Energy Biosciences Institute

# EBIBULLETIN

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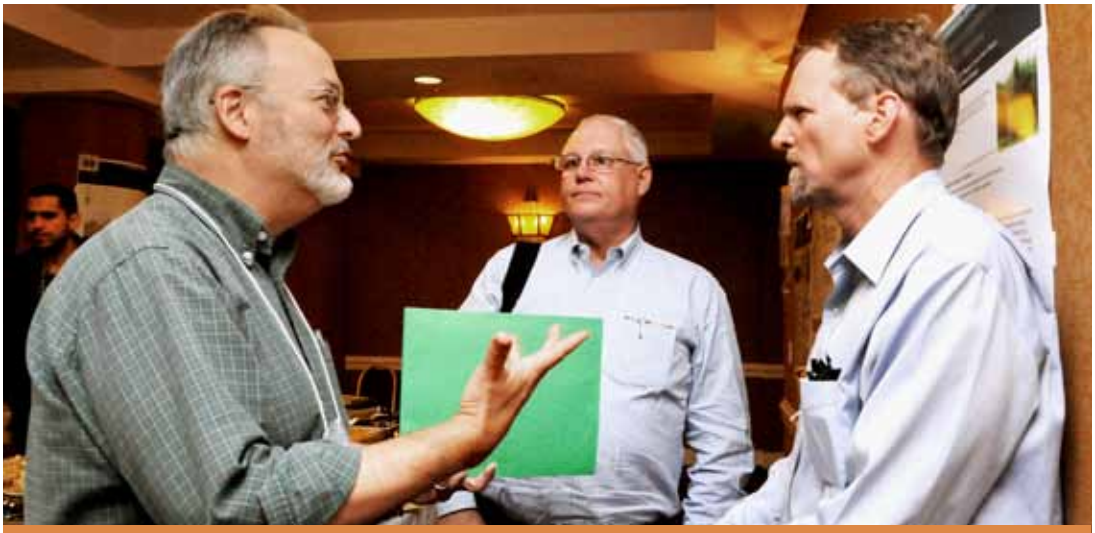


UC BERKELEY INVESTIGATORS KEITH GILLESS (LEFT) AND BILL STEWART (RIGHT) TALK SHOP WITH UNIVERSITY OF ILLINOIS AGRONOMIST TOM VOIGT AT THE 2011 EBI RETREAT.

## IN THIS ISSUE



# BRINGING IT ALL TOGETHER: EBI RETREAT PROVIDES FOCUS TO THE MISSION

They came out of their laboratories and their offices, more than 300 scientists and students and staff from the Energy Biosciences Institute, converging at a hillside retreat in Berkeley in August for three days to learn about the "big picture."

That was the theme of this second all-hands EBI conference arranged by management to bring together the disparate groups of investigators that make up this unique partnership between university and industry. Now in its fourth of 10 scheduled years of work, the institute paused to take inventory and talk about the path forward in the context of a unified vision.

The mission to apply biological research to the problems of energy has occupied more than 400 researchers in a half-dozen defined workgroup areas in Berkeley and in Illinois, most of them comparing notes at multiple common-interest workshops each year. But, as EBI Director Chris Somerville said in the retreat's welcoming address, "seeing the whole picture is also very important."

That picture includes a variety of "Grand Challenges," most of them dependent upon cross-disciplinary understanding to solve. Hence the goal of the meeting at the Claremont Hotel—to bring clarity and context to everyone's role in the EBI program, and to enable essential collaborations through interaction and conversation.

By any measure, the unusual three-campus partnership with funding agent BP has been a success since its inception in 2007: nearly 400 academic papers published and a list of disclosures—public notices by researchers about their work—that has surpassed 1,000.

Yet challenges remain, and as a first exercise in the Claremont Ballroom, all attendees were asked to identify what they considered the most important hurdles yet to clear in their quest to find and deploy biology-based alternatives to fossil fuels. It was a smash-up of disciplines, 8 to 10 people per table, 23 groups drawing on paper tablecloths their visions of the problems at hand. Some worksheets looked like chaotic universes of orbiting ideas, others like a center-city traffic jam of interconnecting roads and colliding vehicles.

The point was made: connections are everywhere. No matter the challenge—the economics of production, policy incentives, conversion efficiency, feedstock availability—it seemed the lines of individual responsibility came together in a nexus of common ground.

(cont. on page 10)

# STATE OF THE INSTITUTE 2010: HOPEFUL ENERGY, PRODUCTIVE FIRST STEPS

The Energy Biosciences Institute completed its third year of work in 2010, a noteworthy period of research captured in image and word in the recently published EBI Annual Report. The institute's directorate—Chris Somerville, Steve Long and Paul Willems—in the report's introductory message marked the milestone this way:

"The EBI's initial three years have been filled with a hopeful energy, tireless dedication of researchers and support staff, and productive first steps on our journey. We are privileged to lead the EBI forward… Many brilliant and engaged people who had not previously worked on topics related to energy biosciences are now testing their ideas, and we expect an outpouring of innovation and creativity."

The 82-page volume, which includes brief progress reports of all 68 programs and projects active during the year, is available at EBI headquarters in Berkeley (Calvin Laboratory) and Illinois (the Institute for Genomic Biology), and will be mailed upon request by writing to sleblovic@berkeley.edu. It can also

(cont. on page 9)

# EBI MODEL SHOWS SWITCH FROM CORN TO GRASS REAPS BENEFITS

Using a computer model to simulate how soil and environmental conditions would change if corn plantings were replaced with dedicated energy crops, EBI researchers have concluded that farmers could increase overall corn yields and ethanol output while improving water quality and reducing greenhouse gas emissions.

UNIVERSITY OF ILLINOIS RESEARCHERS EVAN DELUCIA AND SARAH DAVIS.

Principal investigator Evan DeLucia at the University of Illinois at Urbana-Champaign headed the research team that included EBI bioenergy analyst Sarah Davis and scientists from Colorado State University and the USDA Agricultural Research Service. Their report appears in the journal *Frontiers in Ecology and the Environment*.

A move from corn to next-generation feedstocks Miscanthus and switchgrass could switch the corn belt of the Midwest from a net greenhouse gas source to a sink, according to the study. Pointing out that 30 percent of the 2009 corn crop was dedicated to ethanol, the authors argue that redirecting the least productive land on which that corn was planted could have a significant impact on domestic land use without triggering major food and feed market changes.

