bacteria

THERMOCHEMICAL ENGINEERING

(Chen) *Research objective seeks to synthesize polymeric ILS and cellulose nanocomposites in order to recycle ILS used in cellulose conversion. Project will develop recyclable metal catalysts for efficient glucose-to-fructose isomerization involved in HMF production from cellulosic biomass.*

Chen, Eugene - CSU • Green and Cost-Effective Thermochemical Biomass Conversion to Fuels or Fuel Components

THERMOCHEMICAL ENGINEERING

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Crans, Debbie - CSU • Processing Lipid Biomass Using DeCarboxylation Reactions

Desjardins, Olivier - CU • Enabling the Use of Detailed Chemistry in Numerical Simulations of Biomass Conversion Through Novel Tabulation Strategies

Herring, Andy - CSM • Facilitating Pyrolysis Oil Reformation Through Feedstock Quality

Medlin, Will - CU • Bimetallic Catalysts for Selective Reduction of Acids with Retention of Olefins

Qian, Xianghong - CSU • Catalytic Conversion of Glucose to 5-Hydroxymethylfurfural

PROCESS ENGINEERING

Bedinger, Pat - CSU • Testing a Potential Cell Wall Anchor Protein Motif

Liang, HJ - CSM • Development of a Nanoparticle-based Coagulation Method for Cost-effective Microalgae Harvesting

Liberatore, Matt - CSM • Processing and Viscosity of High Concentration Algal Suspensions

Noble, Rich - CU • Surface Modified Zeolite Membranes for Alcohol/Water Separations

PRODUCT ENGINEERING

Marchese, Anthony - CSU • Demonstration of Bio-Alcohol/FAME Blends with Tailored Low Temperature Chemistry for Enhanced Homogenous Charge Compression Ignition (HCCI) Engine Performance

Weimer, Alan - CU • Pyrolysis of Biomass to Produce Biochar and Syngas

SYSTEM ASSESSMENT AND ANALYSIS

Bradley, Thomas - CSU • Lifecycle Sustainability Assessments for Microalgal Biofuel Production

Braun, Rob - CSM • Evaluating the Cost and Performance Benefits of Integrating Emerging Technologies into Thermochemical-based Biorefineries

Braun, Rob - CSM • Assessing the Impact of Biorefinery Economy-of-Scale and Feedstock Resource Mix on Biofuel Supply Chains Through Integrative Systems Modeling

(Desjardins) Seeks to enable the use of detailed chemical kinetics in numerical simulations of biomass thermochemical conversion. A novel tabulation strategy will be developed in order to reduce the cost of chemistry integration by several orders of magnitude. The tabulation will be based on a few quantities that form a low-dimensional manifold (LDM) capable of representing the full composition space with good accuracy.

(Herring) This proposal aims to demonstrate the use of more homogeneous substrates to produce simpler pyrolysis oils that can be used with robust catalysts for the steam reforming of pyrolysis oil that will minimize coke formation and maximize syngas yield.

(Medlin) Investigators propose to test bimetallic catalysts for the selective reaction of unsaturated organic acids to produce unsaturated alcohols. The bimetallic compositions involve an oxophilic metal alloyed with a Pt-group metal, and have previously been shown to be effective for similar reactions of unsaturated aldehydes.

(Liang) This project will develop a novel coagulation method for microalgae harvesting that is: 1) Cost-effective: the involved coagulation materials can be mass-produced at low cost, re-usable, and requires no post-harvesting processes to be removed; 2) Efficient: the de-watering process is quick, has high concentration factor, requires low maintenance, consumes little energy, and can be operated continuously; and 3) Inert to downstream processes: the involved coagulation materials impose no interference to downstream processes and can be conveniently recovered.

(Liberatore) First, the processing of algal suspensions will be explored by characterizing the viscosity of algae suspensions under conditions likely to be encountered in microalgae-to-fuels processes. Second, this research will identify critical shear rates required to (1) decrease aggregation of algae, (2) lyse the algae cells, and (3) "temporarily" lyse without killing the cells.

(Marchese) FAME/alcohol blends will be formulated with various octane number, cetane number, and volatility characteristics. Experiments will be performed in a rapid compression machine (RCM) which enables the single shot compression ignition of a homogeneous fuel/air mixture. Experiments will also be conducted with primary reference fuels (PRF) to determine if all fuels with the same octane number and cetane number behave similarly in HCCI mode. All experiments will be accompanied by CHEMKIN modeling using available methyl ester, alcohol and PRF mechanisms.

