FALL 2010

C2B2

Colorado Center for Biorefining and Biofuels

ABUZZ ABOUT ALGAE

Colorado State University emerges as a national hub for algal fuels research

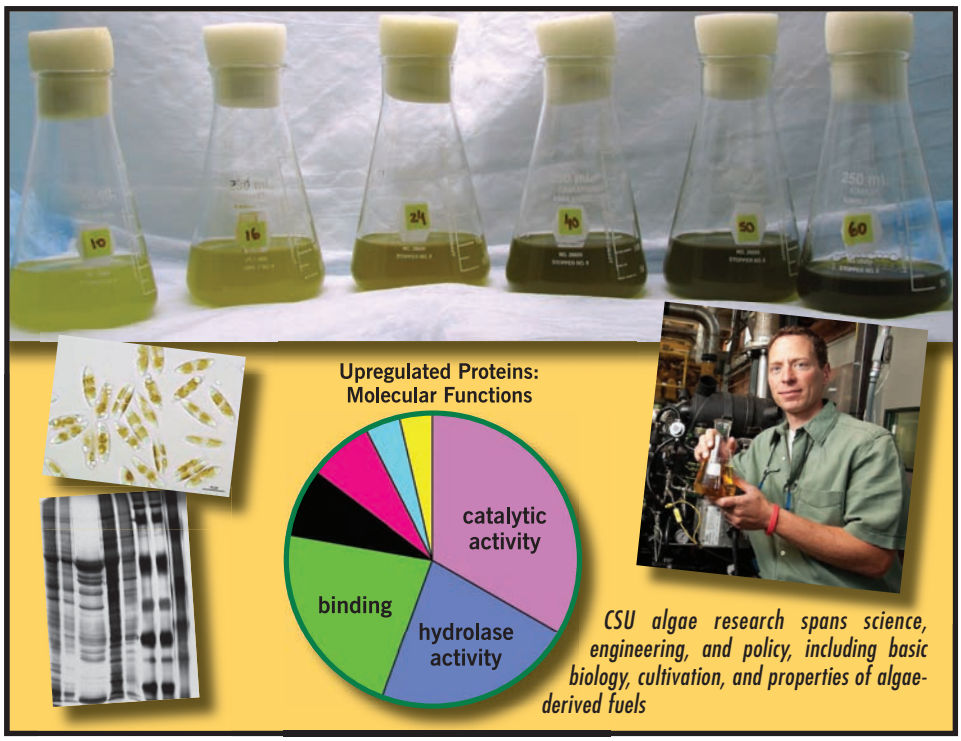
If it's an algae-to-biofuels project with ties to Colorado, chances are Ken Reardon and his colleagues at Colorado State University (CSU) are involved. From feedstock cultivation and evaluation to end-use technology development; from narrowly focused local collaborations to sprawling federally funded projects, CSU's team has its hands in virtually every aspect of the effort to develop technologies and processes for the conversion of micro-algae to biofuels.

Reardon, a professor in the Department of Chemical and Biological Engineering, says he welcomes all the activity because it means he and his CSU colleagues are playing an important role in pushing algae-derived fuels closer to commercial viability. To get there, he adds, "the algal biofuels industry needs advances in all [key] research areas."

There are four key areas in which CSU researchers are actively pursuing projects:

- **algal biotechnology and cultivation**, where, according to Reardon, researchers from the school are working on characterizing and modifying micro-algal strains to improve biofuels characteristics. They are also working to develop better cultivation systems, as well as new ways to monitor algae "crops" and extract lipids. Team members June Medford, Bryan Willson, Chuck Henry, Christie Peebles, Anthony Marchese, Gordon Smith and Stephen Chisholm are involved in these projects, along with Reardon.
- **conversion**, where Reardon and colleague Debbie Crans are leading efforts to develop methods to produce different fuels and chemicals from algal biomass.
- **product science and engineering**, where Marchese and his group at the Advanced Biofuel Combustion and Characterization (ABC^2) Laboratory [part of CSU's Engines and Energy Conversion Laboratory (EECL)] are analyzing the emissions properties of algae-derived biofuels. In addition, Shawn Archibeque is evaluating the suitability of residual algal biomass for livestock feed.
- **environmental assessment and public policy**, where CSU's Tom Bradley and Jenna Bloxom have developed life-cycle assessment models and are studying public policies related to algal biofuels.