Such a switch could achieve 82 percent more etha-

(cont. on page 9)

UNIVERSITY OF MARYLAND POSTDOC JOEL GRAHAM COLLECTS SAMPLES NEAR DECOMPOSING WOOD IN A BOILING HOT SPRING IN NEVADA.

# SOME (MICROBES) LIKE IT HOT…AND STILL DIGEST CELLULOSE

Energy Biosciences Institute bioprospectors recently found a microbe in a Nevada hot spring that happily eats plant material—cellulose—at temperatures near the boiling point of water. And that's good news for EBI researchers, since industrial processes like biofuel production often require extreme heat which frequently renders enzymes recalcitrant or impotent.

The newly discovered hyperthermophilic microbe includes a cellulase—an enzyme that digests plant fiber—that is the most heat-tolerant of any previously found, including archaea and bacteria. The cellulase is most active at a record 109 degrees Celsius (228 degrees Fahrenheit). By comparison, the enzymes currently being used to break down plant cellulose are comfortable at around 50 degrees C and become unstable at higher temperatures.

"These are the most thermophilic archaea discovered that will grow on cellulose and the most thermophilic cellulase in any organism," said principal investigator Doug Clark of UC Berkeley, a co-author of the paper that appeared in *Nature Communications*. "Our hope is that this example or examples from other organisms found in extreme environments—such as high-temperature, highly alkaline or acidic, or high salt environments—can provide cellulases that will show improved function under conditions typically found in industrial applications, including the production of biofuels."

Clark and UC Berkeley colleagues Melinda Clark, Dana Nadler, Sarah Huffer, Harshal Chokhawala, and Harvey Blanch, along with Frank T. Robb and

(cont. on page 11)

EBIBULLETIN
SUMMER 2011 // VOLUME 5, ISSUE 2

REGULATORY ASSOCIATE TIMOTHY SLATING (LEFT) WITH ILLINOIS LAW PROFESSOR JAY KESAN.

# EBI-ILLINOIS RESEARCHERS FIND REGULATORY HURDLES HINDER DEVELOPMENT OF BIOFUELS MARKET

At the same time that the United States has established ambitious production mandates for biofuels, it maintains a regulatory system that by its nature serves as a disincentive toward commercialization of emerging alternative fuels, according to a recent EBI study.

University of Illinois law professor Jay Kesan and regulatory associate Timothy Slating argue in their report that regulatory innovations are needed to keep pace with technological innovations in the biofuels industry.

"Getting regulatory approval for new biofuels is currently a time-consuming and costly process," said Kesan, program leader of the Biofuel Law and Regulation Program in the EBI. "By removing some of the uncertainty and some of the expense without compromising on the regulatory concerns, you are also removing some of the disincentives to entering the biofuel market, where we need more competition."

Their sample case was biobutanol, an alternative fuel which has a higher energy content than ethanol. Biobutanol is compatible with existing vehicle engines as well as with existing fuel distribution infrastructure and thus holds promise as a transportation fuel of the future.

Kesan and Slating's study describes and elaborates on the effects of the federal Renewable Fuel Standard and the Clean Air Act's regulatory framework for the commercialization of new fuels and fuel additives like biobutanol.

"Since biobutanol can help us meet the Renewable Fuel Standard's mandates much more quickly and effectively, it makes good economic and policy sense to line up our regulatory processes to facilitate its commercialization," said Kesan.

According to Kesan, it's not clear that the U.S. can meet all the renewable fuel mandates required under the Renewable Fuel Standard. By 2022, the standard mandates the production of 21 billion gallons of advanced biofuel, excluding corn-based ethanol. Biobutanol is among the options to help meet the mandate.

Kesan and Slating note that under existing regulations, biobutanol can lawfully be blended with gasoline in a concentration of only 11.5 to 12.5 percent by volume. Although regulations provide a mechanism for fuel manufacturers to seek a waiver from the U.S. Environmental Protection Agency to allow higher blending limits, the process is onerous.

"One of the things we're suggesting is to update the regulations to allow higher blending limits for biobutanol," Slating said. "The interesting thing here is that the EPA could actually do this on their own. The regulation that effectively sets the default blending limit for biobutanol is simply an agency interpretation of an undefined phrase enacted by Congress."

Also, he said, the Clean Air Act prohibits fuel manufacturers from commercializing a new fuel that is not "substantially similar" to the fuel that the EPA uses in its emissions certification process. As Congress did not specify what constitutes "substantially similar," the task is left to the EPA's discretion.

Other recommendations the authors make to streamline the approval process include allowing all fuels to be evaluated based upon the same oxygen-content requirement that the EPA has already, and creating a fast-track review process for new fuel waivers for biofuels that have been designated as compliant with the Renewable Fuel Standard.

"If the RFS is going to achieve its goal of incentivizing the deployment of second-generation biofuels, then manufacturers need to be assured that there will be no unnecessary delay in the fuel waiver process," Slating said.

Their research will be published in a forthcoming issue of the *Wisconsin Law Review*.

*-- Phil Ciciora*

# EBI LOOMS LARGE IN *TIMES* STORY ON CELLULOSE

When the *New York Times* story appeared in the July 13 issue, there was former Energy Biosciences Institute researcher Matthias Hess at the top, front and center, introducing what writer Paul Voosen called "one of the defining scientific pursuits of our time"—the campaign to crack the cellulosic challenge.

The EBI would appear later in the story, too, under the sub-headline "BP's $500 million bet." In that section, Voosen describes what he calls "one of the biggest recent steps toward cellulosic ethanol"—solving the problem of using all sugars during fermentation by engineering new yeast strains.

Voosen visited EBI's research sites at Berkeley and Illinois in the spring to gather data for the story, interviewing Hess (while he was removing a nylon mesh sack from a cow's rumen), EBI Director Chris Somerville, and DOE Joint Genome Institute Director Eddy Rubin, among others.

"It may sound strange," he wrote, "but the cellulose work is like Lewis Carroll's 'Through the Looking-Glass,' said Rubin. At one point, Alice hustles after the Red Queen, but she never gains any ground. Likewise, plants have kept their lead over all comers, Rubin said.

"It's been this Red Queen kind of event," he said. "This molecule has evolved ways to prevent water and pests from getting in. It's kept on building new structures … These plants have evolved for tens of millions of years to prevent their breakdown."

"If biofuels are going to make up a large amount of the U.S. fuel mix, scientists will need to chase down this Red Queen. Only then can the incipient bioeconomy, now based on energy-intensive corn, decouple from its competition with food. At their most hopeful, scientists envision fields of ultra-productive grasses and trees growing on degraded land, the plants providing the energy for a new American century.

