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NEW YEAST DEVELOPMENT SHOWS HOW EBI COLLABORATIONS WORK

One of the keystones of the architecture that became the Energy Biosciences Institute was the idea of collaborations, of scientists from multiple disciplines and, in some cases, different locations bringing their particular expertise to bear on a problem. And as the EBI entered its fourth year of research, the most dramatic illustration of this concept emerged from the laboratories at Berkeley and Illinois.

All four partners had a hand in what is arguably the most significant breakthrough to date by this historic public-private enterprise. Through painstaking biotechnical experiments that tested and proved bold new theories, researchers figured out a way to get yeast to co-ferment two cellulosic sugars, an unprecedented reaction that promises to save time and money in the search to make biofuels competitive in the energy market.

Targeting Two Sugars

In the world of biofuels, the goal is to optimize the fermentation of sugars to make ethanol. But while corn seeds are about 65 percent starch, which is easily converted to glucose, potential cellulosic feedstocks such as switchgrass and Miscanthus are comprised of about 30 to 40 percent glucose and 30 percent xylose sugar.

Yeasts feed on sugar and produce various waste products, some of which are useful to humans. One type of yeast, *Saccharomyces cerevisiae*, has been used for

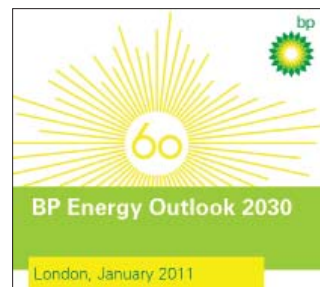
centuries in baking and brewing because it efficiently ferments sugars and in the process produces ethanol and carbon dioxide. The biofuel industry uses this yeast to convert plant sugars such as starch or sucrose to bioethanol. And while *S. cerevisiae* is very good at utilizing glucose, it cannot use xylose. Most yeast strains that are engineered to metabolize xylose do so very slowly, which also adds significantly to the cost of biofuels production.

“Most yeast cannot ferment xylose,” said Yong-Su Jin, a professor of food science and human nutrition at Illinois. Jin and his colleagues wanted to induce yeast to quickly and efficiently consume both types of sugar at once, a process called co-fermentation. Once Jin’s team engineered such a yeast strain, they discovered another challenge: the engineered yeast had a marked preference for glucose over xylose.

Building a Pathway

Meanwhile, in Berkeley, microbiologist Jamie Cate and his team had successfully developed a metabolic

BIG GROWTH PREDICTED FOR RENEWABLES BY 2030, BUT OIL STILL KING



Over the next 20 years, renewable energy and biofuels will be the fastest growing segment in energy.

That is the prediction from the annual report on energy released recently by BP, the EBI’s funding and research partner. “BP’s Energy Outlook 2030,” a view of future trends based upon its 60-year-old “Statistical Review of World Energy,” was shared for the first time with the public to contribute to the global energy debate.

According to the report, renewables (including biofuels) are expected to grow 8.2 percent annually between 2010 and 2030, representing 18 percent of the growth in total energy during that period.

“The rate at which renewables penetrate the global energy market is similar to the emergence of nuclear power in the 1970s and 1980s,” the BP report states.

STUDIES ON BIOMASS AGRICULTURE AMONG 7 PROJECTS ADDED TO EBI PORTFOLIO

The Energy Biosciences Institute has added seven new projects to its broad research agenda, including several designed to assess the feasibility and ecological impacts of potential biofuel crops. The new group brings the total of programs and projects funded by the EBI since its formation in 2007 to 89.

The most recent research grants went to investigators at the University of California, Berkeley, and the University of Illinois at Urbana-Champaign, two of the three partners in the 10-year public-private collaboration.

Project funding is the result of an external peer review process following the solicitation of formal proposals for defined research activities. Pre-proposals were vetted by a 13-member EBI Executive Committee, which then requested formal proposals from select submissions and made final decisions based upon review input and programmatic need.

The new projects are:

- *Potential Ecological Impacts of Agricultural Intensification: Invasiveness of Miscanthus Species*: Tom Voigt, Principal Investigator of EBI's Agronomy Program at Illinois, will head a team that will study the promising perennial grass *Miscanthus* and conditions in which farmers can increase yield while preventing its escape, through pollen and seeds, into natural areas. Data will be gathered and fed into models to predict suitable and unsuitable habitats. The studies will benefit developers, growers and land managers to ensure access to a beneficial crop while reducing potentially negative consequences.