The list of partners and projects in which CSU's team of researchers is involved is a lengthy one. Among the most notable projects on the list is a new U.S. Department of Energy (DOE) funded, algae-focused consortium, the National Alliance for Advanced Biofuels and Bioproducts, in which CSU and Solix Biofuels, Inc. (C2B2 Sponsor) are Colorado representatives. Solix, a private spin-off of the EECL, and CSU researchers are collaborating in the consortium effort to identify algae strains, growth conditions, and processing methods that offer the most promise for large-scale cultivation to produce oil for conversion to biofuel. In a separate but related project, EECL and Solix researchers are working to develop photobioreactors for the mass production of algae as a biodiesel feedstock, says Reardon.



CSU algae research spans science, engineering, and policy, including basic biology, cultivation, and properties of algae-derived fuels

Also under the auspices of the new DOE consortium, Marchese is analyzing the properties of algae-produced fuels to determine their viability as replacements for conventional petroleum-based fuels. In addition, Reardon and Archibeque are investigating methods for

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C2B2 2010 SEED GRANT/POSTDOCTORAL FELLOWSHIP 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 2010 C2B2 Seed Grant/Postdoctoral Fellowship Program. Proposals undergo an academic review and are selected for funding with the help of C2B2 Sponsoring Members.

Proposal titles below have been organized according to C2B2 Research Thrust and listed alphabetically by lead principal investigator. The five proposals selected for funding beginning in January 2011 are delineated in **BOLD**.

(Broz/Bush) *This research will further understanding of the ways in which water deficit influences both physiological and molecular parameters that control overall productivity and cell wall composition of switchgrass. The information gathered on biomass production and feedstock quality is expected to be important for energy crop producers and the lignocellulosic ethanol processing industry. Due to the perennial nature of switchgrass, early indicators of drought stress tolerance and feedstock quality will be instrumental in accelerating attempts to improve this new energy crop.*

(Darzins) ***This project is a renewal of a previous seed grant whereby C2B2 supported the successful establishment of a bioenergy focused collection of diverse microalgae. The objective of this further research is to identify and characterize the most promising lipid producing algae in the C2B2 culture collection and to evaluate high-throughput techniques to rapidly assess the biofuel feedstock's potential for previously uncharacterized microalgal isolates.***

(Leach) *The goal of this research is to adapt a high-throughput screening protocol to enable rapid and inexpensive screening of large plant populations for quantitative and qualitative differences in cell-wall composition. Plant cell-wall composition can influence the efficiency of downstream processing of feedstock into biofuels. This high-throughput protocol will enable crop breeding programs to optimize cell-wall composition for multiple processing methods. Furthermore, the protocol will facilitate identi fication of genes that govern variation in feedstock composition, expediting targeted future improvement.*

(Reardon) *Many species of microalgae are capable of accumulating high levels of lipids, which can be extracted and converted to biodiesel or green diesel. However, there is a need to improve the economics of algae-to-biofuels processes through the production of additional products from the algal biomass. Successful completion of this project will result in the generation of algal strains that express a valuable industrial enzyme. The research also aims to produce an analysis of the metabolic impact of recombinant protein production on the algal growth rate and the characterization of the cost-benefit ratio of extracting a recombinant protein in addition to lipids.*

(Vivanco) *This research proposes to map the specific proteome profiles of adapted (native) and non-adapted soils for several model biomass-soil microbe environments in order to determine the baseline degradative enzymes common to general biomass degradation. The research also plans to correlate specific enzyme activities to biomass types and cell wall features. Understanding the mechanisms behind this ecological concept will lead to the development of less expensive methods to degrade lignocellulosic biomass for biofuels production.*

(Kaar) *The goal of this project is to develop an approach to activate cellulases in Ionic Liquids (ILs) and thus improve the efficiency of cellulase hydrolysis using reverse micelles as hosts for celluase in ILs. Conventional solvent systems that dissolve cellulose are toxic and environmentally polluting and thus impractical for large-scale cellulose processing. This proposal seeks to determine the utility of this emerging class of heterogeneous solvent systems to solubilize high concentrations of cellulose while supporting cellulase activity. A major aspect of the proposed work is to elucidate the fundamental relationship between enzyme structure, function, and environment in such solvent systems.*

FEEDSTOCK ENGINEERING, PLANT BIOTECHNOLOGY & CROP SCIENCE

Ali, GulShad – CSU • In Planta Degradation of Lignin Using Fungal Enzymes

Bhaskar, Singh/Sharvelle, Sybil - CSU • Anaerobic Digestion of Glycerol Obtained as Byproduct from Biodiesel Synthesis and Wastewater Containing Oil for Generation of Methane

Broz, Amanda/Bush, Daniel - CSU • Impact of Water Deficit on Biomass, Feedstock Quality and Gene Expression in Multiple Varieties of Swtichgrass