(Weimer) Aims to develop an understanding of the effect of pyrolysis temperature on the product distribution (i.e. biochar and syngas compositions/yields) from *Miscanthus x giganteus*. Will determine the effect of biomass source using 20 different rice lines on the product distribution. Examines the impact of *Miscanthus x giganteus* biochar addition on the yield of *Miscanthus x giganteus* over one growing season and develops a preliminary AspenPLUS process model for biomass pyrolysis to develop material and energy balances for various yields of biochar vs. syngas. A preliminary process economic evaluation will be conducted to assess the impact of biochar carbon credits on the distribution of biochar/syngas products.

(Bradley) This effort proposes to extend the existing model for evaluation of energy and GHG emissions through the addition of modules to capture the effects of plant construction, N₂O output, co-products allocation, land use change and water footprint. The result of this effort will be a more comprehensive lifecycle environmental impact assessment model for the algae biofuel process.

(Braun) Project will evaluate the potential cost and efficiency performance benefits of integrating emerging syngas cleanup (warm desulfurization), process purification (ITM and membrane reactors), and power production (fuel cells) technologies with thermochemical-based biorefineries. The research will be carried out through modeling and simulation in ASPEN Plus™ and will draw upon synergistic research efforts being carried out at CSM in collaboration with various research partners.

C2B2 is a cooperative research and educational center devoted to the conversion of biomass to fuels and other products, supported by state, institutional, and industry funds. The mission of C2B2 is to become the world's leading center in biorefining and biofuels research and education.

We provide private industry with one-stop access to researchers, laboratories, students, and educators from four innovative institutions, each having unique strengths in biofuel and biorefining application areas.

Colorado Renewable Energy Collaboratory

Created to develop energy technologies for rapid commercialization, the Collaboratory consists of the following institutions:

University of Colorado at Boulder

Ranked in the top 25 nationally in Chemical and Biological Engineering, Molecular and Cellular Biology, and Biochemistry.

Colorado State University

Ranked in the top 10 nationally in Agricultural Sciences with an internationally renowned Engines and Energy Conversion Laboratory.

Colorado School of Mines

One of the few universities uniquely focused on energy research.

National Renewable Energy Laboratory

The only national laboratory dedicated to renewable energy and energy efficiency research and development (R&D).

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One advantage membrane extraction holds over more conventional extraction methods in this setting, is its ability to minimize the transfer of organic-phase compounds into the hydrolysate that can be toxic to the microorganisms used in the fermentation process. Not only did the membrane extraction show an ability to remove the targeted toxic compounds, it increased the pH level in the hydrolysates from 1.0 to 5.0. This reduces the amount of ammonium hydroxide needed to raise the pH of the hydrolysate to near 6, which is the value required for the fermentation process.

Soon, the CSU team's focus will shift to cost modeling in order to explore the practical economic feasibility of incorporating the extraction approaches pioneered by the team. It's unclear, says Wickramasinghe, whether this method of membrane extraction alone will be adequate to prepare the feedstock for conversion, or whether additional detoxification steps are needed. That in turn raises questions about the economic viability of such an approach, he says. "Really, it's about finding ways to improve the cost-effectiveness of this specific aspect of biorefining."

"The push to develop commercially viable renewable fuels is international in scope. Albeit on a smaller scale, so too is the search for improved extraction techniques in the bioethanol production process," notes Wickramasinghe. While his CSU team continues its work in Colorado on behalf of NREL, it is also collaborating with the engineering school at the University of São Paulo in Brazil as part of a reciprocal relationship in which researchers from the two

schools are jointly exploring various detoxification approaches using membrane technology. The thrust of the collaborative effort, he explains, is to identify an extraction method that is flexible enough to detoxify other types of biomass, such as sorghum, coffee husks and sugar cane, a popular feedstock in Brazil's bustling biofuels industry.

Ultimately, says Wickramasinghe, the current work at CSU and NREL could lead in promising new directions. For example, results of the project thus far suggest that in the production of bioethanol, it may be worthwhile to explore combining a fermentation process using membrane extraction with a thermochemical reaction process. "That could make [producing bioethanol] much more efficient," he says.

There is also the potential to develop an extraction process capable of isolating and concentrating the extracted toxic compounds so that they can be packaged and sold into the market instead of handled and discarded as toxic waste material. "For example," he notes, "acetic acid, besides being a key ingredient in household products like vinegar, also has applications in printing processes and in the production of photographic film, textiles, plastics and solvents."

It is yet another example of how seemingly esoteric research, conducted with little fanfare at venues such as NREL, CSU and C2B2, can prove promising enough to make a splash in the commercial mainstream.