- *Best Practices for the Multi-Use of Miscanthus Feedstock Production Landscapes*: UC Berkeley Assistant Professor Matthew Potts leads this project, which will develop a landscape management tool to help determine the best ways to grow *Miscanthus* while conserving the biodiversity and ecosystems of a given region. The model will assist stakeholders in assessing tradeoffs between crop expansion and environmental and economic impacts, developing optimal landscape configurations, and predicting socio-economic effects of introducing *Miscanthus* cultivation.
- *Effects of Biofuels Production on Biodiversity and Landscape Connectivity*: University of Illinois Assistant Professor Robert Schooley, with co-principal investigators Jeffrey Brawn and Patrick Weatherhead, hope to gather data on the biodiversity values of various biofuel feedstocks, in particular *Miscanthus* and switchgrass. The goal is to ensure that introduction of such crops into agroecosystems will not have adverse effects on wildlife habitat, animal movements, or demographic and genetic connections between landscapes.
- *Socio-Economic Issues Associated with Biofuels or Sequestration*: UC Berkeley Professor Peter Berck and colleague Maximilian Auffhammer of the Department of Agricultural and Resource Economics will develop a model for predictions of land use by crop at specific locations within counties, based upon land characteristics, acreage shares per crop, weather data, and crop yields.

- *Adding Biofuel Feedstock Options to Regional Forest Product Market Models*: J. Keith Gilles, Professor of Forest Economics and Management at UC Berkeley, plans to develop tools to predict the volumes of tree-based biofuel feedstocks that will be available in regional markets for wood products. The Pacific Coast and the Southeastern U.S. will be modeled along with their relevant global trading regions to determine how woody biofuel feedstocks might complement or compete with other forest product markets.
- *Practical Synthesis of Thermoresponsive Cellulase-Polymer Bioconjugates*: The goal of UC Berkeley Associate Professor Matthew Francis' project is to generate cellulase enzymes with enhanced physical properties that will increase their ability to be reused through several cycles as they break down cellulose into sugars. His proposed method of "thermorecycling" may provide a useful design concept that could be applied to other enzymatic transformations in feedstock processing.
- *Microbial Chlorate Reduction As A Control Of Biotogenic Hydrogen Sulfide Production In Oil Reservoirs*: UC Berkeley Associate Professor of Microbiology John Coates, a co-leader of the EBI team in Microbially Enhanced Hydrocarbon Recovery (MEHR), will focus on sulfur metabolism by bacteria in oil wells. The generation of hydrogen sulfide in reservoirs presents several oil recovery problems, and Coates hopes to introduce microbes that will inhibit and reverse the undesirable sulfate reaction.

For information on the EBI and its portfolio of programs and projects, visit the institute's website www.energybiosciencesinstitute.org



FUNGAL BIOLOGIST GETS PRESTIGIOUS PROFESSORSHIP TO ADVANCE EBI RESEARCH

UC Berkeley microbiologist N. Louise Glass, a leader in the effort to apply the plant deconstruction properties of the filamentous fungus *Neurospora crassa* to the process of cellulosic biofuel production, has won a professorship from the Miller Institute

for Basic Research in Science—a hub for young scientists exploring the frontiers of research.

"The purpose of the Professorship is to release members of the faculty from teaching and administrative duties and allow them to pursue research," according to the institute. Glass' award will be for six months, beginning next January, to pursue her Energy Biosciences Institute project on genome-scale metabolic-regulatory network models for *N. crassa*.

She and collaborator Nathan Price of the University of Illinois at Urbana-Champaign have formed a unique partnership that pairs a fungal biologist with a computational biologist to share their experimental data and knowledge bases in orchestrating the construction of a metabolic-regulatory network model for filamentous fungi.

"Our ultimate objective is to understand how the organism remodels its metabolism to deconstruct

plant biomass," said Glass. "From this metabolic-regulatory network model, we hope to map out a program of rational engineering of fungi to optimize lignocellulose utilization for biofuel production."

They will develop potentially important tools to accelerate actualization of the use of plant biomass in producing biofuels. A rapid and user-friendly network interface will be accessible to biologists and publicly available.

The Miller Institute, based at UC Berkeley, is "dedicated to the encouragement of creative thought and the conduct of research and investigation in the field of pure science and investigation in the field of applied science in so far as such research and investigation are deemed by the Advisory Board to offer a promising approach to fundamental problems." The institute, named after alumnus and noted economist Adolph C. Miller and his wife Mary, issued its first awards in basic science in 1957.

ANOTHER PROMISING BIOFUEL FEEDSTOCK GETS CLOSER LOOK—AGAVE

Although it is clear that more research is needed to resolve uncertainties about the amount of liquid fuel that can be produced from it, the agave plant—long a source of tequila and fiber—has the potential to be a productive feedstock for biofuels in the future.