"It's a vision that has existed for decades. And a dream that won't be easily reached."

Former EBI postdoc Hess, now a Washington State University microbiologist, was featured for his work identifying the microbes that digest switchgrass harvested from a cow's stomach at the University of Illinois' dairy barn. He worked with Rubin and UI's Rod Mackie in the EBI project "Analysis of Bovine Rumen Microbiota Under Different Regimens for Identification of Feedstock-Targeted Cellulolytic Genes."

The team sequenced and analyzed metagenomic DNA generated directly from the microbes. It identified 27,755 candidate genes encoding putative carbohydrate-active enzymes and synthesized 90 candidate genes selected from this pool. The researchers tested the activity of the expressed proteins against 10 substrates, including the potential biofuel feedstocks Miscanthus and switchgrass.

"Perhaps most impressively from the point of pure science," Voosen writes, "the team was able to divine nearly complete genomes for several microbial species, out of an estimated 1,000 bugs expected in the rumen."

Voosen explained the EBI's breakthrough in yeast fermentation and quoted Somerville: "We've figured out how to engineer strains to simultaneously (use) all the sugars with very high rates. It's good enough that the company moved it into commercialization already." Berkeley doctoral student John Galazka and Illinois professor Yong-Su Jin are referenced for their work on the program.

BP's cellulosic ethanol refinery, planned for opening in 2013 in Highlands County, FL, is mentioned, a $400 million facility that will produce annually 35 million gallons of cellulosic ethanol from energy cane. Cautioning that the cellulosic biofuel field may not be profitable until 2020, Somerville is quoted as saying, "You've got to understand that it's a big new industrial process. It's a really expensive proposition to put that much steel in the ground. And once you put it in the ground, it's there. You can't just pick it up and put the money somewhere else."

The Joint Bioenergy Institute (JBEI) in Emeryville, CA, a Department of Energy research center that is exploring aspects of the biomass conversion challenge, is also featured in the *Times* story.

The complete article, "No Eureka Moments in Long U.S. Campaign to Crack Cellulosic Code," can be accessed via a link on the "EBI in the News" page under the "News Center" category of the EBI website (www.energybiosciencesinstitute.org).


MELVIN CALVIN, TOP LEFT, AND HIS FOREVER STAMP SCIENTIFIC COLLEAGUES.

# STAMP OF APPROVAL: CALVIN IMMORTALIZED 'FOREVER'

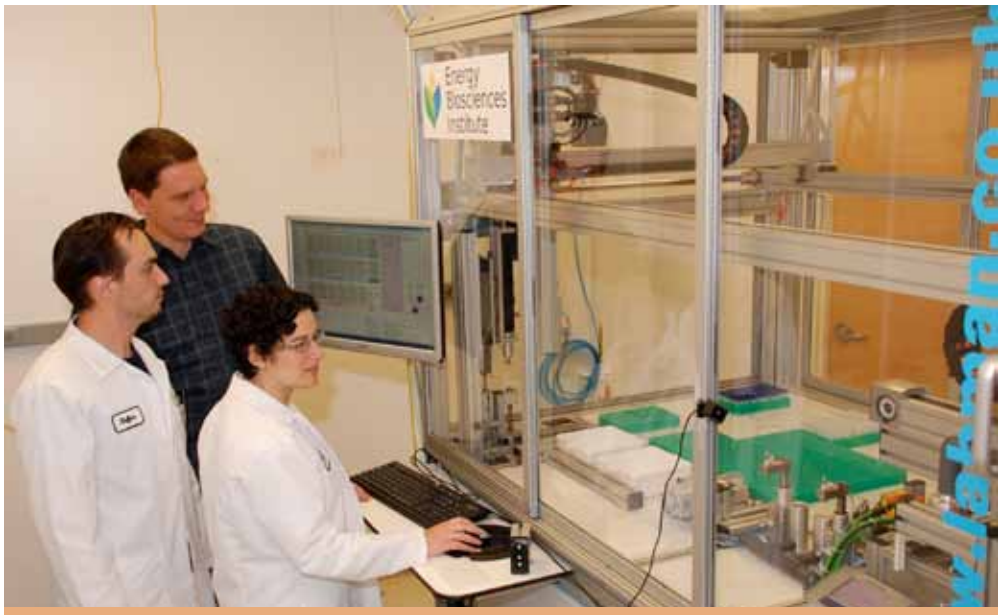
Melvin Calvin, the Nobel Prize-winning namesake of the Berkeley home of the Energy Biosciences Institute, has his famous name on another landmark site—a U.S. Postal Service "Forever" stamp.

The EBI's Calvin Laboratory building was the location in which UC Berkeley professor Calvin became the first scientist to trace in detail the process of photosynthesis, and he conducted pioneering research on using plants as an alternative energy source. He won the Nobel Prize in chemistry in 1961.

He is one of four American scientists honored with the Forever stamp which, when purchased for first-class postage today, will be usable at any future time for one-ounce postage even if the price goes up. Others so honored were botanist Asa Gray, physicist Marie Goeppert Mayer, and biochemist Severo Ochoa. This is the third set in the Postal Services' American Scientists series.

The stamps are on sale nationwide at local Post Offices, online at usps.com and through the toll-free line, 1-800 782-6724.

# SAMPLE PREPARATION FOR EBI SCIENTISTS HAS A SUPERHERO NAMED 'GRASSHOPPER'




LABORATORY MANAGER MARA BRYAN AT THE CONTROLS OF GRASSHOPPER WITH STEFAN BAUER (LEFT) AND MARKUS PAULY LOOKING ON.

One of the newest, fastest and most productive members of the EBI scientific team in Berkeley goes by the name of Grasshopper and does the work of several researchers in the laboratory. Grasshopper is efficient, accurate and precise, and serves the needs of many scientists in EBI programs.

Grasshopper is a robot. The custom-built glass-encased system of mechanical instruments, electronics and software takes up much of Room 143 in Calvin Laboratory. Since April, it has been automatically grinding and weighing plant material in source vials at speeds and accuracy which humans find difficult to match.

The EBI Grasshopper—the name was coined by Berkeley Research Operations Manager Mara Bryan, Analytical Chemistry Group Manager Stefan Bauer, and biochemist Markus Pauly based on the robot's input materials and on the "hopper" storage container used for powder dispensing in another EBI robotic system—is actually a third-generation model. But its upgrades are unique to the industry.