Darzins, Al - NREL • Renewal for 'Characterization and Optimization of Lipid Accumulation in Oleaginous Algae from the C2B2 Culture Collection'

Leach, Jan - CSU • Optimizing Biofuel Feedstocks: Rapid Screening and Identification of Crops Tailored for Downstream Processing

Reardon, Ken - CSU • Recombinant Protein Production in Algae for Improved Biofuel Economics

Vivanco, Jorge - CSU • Co-adapted Soil Microbes Play an Efficient Role in Biomass Degradation

BIOCHEMICAL ENGINEERING

Kaar, Joel - CU • Biocatalytic Conversion of Biomass in Water-in-Ionic Liquid Microemulsions for Efficient Bioethanol Fuel Production

BIOCHEMICAL ENGINEERING continued

Maupin, Mark - CSM • Evaluations of the Enzymatic Depolymerization of Cellulase by Means of Reactive Molecular Dynamics

(Maupin) *Several major obstacles remain with regard to the viability of cellulosic ethanol including feedstock production and logistics, conversion science and technology, and the distribution infrastructure, which all contribute to its current high production cost. This research aims to reduce this high production cost by determining a more efficient means of hydrolyzing enzymes (conversion science and technology). The options being investigated are more economic production of the enzymes (typically harvested from bacteria), discovery of new and more efficient enzymes, and/or engineering a more effective enzyme using rational design and directed evolution.*

Randolph, Ted - CU • Pressure-Swing Desorption for Cellulase Recycling

(Randolph) *This research is focused on the cost of enzymes lost to adsorption and/or unfolding during the biomass saccharification processes. The combination of the novel processing technique of pressure-swing enzyme desorption and new, state-of-the-art spectroscopic techniques to examine enzyme structure in lignocelluloses slurries will provide not only higher saccharification yields, but also insight into the role that adsorption and surface-induced unfolding of enzymes may play in decreasing yields. The ability to desorb and refold enzymes offers great potential for improving process yields and economics.*

THERMOCHEMICAL ENGINEERING

Aravindan, R/Wickramasinghe - CSU • Detoxification of Biomass Hydrolysates by Reactive Membrane Extraction in Hollow Fibre Reactor and Evaluation of its Acetone-butanol-ethanol (ABE) Fermentation Efficiency

Hrenya, Christine - CU • Modeling of Biomass Gasifiers

Iisa, Kristiina - NREL • Catalytic Pyrolysis of Lignin

(Iisa) ***The aim of this seed grant is to determine the efficacy of catalytic pyrolysis for producing high-quality bio-oil from lignin. In addition to standard inert atmospheric conditions, the impact of hydrogen and slightly elevated pressures on the catalytic conversion of lignin will be evaluated. The expected outcomes from the research are quantification of the impact of temperature, pressure, and gas atmosphere (H2) during catalytic pyrolysis of lignin, and development of an efficient process of converting lignin to transportation fuels.***

Liang, Hongjun - CSM • Development of Novel Core-corona Structured Block-copolymer Beads as Solid-phase Flocculants for Cost-effective Microalgae Harvesting

Marchese, Anthony - CSU • A Novel, Energy Efficient Method for Lipid Extraction from Microalgae Combining Algicidal Cell Lysis and Subcritical CO2.

Neltner, Brian/Weimer, Al - CU • Catalysts for Upgrading Pyrolysis Oils

(Neltner/Weimer) ***This project proposes the formation of several new catalysts deposited using atomic layer deposition (ALD) on various substrates. The goal of this research is to produce inexpensive, highly active, and stable catalysts for the conversion of pyrolysis oil into higher value hydrocarbon products.***

Pellegrino, John - CU • Lipid Fractionation from Wet Algae

(Pellegrino) ***This proposal addresses C2B2 sponsor topics related to technology for algal biomass processing with an emphasis on lipid extraction and other biomass fractionation. A two step extraction will be studied using mixed solvents and membrane-based separations. Use of solvent-resistant nanofiltration (SRNF) as the final purification step enables the energy efficient recycle of the mixed solvents while recovering the lipid fraction. This joint project combines membrane separations expertise (CU) with state-of-the-art algal biomass knowledge and analytical resources (NREL).***

Richards, Ryan - CSM • Nanostructured Catalyst Engineering for Thermochemical Biomass Conversions to Fuels

(Richards) *The proposed project is an extension of a 2008 C2B2 seed grant. The initial research was focused on examining a portfolio of nanostructured catalysts as potential models for future biomass conversion technologies (syn gas tuning and pyrolysis). This seed grant proposal is directed towards utilizing the results from the initial investigations (and parallel projects at NREL) for development of second generation catalyst systems that may exhibit significantly enhanced properties.*