"The bottleneck for every quantitative plant biochemist is the grinding of plant material and weighing it out," says Pauly, who might be considered one of Grasshopper's forefathers. Pauly, principal investigator of the EBI program on understanding plant cell wall structure, helped to design a similar machine at Michigan State University before he moved to Berkeley in 2009.

Preparing plant sample material for quantitative analysis can be tedious and inaccurate, Pauly says, reflecting the traditional method of grinding feedstock into a powder with a mortar and pestle or steel ball milling, then meticulously measuring out exact quantities on a weighing balance. Determining the precise composition of plant materials requires accuracy and sample purity.

Enter grasshopper.

"You can dispense as little as 2-4 milligrams of Miscanthus, or any biomass," says Bauer. "We can use pre-ground materials and weigh them out in about one to two minutes , or use the grinder in the Grasshopper and process each sample in about six minutes. Now, it might take one person to do a range of 20 to 40 samples per week. My goal is to do 100 samples a day."

With the new biomass dispensing and weighing robot, such a goal is viable, and researchers like Pauly and others in the EBI who are analyzing feedstock composition will benefit from the high-production capabilities that Grasshopper provides.

Bryan notes that the unit can work with individual vials or multiple formats of microtiter plates, for people who might, for example, be doing enzyme activity assays on solid substrates. It runs with a simple drag-and-click software interface and can operate unattended for 24 hours, leaving researchers to pursue endeavors other than manual grinding and weighing. The robot can grind individual samples or use pre-ground materials, and it can weigh out milligram-scale subsamples in 1.5-2 minutes, using materials up to 120 microns in size (not just ball-milled material, which is smaller). In addition, the tubes and plates used in the Grasshopper are compatible with the other automated systems at the EBI, such as the Biomek liquid handling robots, so analyses and experiments can go between multiple robotic systems.

Pauly actually developed the idea for such an automated system when he was a scientist at the Max Planck Institute for Plant Physiology in Germany in 2006. There, colleagues worked with a laboratory equipment company in England named Labman to design a basic machine that would grind and weigh plant samples. Its limitation was, because it was used for metabolomics, the robot had to work in minus-80C temperatures to freeze the samples.

When Pauly moved to Michigan and worked with the Department of Energy's Great Lakes Bioenergy Research Center in 2008, he suggested getting the robot out of deep-freeze and expand its utility and throughput capacity. Labman also built the second-generation machine, the first of its kind working with milligram-size samples.

After discussions with the three EBI colleagues in Berkeley, the company enhanced its robot with the addition of a vacuum system to eliminate potential contamination and by tweeking technologies to accommodate higher throughput and a finer grind. Bar-coded vials are tracked by computer through the process, and there are several different types of vials or tubes to which biomass powders can be dispensed.

"It can also be used to prepare samples for quantitatively measuring lipids, starches, or other metabolites," says Pauly, whose program represents about 20 percent of Grasshopper's usage. Bauer and his team of three analytical chemists utilize the robot to prepare samples for characterization of lignocellulosic components and other chemical substances found in the biomass. The quality of the analysis depends in large part on the normalized preparation of the samples.

Other EBI researchers who wish to benefit from Grasshopper's skills should contact Bauer at Stefan.bauer@berkeley.edu

# BP BIOFUEL CEO: PLANT-BASED FUELS ONLY VIABLE OPTION TO CRUDE

EBI Associate Director Paul Willems called Phil New one of the early crusaders for biofuels within energy giant BP, a visionary who saw a future of fuel demand that would outstrip the world's ability to meet it with current resources and reduce global warming at the same time. Today, that crusade is very much alive and well in the company.

"The world's population will surpass 8 billion people by 2030," New told a jam-packed audience of researchers and students in the Calvin Lab conference room in May. "(Transportation) fuel demand will likely be up by 25 percent, declining in OECD areas but increasing with the GDPs of developing (non-OECD) countries. Where is this growth going to come from?"

Not fossil fuels, with the prospect of increasingly expensive supplies, he said. "The world is looking for energy diversity. There is only one viable substitute for crude oil in transport, and that is biofuels."

BP has taken New's prophecy seriously. In addition to funding the Energy Biosciences Institute partnership for 10 years, the company has invested in a major ethanol project in Brazil, a biobutanol joint development program with Dupont, and what will be the world's largest commercial cellulosic biofuel production plant in 2013 in Florida.

Since becoming active in the field in 2006, BP has "made some reasonably interesting progress" in alternative fuel development, according to New, but "we're lonely at the moment. There are not that many folks (investing in advanced biofuels). But we have our game plan, we're putting our heads down and marching forward."

He said in order for biofuels to be successful as an energy source, they will have to meet four main criteria—cost-competitive with fossil fuels, scalable to commercial requirements, sustainable over time, and vastly reduced carbon emissions. New said there are "three levers to pull" to reduce costs—better-yielding feedstocks, rational capital investments, and a less expensive and more efficient conversion process. "And so much of the work done here (at the EBI) will be fundamental to getting those economies down," he told the group. New follows the EBI's progress as a member of the institute's Governance Board.

Brazil "is the only source of sugars in the world that can fit all four categories," he said, so BP has entered the field there in a big way. Recently purchasing two producing ethanol mills and another under development, BP will be studying the operations of 2,500 workers on 50,000 hectares of sugarcane as a potential model for future development.

"We see this as a fully integrated analog of the upstream oil business," New said. "It will be roughly the same as an 8,000-barrel-a-day oil well, but with biomass you have an infinite well that does not deplete over time." BP will be assessing both yield and logistics, in particular the transport connections between field and refinery.

The forthcoming lignocellulosic plant in Highlands County, FL, will also be an important test bed. To be located over the next two years on 30,000 acres of undeveloped land on which will grow energy cane and napier grass, "CP-1" as it's called will produce 36 million gallons of biofuel a year. BP's plan calls for subsequent development of "CP-2," a plant twice that size on the same site, followed by CP-3 and CP-4 along the Gulf Coast. At each step, New said he expects improvements in technology, costs and scale.

"We believe this will be production in a market that's desperate for it," he added. "CP-1 will be a game-changer for energy production in the United States. But it is only the first step in the journey."

Also critical to that journey will be national policy that enables biofuels to develop competitively. New referenced America's Renewable Fuel Standard 2, with its 36-billion-gallon target of renewable transportation fuels by 2022, as "an absolute cornerstone piece of regulation for setting expansion of biofuels in the U.S. We think cellulosic (fuel) will fill a lot of that."

But he warned, "Although the legislation is out there, industry hasn't risen to the challenge. And if we (as an energy community) don't get going, then RFS2 might go, and if that happens, I don't have high hopes for the biofuel industry. Mandates give the investor security to know that there will be a market there. If you are building a whole new crop, it's nice to know that there will be a demand."

PHILLIP NEW

BP is at the forefront of trying to create that market, leading with the first commercial plant in the U.S. And with the promise of advances from research centers like the EBI in feedstock development, biomass conversion, and fuel production, others are sure to follow.

# EBI GOES 'DOWN UNDER' TO COLLABORATE ON BIOMASS PLANT RESEARCH

EBI DIRECTOR CHRIS SOMERVILLE & BIOMASS FEEDSTOCKS (CLOCKWISE): MISCANTHUS, SWITCHGRASS, WOODY CROPS, SUGARCANE, PRAIRIE GRASS.

Illustrating how the Energy Biosciences Institute is truly international in scope, Director Chris Somerville traveled to Melbourne, Australia in late July to extend collaborations between the EBI and the Australian Research Council's (ARC) Centre of Excellence in Plant Cell Walls.

Somerville was a keynote speaker at the 18th International Botanical Congress at the University of Melbourne.

"The two Centers have concentrations of expertise that are unique," Somerville said. "The agreement between EBI and the ARC Center facilitates sharing of these intellectual assets. Additionally, discovering ways to produce sustainable low-carbon fuels is a world problem which demands a world solution, so we see it as desirable to participate in a network of global research centers."

Under the agreement, the EBI will gain access to the biological and technical expertise of the Centre, and the Centre will gain access to expertise and facilities at the EBI, including a team of economists and modelers who will assess Australia's natural resources and infrastructure capacity for producing biofuels. University of Illinois agricultural economist Madhu Khanna will be visiting Australia in the fall to begin that work.

Somerville and his Australian colleagues, Geoffrey Fincher, director of the Centre, and deputy director Tony Bacic, hope that future research will be able to fine-tune the cellulosic conversion process and increase its efficiency. According to a news release issued by ARC during the meeting, the two researchers are in no doubt of the capacity of biofuels to play a significant role in satisfying the world's future transportation fuel needs.

"At present Brazil produces about 40 per cent of its transport fuels from sugarcane grown on about 4 million hectares," Somerville said. "They are planning to expand that to 64 million hectares and eventually to use the bagasse in addition to the sugar. That's why they will become the Saudi Arabia of cellulosic fuels."

The EBI Director also expressed confidence during his visit that the U.S. will meet its target of producing 30 percent of its transport fuels from plants by the year 2030.

The ARC Centre of Excellence in Plant Cell Wall Biology will receive $19.25 million over seven years from the Australian government. Its scientists investigate the central roles of cell walls as renewable sources of transport fuels, as functional foods to improve human health, and as a source of raw materials for industrial processes. The Centre is building an international team with scale and focus at the scientific, technical and training levels to generate outcomes that will significantly enhance biotechnologies that underpin Australian crop industries valued at over $8 billion per year, associated food industries valued at about $40 billion per year, and massive emerging industries related to renewable transport fuels and biomaterials.

ARC Centres of Excellence are prestigious hubs of expertise through which high-quality researchers maintain and develop Australia's international standing in research areas of national priority.

Other international centers with which the EBI has connections include the B-Basic consortium for bio-based sustainable industrial chemistry in Delft, the Netherlands; Peking University in Shenzhen, China; BIOEN in Brazil; and the Indian Institute of Technology-Karaghpur.

# EBI INPUT INCLUDED IN REPORT ON CALIFORNIA'S ENERGY FUTURE

Energy Biosciences Institute bioenergy analyst Heather Youngs is one of the lead authors of the recent report, "California's Energy Future—the View to 2050," and EBI Director Chris Somerville served on the study's working committee. UC Berkeley professor Dan Kammen, an original member of EBI's Executive Committee, and EBI Advisory Group member Lynn Orr of Stanford University were also on the working group.

The report, released in May by the California Council on Science and Technology, outlined the steps needed for the state to be able to meet the requirements of a 2005 executive order that called for reducing the state's emissions 80 percent below the 1990 level by the year 2050.

Bottom line: the technology required to get to the 60 percent mark from electricity and fuel production is either in demonstration or is already in use, according to the study. But getting to the full 80 percent reduction will require significant levels of "research, development, invention and innovation."

The report's conclusion recommends the development of multiple solutions, including aggressive efficiency measures for building, industry and transportation; electrification of transportation and heat where possible; developing emission-free electricity production; and finding low-carbon fuels to supply transportation and heating use. It notes that biofuels are important part of California's future, along with low-carbon fuels that do not require biomass.


EBI BIOENERGY ANALYST HEATHER YOUNGS (CENTER) IS SHOWN AS PART OF A MEDIA APPEARANCE AT THE ANNUAL BIO CONVENTION IN WASHINGTON D.C. IN JUNE. SHE DID TELEVISION INTERVIEWS WITH PANEL COLLEAGUES JIM LANE (LEFT), EDITOR OF BIOFUELS DIGEST, AND PAUL GILNA, DIRECTOR OF THE BIOENERGY SCIENCE CENTER AT OAK RIDGE NATIONAL LABORATORY.

To read the entire report, go to http://www.ccst.us/publications/2011/2011energy/php

A video of Youngs discussing California's energy future can be seen on the California Academy of Sciences web site, http://www.calacademy.org/sciencetoday/the-future-of-biofuels/

# EBI'S ARKIN TO LEAD PLANT AND MICROBE 'KNOWLEDGE BASE'

EBI principal investigator Adam Arkin of Lawrence Berkeley National Laboratory has been selected to lead a U.S. Department of Energy consortium that will assemble "Kbase," an integrated cyber-knowledge base of biological information focused on plants and microbes.

The genomic revolution over the past decade has resulted in the generation of quantities of raw data so immense that they threaten to overwhelm researchers' ability to make sense of them. Thus the DOE's multi-institutional effort is designed to combine information about plants, microbes, and the complex biomolecular interactions that take place inside these organisms into a single resource that will greatly enhance scientists' ability to access and share data, and use it to improve the production of biofuels and other useful products.

The entire Kbase effort, spanning plants, microbes, and metacommunities (microbes in the context of the vast communities in which they live, both in the environment and within other living things) will be led by Arkin, who heads the EBI's Microbial Characterization Facility program.