Richards, Ryan - CSM • Nanostructured Catalysts for converting algal biomass to fuels

Zhang, Wei - CU • Efficient Extraction of Algal Oils with Recyclable and Tunable Ionic Liquids

(Zhang) *The aim of this proposal is to explore the use of structurally tunable ionic liquids (ILs) to partially dissolve algae thin cell walls (a few m thick) and release algal oil from its wet slurry in an energy efficient and environmentally friendly way. The extraction process will be conducted with gentle heating by sunlight or microwave. Design and synthesis of ILs as well as the oil extraction process development and optimization will be key research elements of this project.*

PRODUCT ENGINEERING

Dorgan, John - CSM • Enhanced Utilization of Lignin: Novel Impact Modifiers for use in the Plastics Industries

(Dorgan) ***Lignins available from both traditional pulping and the organosolve process will be investigated for use as impact modifiers in plastics. Impact modifiers are compounds added to plastics in order to increase their fracture toughness (i.e. impact strength). The plastics industry is interested in improving sustainability and many customers are seeking "green" plastic products incorporating renewable content. This seed grant represents a step in both directions.***

SYSTEM ASSESSMENT AND ANALYSIS

Marchese, Anthony - CSU • Experimental Verification of an Economic Model for Scalable Lipid Extraction Using Supercritical CO2.

(Marchese) *Midstream processes of algae harvesting, dewatering and lipid extraction remain as critical path issues if algal biofuels are to achieve substantial penetration into the mandated advanced biofuels portfolio. One of the major issues confronting the development of a commercially viable algae-to-fuel industry is extraction of triacylglycerides (TAG) from the algae. Preliminary studies on supercritical CO2 extraction suggest that this approach has the potential as a viable lipid extraction technology. However, additional data is needed to develop a detailed economic model to verify that this technology is commercially scalable.*

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C2B2

C2B2 is a research center of the Colorado Renewable Energy Collaboratory
This cooperative research and educational center is 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.

C2B2 provides 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.

The Colorado Renewable Energy Collaboratory was 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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adding value to the byproducts from the algae-to-oil process. Specifically, Archibeque's research is focusing on the potential use of biomass leftover from the algae-to-oil conversion process as cattle feed, while Reardon is investigating whether leftover biomass can be used to make alcohol that ultimately can be converted to another usable fuel such as ethanol or butanol. "The grant has only recently started," he says, "but the research is moving quickly." Marchese, meanwhile, is working at the EECL to characterize these fuels to determine if they're suitable for commercial engines.

As CSU's Site Director and liaison to C2B2, Reardon says he recognizes that the DOE-funded work he and other researchers are conducting in Fort Collins is inextricably linked to the activities of C2B2 and to the overall success of the biomass-to-biofuel research, development and commercialization effort, both domestically and internationally. Indeed, he notes, previous work on a C2B2-funded seed grant ("Bioconversion of Extracted Algal Biomass into Ethanol") helped pave the way for Reardon and his colleagues to land funds from the DOE consortium.

Some of those funds, adds Reardon, are helping bolster the fuel characterization capabilities of the Marchese-run ABC^2 Laboratory within the EECL. Marchese is working on a study of emissions from engines running on biofuels produced from algae. That bodes well, he says, for C2B2 members who want to have any biofuel they're developing evaluated for combustion chemistry, pollutant formation, engine emissions and fuel/lipid/feedstock properties.

Reardon and his colleagues are busy with non-DOE micro-algal projects as well. Several CSU researchers are involved in an effort funded by the National Science Foundation to develop an interdisciplinary bioenergy program for doctoral students. Additionally, C2B2 Sponsoring Member Cobalt Technologies has enlisted CSU's newly formed Sustainable Bioenergy Development Center (SBDC) to test engines running on a gasoline-butanol blend made with biobutanol produced from beetle-killed lodgepole pine trees. Focus areas for the SBDC include sustainable crops and agricultural strategies, biomass processing technologies, biofuel characterization and engine adaptation, byproducts for sustainable biorefining and sustainability assessments.

The overarching goal of all these projects is to advance the commercial viability of algae-derived biofuels. Algae is recognized as a particularly promising biofuel feedstock because it doesn't compete with the global food supply as do other feedstocks such as corn and soybeans. What's more, it has the ability to yield fuel in much greater volumes while using much less energy than other biomass options.

Still, a great deal of progress must be made in several research and development fronts before that capability is achieved. Fortunately, says Reardon, "we have a bunch of really good people working in a lot of different areas," toward the goal of bringing algae-derived biofuels to commercial prominence in the United States.