The new cybercenter will enable scientists to discover currently unknown relationships that exist between species and between groups of species and the surrounding environment—interrelated and interdependent communities of microbes and plants, in this case.

Kbase will be accessible to scientists everywhere.

# SUMMER 2011 EBI PUBLICATIONS

The following journal articles featuring EBI research were published online this summer. Papers can be accessed via the EBI web site—www.energybiosciencesinstitute.org—under the category "Publications":

## JUNE

*O-Glycosylated Cell Wall Proteins Are Essential in Root Hair Growth*, Markus Pauly, Chris Somerville, Sascha Gille, Jose Estevez, et. al., ***Science***, June 17, 2011.

*Systematic Search for Cultivatable Fungi That Best Deconstruct Cell Walls of Miscanthus and Sugarcane in the Field*, Prachand Shrestha, Timothy Szaro, Thomas Bruns, John Taylor, ***Journal of Applied Environmental Microbiology***, June 17, 2011.

*Discovery and Characterization of Heme Enzymes from Unsequenced Bacteria: Application to Microbial Lignin Degradation*, Margaret Brown, Mark Walker, Toshiki Nakashige, Anthony Iavarone, Michelle Chang, ***Journal of the American Chemical Society***, June 14, 2011.

*Biochemical Analysis of Multiple Endoxylanases from the Rumen Bacterium Ruminococcus albus and their Synergistic Activities with Accessory Hemicellulose Degrading Enzymes*, Young Hwan Moon, Michael Iakiviak, Stefan Bauer, Roderick Mackie, Isaac Cann, ***Applied and Environmental Microbiology***, June 10, 2011.

*Xylitol Does Not Inhibit Fermentation by Engineered Saccharomyces cerevisiae Expressing xylA as Severely As It Inhibits Xylose Isomerase Reaction In Vitro*, Suk-Jin Ha, Soo Rin Kim, Jin-Ho Choi, Myeong Soo Park, Yong-Su Jin, ***Applied Microbiology and Biotechnology***, June 8, 2011.

## JULY

*Dissecting Force Interactions in Cellulose Deconstruction Reveals the Required Solvent Versatility for Overcoming Biomass Recalcitrance*, Hyung Min Cho, Adam Gross, Jhih-Wei Chu, ***Journal of the American Chemical Society***, July 28, 2011.

*The Role of Alcohols in Growth, Lipid Composition, and Membrane Fluidity of Yeast*, Sarah Huffer, Melinda Clark, Jonathan Ning, Harvey Blanch, Douglas Clark, ***Applied Environmental Microbiology***, July 22, 2011.

*A Quantitative Proteomic Approach for Cellulosic Degradation by Neurospora crassa*, Chris Phillips, Anthony Iavarone, Michael Marletta, ***Journal of Proteome Research***, July 11, 2011.

*Identification and Characterization of a Multidomain Hyperthermophilic Cellulase from an Archaeal Enrichment*, Joel Graham, Melinda Clark, Dana Nadler, Sarah Huffer, Harshal Chokhawala, Sara Rowland, Harvey Blanch, Douglas Clark, Frank Robb, ***Nature Communications*** , July 5, 2011.

*Solubility and Rate of Dissolution for Miscanthus in Hydrophylic Ionic Liquids*, Sasisanker Padmanabhan, Michael Kim, Harvey Blanch, John Prausnitz, ***Fluid Phase Equilibria***, July 5, 2011.

(cont. on page 11)

## STATE OF THE INSTITUTE 2010: HOPEFUL ENERGY,PRODUCTIVE FIRST STEPS

(cont. from page 1)

be accessed in electronic form on the EBI web site (www.energybiosciencesinstitute.org).

The Directors point to one program in particular that illustrated the successful collaboration structure that is a hallmark of the EBI. A series of published papers reported a multidisciplinary team's development of new yeast strains that can simultaneously utilize all the major sugars that comprise lignocellulose.

"Aside from the importance of the scientific advance," they write, "we consider this body of work to be an outstanding example of what we had hoped to accomplish in the EBI—based initially on a curiosity-driven discovery that is typical of the best university research, and involving a multidisciplinary collaboration supported by a very high degree of collegiality among many labs at different institutions. It capitalized on inherent scientific capabilities in the institute, and it benefited directly from the involvement of BP scientists."

Details on this effort, and on the many studies under way in six different research areas, can be found in *Bioenergy—Exploring the Applications of Modern Biology to the Energy Sector*, the third edition of the EBI Annual Report. Work is focusing on feedstock development, biomass deconstruction, biofuels production, fossil fuel microbiology, environmental services, and the economic and societal impacts of biofuels. The institute's present goal is to probe all aspects of lignocellulosic fuels in order to build a coherent understanding of the overall topic. In deep earth ecology, the goal is to understand the properties of oil reservoirs and their microbes in order to evaluate the potential to enhance fossil fuel recovery, processing, transport and storage.

Since the EBI's inception in late 2007, more than 750 researchers have contributed work to the enterprise, more than 120 papers have been published in scientific journals, and nearly 80 programs and projects have been launched. The Annual Report describes the facilities, support services, and operational procedures that facilitate the work of the institute.

One feature of the report is an overview of current trends in commercial biofuel production, by EBI Bioenergy Analysts Caroline Taylor and Heather Youngs. Among the subjects they review are target fuels beyond ethanol, feedstock options, and social and environmental issues in biofuel development.

"We still have a long road ahead to wide-scale commercial implementation," the analysts conclude, "but progress has spanned the value chain from feedstock production through conversion technology and extending into environmental impact and policy considerations." They note that integration of research across varied disciplines will be critical to developing a commercial-scale industry.

Six profiles of faculty investigators in the EBI are also presented in the report—agricultural entomologist Michael Gray, microbial genomics scientist Yong-Su Jin, and environmental plant biologist Carl Bernacchi, all from the University of Illinois at Urbana-Champaign; biochemist Michelle Chang and resource economist Brian Wright of the University of California, Berkeley; and microbial ecologist Terry Hazen from Lawrence Berkeley National Laboratory.


The fourth partner in the collaboration and source of institute funding, the global energy company BP, has been instrumental in the EBI's success so far, according to the directors. BP scientists and engineers are co-located with their university counterparts at Berkeley, studying the industrial perspectives of energy biosciences.

"Unlike the academic members of the EBI, who are focused on fundamental academic questions, the BP fellows are engaged in envisioning how knowledge might be used in practice. The dialectic between those engaged in basic research and those charged with applied research is fertile ground for the development of new questions and a highly efficient way to translate basic research," the directors write.

The EBI is funded for 10 years. Most of the research teams in Berkeley and the institute's central administrative units will move late next year to a new building now under construction just west of the campus in downtown Berkeley.

## EBI MODEL SHOWS SWITCH FROM CORN TO GRASS REAPS BENEFITS

(cont. from page 2)

nol and 4 percent more grain for food, while reducing nitrogen leaching by 15 to 22 percent and greenhouse gas emissions by 29 to 473 percent, the model showed.

"Globally, agriculture contributes about 14 percent of the greenhouse gases that are causing global warming to the atmosphere," said DeLucia, who heads the EBI's ecology services program. "The whole Midwest has been, since the advent of modern agriculture, a source of greenhouse gases to the atmosphere.'

Miscanthus, a perennial grass highly regarded for its productivity and sustainability, does well on marginal land without being fertilized, Davis said. Nitrous oxide, a byproduct of the fertilizers used in cornfields, 'is actually a more potent greenhouse gas than carbon dioxide," she said.

Of course, before these grasses can replace corn as an ethanol source, various technical and economic challenges have to be solved, not the least of which is breaking down and then converting the sugars in cellulose to fuel. "We know that these grasses are enormously productive, we know the agronomy works, we know the ecology works," DeLucia said. "So the next step is to break down the economic barriers by making an efficient conversion chain from lignocellulosics to ethanol."

More than 100 EBI researchers at three public institutions—Illinois, UC Berkeley and Lawrence Berkeley National Laboratory—are trying to do just that through dozens of intensive multidisciplinary programs and projects.

The authors conclude: "We expect the bioenergy debate to shift from the question, 'Are biofuel crops beneficial?' to that of 'Which biofuel crops are beneficial in a given location?'"

*-- Diana Yates*

MIXED PRAIRIE ON THE ENERGY FARM

(CLOCKWISE FROM TOP LEFT) CHRIS SOMERVILLE WELCOMES THE RETREAT PARTICIPANTS; RESEARCHERS FROM BERKELEY AND ILLINOIS SHARE SPACE AND IDEAS; ONE TABLE GROUP'S VISION OF THE BIOENERGY CHALLENGE, GRAPHICALLY RENDERED ON PAPER; JOHN PIERCE, CHIEF BP BIOSCIENTIST, ADDRESSES DINNER GUESTS.

## BRINGING IT ALL TOGETHER: EBI RETREAT PROVIDES FOCUS TO THE MISSION

(cont. from page 1)

The retreat was off and running, and the institute's great minds went to work … together.

### ATTACKING PROBLEMS WITH KNOWLEDGE

Somerville laid out what he considers the next priorities in the EBI research portfolio: enable feedstock availability by region and species, reduce capital and operating costs in biofuel processing, develop strategies for making cellulosic diesel fuel, and understand the social, economic and environmental implications of land use for biomass. Seeking more efficient oil recovery from reservoirs using microbial tools continues to be an emphasis, he added.

"We offer a holistic view, with academics who can see all aspects of the problem in a coherent way," he told his audience of colleagues. "That's what is special about the EBI. Knowledge is what's got real value, not just inventions that are made. And it's knowledge of the whole field. Then we can see the whole picture."

Participants spent much of the retreat's final day focusing on research themes within their areas of expertise —feedstocks, depolymerization, conversion to fuels, fossil fuel microbiology, environmental impacts and ecology, and socioeconomic, legal and policy impacts. Discussion leaders summarized capabilities and challenges within their disciplines and described their search for solutions.

Earlier, in break-out sessions on discussions about grand challenges, randomly chosen clusters of researchers expressed their interests in better communications both within the institute and publicly; improved collaborations and integration across research areas, and especially between campuses; and more clarity on BP's and EBI's preferences for process pathways.

Somerville reminded the group that when the EBI was established, the call was for novel ideas and strategic insights, not to create "widgets." To illustrate, he referenced the EBI discovery of cellobiose transporters and an enabling yeast strain to digest sugars. "It came out of blue sky," he said. "We got the idea from (graduate student) Will Beeson, who took it to (Berkeley faculty members) Louise Glass and John Taylor. They thought maybe it would be interesting to figure out systematically how Neurospora (a fungus) grows on biomass. Cellobiose would not have jumped on our priority list (when EBI began).

"Now 11 different groups are somehow involved with that work. Other things are coming from that platform that have opened up new ideas for where we might go into the future. So as management, we do have goals, but reaching those goals is not a straightforward process. We are $150 million into this, and we really learned some great things."

### POSITIVE REVIEW FROM BP

From BP's perspective, the EBI has been doing just fine. "So far, we have been quite happy with the way the EBI has been developing, the look and feel of the whole place," said EBI Associate Director Paul Willems, BP's technology vice president for bioenergy sciences. "We are pleased with how it works and how it delivers."

He did point out that the barriers to a successful lignocellulosic biofuels industry have yet to be scaled. The requirements include favorable policy and regulatory environments, societal acceptance of biofuels' proven sustainability, a low-cost regular supply of feedstock, efficient conversion technology, and target fuel molecules that are compatible with today's vehicles. Capital and operating costs for second-generation biofuels will have to be reduced by 50 percent before they will be competitive with fossil fuels, he added.

Second night dinner speaker John Pierce, BP's chief bioscientist and a member of the EBI Governance Board, echoed those challenges and reiterated the company's commitment to biofuels development. He said the field holds the biggest percentage of BP's R&D investment funding, and the EBI is by far largest of its 16 university partnerships.

"Looking at the EBI, it's been great to see the evolution," he said. "EBI and BP have a unique opportunity to make a difference. I hope to come back a couple of years from now and see amazing things."

In closing, Deputy Director Steve Long of the University of Illinois noted that the purpose of the retreat had largely been achieved. "The unique feature of the EBI is horizontal integration, from molecular biology to law and policy," he said, "and you've showed us links that you've identified as important to you. I urge you to follow up on them, and expand the links between Illinois and Berkeley."

## SOME (MICROBES) LIKE IT HOT…

(cont. from page 2)

Joel Graham at the University of Maryland School of Medicine in Baltimore, are gathering and analyzing microbes scooped from hot springs and other extreme environments around the United States. They are in search of these "extremophiles" because they can withstand the harsh conditions found in industrial processes. They have not, however, been optimized for these purposes.

"The discovery is interesting because it helps define the range of natural conditions under which cellulolytic organisms exist and how prevalent these bugs are in the natural world," Clark said. "It indicates that there are a lot of potentially useful cellulases in places we haven't looked yet."

The new enzyme is so stable that it works in hot solutions approaching conditions that could be used to pretreat feedstocks like Miscanthus to break down lignocellulose and liberate cellulose. This suggests that cellulases may someday be used in the same reaction vessel in which feedstocks are pretreated—a very economical step.

UNIVERSITY OF MARYLAND POSTDOC JOEL GRAHAM EXAMINES A PERFORATED PLASTIC TUBE OF PULVERIZED MISCANTHUS, WHICH SERVES AS BAIT FOR DECOMPOSING THERMOPHILES.

Clark said the newly discovered cellulase actually works at too high a temperature for some processes. By collecting more hyperthermophilic cellulases, protein engineers may be able to create a version of the enzyme optimized to work at a lower temperature, but with the robust structural stability of the native enzyme. Engineering new cellulases will be a key to developing a more efficient and cost-effective biofuel production process.

The research paper, "Identification and Characterization of a Multidomain Hyperthermophilic Cellulase from an Archaeal Enrichment," can be read at the EBI web site (www.energybiosciencesinstitute.org) in the "Publications" section under 2011 Biomass Deolymerization listings.

*-- Robert Sanders*

## SUMMER 2011 EBI PUBLICATIONS

(cont. from page 8)

*Tuning Structural Durability of Yeast-Encapsulating Alginate Gel Beads with Interpenetrating Networks for Sustained Bioethanol Production,* Chaenyung Cha, Soo Rin Kim, Yong-Su Jin, Hyunjoon Kong, ***Biotechnology and Bioengineering***, July 5, 2011.

*Beyond the 'Food or Biofuel' Dilemma,* Steve Sexton and David Zilberman, ***Biofuels***, July 2011.

*Multicriteria Comparison of Fuel Policies: Renewable Fuel Standards, Clean Fuel Standards, and Fuel GHG Tax,* Deepak Rajagopal, Gal Hochman, David Zilberman, Working Paper Series, ***UC Center for Energy and Environmental Economics***, WP-012, July 2011

*Impact of Second-Generation Biofuel Agriculture on Greenhouse-Gas Emissions in Corn-Growing Regions of the U.S.,* Sarah Davis, William Parton, Stephen Del Grosso, Cindy Keough, Ernest Marx, Paul Adler, Evan DeLucia, ***Frontiers in Ecology and the Environment***, July 1, 2011.

*Meeting a Growing Demand for Food and Fuel in a Sustainable Manner,* David Zilberman, Gal Hochman, ***ARE Update***, July 2011.

*Making Regulatory Innovation Keep Pace with Technological Innovation,* Jay Kesan and Timothy Slating, ***ExpressO SelectWorks***, 2011.

### AUGUST

*Agent-Based Analysis of Biomass Feedstock Production Dynamics,* Yogendra Shastri, Luis Rodriguez, Alan Hansen, K.C. Ting, ***BioEnergy Research***, August 3, 2011.

*A Study of Acid-Catalyzed Hydrolysis of Cellulose Dissolved in Ionic Liquids and the Factors Influencing the Dehydration of Glucose and the Formation of Humins,*

## PRIZE WINNERS IN POSTER CONTEST

The 2011 EBI retreat included a poster session that featured 124 posters summarizing program and project research. A set of judges evaluated the entries for their accessibility and clarity and issued the following awards to the most compelling posters:

**Top prize:** "A Chemical Sponge for Converting Cellulose to Glucose;" authors: Alexandre Charmot, Po-Wen Chung (Cedric), Oz Gazit and Alexander Katz, all of UC Berkeley.

**Runners-up:**
"Enhanced Below-Ground Carbon Cycling in Perennial Bioenergy Crops;" authors: Kristina J. Anderson-Teixeira, Michael Masters, Marcelo Zeri, Christopher Black, and Evan DeLucia, all of the University of Illinois.

"Spatial Organization of Cellobiose Catabolism Enzymes;" authors: Hanson Lee and John Dueber of UC Berkeley.

# THREE-DAY INTERNATIONAL BIOENERGY CROP MEETING SET FOR ILLINOIS

More than 100 academics, government officials, industry representatives and end users from around the world will gather at the University of Illinois at Urbana-Champaign, Sept. 21-23, to exchange ideas and collaborate on the development of bioenergy feedstocks for competitive market use.

Sponsored by the Association of Applied Biologists (AAB) and the Energy Biosciences Institute (EBI), "Biomass and Energy Crops IV" will offer up-to-date information on various aspects of bioenergy research and production. It will be a follow-up to the three preceding meetings in the series, at which greater understanding was achieved on the potential of energy crops. This one will shift its attention toward the most effective deployment of biomass in the U.S. and Europe.

Among the list of keynote speakers will be EBI Deputy Director and University of Illinois professor Steve Long, whose Sept. 23 talk will address "Thinking Outside the Lunch Box: How We Could Have Sustainable Bioenergy Without Conflict with Food Production." Also, Steve Koonin, Under Secretary for Science with the U.S. Department of Energy, will be the opening speaker for the three-day conference. Other EBI presenters, all from Illinois, include Haixiao Huang, D. K. Lee, Christian Hillnheutter, Yogendra Shastri, Tom Voigt, Andy Wycislo, Ray Ming, and Dan Wang.

Agricultural and forestry-derived resources offer many opportunities to generate renewable heat, power, gas and liquid fuels to meet requirements to reduce greenhouse gas emissions and increase fuel security. However, the potential scale of development required to make a significant contribution to those objectives raises a number of concerns, in particular:

- the amount of land required and the constraint this places on available biomass resources, and
- the sustainability credentials of biomass and biofuels.

The most appropriate uses of biomass need to be identified to ensure delivery of the greatest benefit in terms of GHG savings and fossil fuel displacement in the power, heat and transport sectors. The meeting participants will discuss the outputs of biomass and bioenergy crops that need to be optimized to reduce the overall land demand; the most appropriate pretreatment and energy conversion technologies to ensure the best integration of technologies and highest levels of energy conversion efficiency; and integrated management systems needed to help reduce costs, reduce waste and identify additional value chains.

The conference will include an optional tour of the 320-acre EBI Energy Farm. More information and registration forms can be found on the AAB web site (http://www.aab.org.uk).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