That is the conclusion of a package of articles in the February special issue of the journal *Global Change Biology-Bioenergy*, whose editors and authors include Energy Biosciences Institute Deputy Director Steve Long and EBI Bioenergy Analyst Sarah Davis, both from the University of Illinois at Urbana-Champaign.

“The articles serve as an introduction to research on agave as a biofuel feedstock,” the authors write. “They clearly demonstrate that there is potential for agave to be produced for very substantial quantities of bioenergy, and that there are environmental and economic benefits to be gained by doing so in some regions of the world.”

Davis notes that Mexico is in a position to initiate agave as an energy feedstock in the near term by using bagasse and residues, while others who are already invested in the commercial production of agave in Brazil and Africa should also be able to begin using agave biomass for energy fairly quickly.

“Combustion technologies could easily provide heat and power from agave biomass in the short term, while conversion to liquid fuel will require greater capital investment,” she says. “More research is required, however, to determine if high-yielding agave varieties can be grown with low inputs in the U.S. Conclusions about the highest yield agave species for the semiarid southwest and for other marginal agricultural areas in the world will take close to a decade to resolve.”

And she adds, “The bright side of this timeline is that cellulosic conversion technology should advance to a commercial scale by then also.”

The issue features eight articles about agave species that have been evaluated as bioenergy feedstocks for semi-arid regions of the world. The articles review a wide variety of topics that are relevant to bioenergy production, including the natural history of agave plants, genomic sequencing, efficiencies of converting biomass to ethanol, and economic analyses of agave-based bioenergy in Mexico.

Mexico has been the primary location for research on agave, which has been grown there commercially for tequila and fiber. Both industries have suffered economic losses in recent years, and a new market for agave plants might supplement those losses.

In an article contributed by Madhu Khanna of the University of Illinois, EBI principal investigator on the economic impacts of biofuels, in collaboration with graduate student Hector Nuñez and Assistant Professor Luis Rodriguez, the economics of initiating bioenergy production as a co-product or as an added commodity is explored. As a bioenergy crop, agave would not yield income competitive with the tequila industry, but with demands for tequila down, there is a surplus of agave biomass that could be used for ethanol feedstock, according to the researchers.

“It is evident that certain varieties are more suited for efficient conversion to ethanol,” Davis says. “Agave traits that are favorable for bioenergy crops already exist, such as high water use efficiency and low lignin content, and there is greater potential still because no attempts have yet been made to optimize traits through breeding or genetic modification.” She notes that the wealth of genetic diversity in the more than 200 species of agave is a valuable resource to be explored. One article describes an experiment that



EBI BIOENERGY ANALYST SARAH DAVIS BELOW AGAVE PLANT

will test the success of several varieties outside their native habitat, in the semi-arid climate of Australia.

In an article authored by Davis and Long in collaboration with former EBI graduate student Frank Dohleman, the land opportunity for agave plantations in Africa was highlighted in addition to the opportunities in Australia and Mexico. Some African nations were at one time leading producers of natural fibers from agave, but as demand diminished due to the rise of synthetic fibers, agave plantations were abandoned. Davis estimates that these abandoned lands represent 0.6 million hectares globally that might be reclaimed for bioenergy crops with minimal environmental impacts.

The special issue was organized by Long and Davis, with editorial contributions from Howard Griffiths of Cambridge University in England, Joseph Holtum of James Cook University in Australia, and Alfonso Larqué-Saavedra of the Centro de Investigación Científica de Yucatán in Mexico. The articles can be accessed online via the “Publications” page of the EBI web site, www.energybiosciencesinstitute.org.



COW RUMEN YIELDS TREASURE TROVE OF BIOMASS-DEGRADING MICROBES

Through massive-scale DNA sequencing, researchers in the Energy Biosciences Institute have characterized the genes and genomes of plant-digesting microbes isolated from cow rumen, an accomplishment that may someday accelerate the large-scale deployment of biofuels. Their EBI project results will offer a window into a major category of organisms that has long resisted the attempts of scientists to grow and study.

The work, reported in a study published Jan. 28 in the journal *Science*, has enabled the investigators and collaborators to mine, at a scale thousands of fold greater than in any prior work, the enzymatic capabilities encoded in the genomes of previously uncharacterized rumen microbes. This has deepened our understanding of nearly 30,000 genes generating enzymes that may possess powerful capabilities for degrading biomass into simple sugars, the essential first step in cellulosic biofuel production.

Eddy Rubin, Director of the Department of Energy Joint Genome Institute (JGI) in Walnut Creek where most of the analysis was completed, was principal investigator (PI) on the EBI project “Analysis of Bovine Rumen Microbiota Under Different Dietary Regimens for Identification of Feedstock-Targeted Cellulolytic Genes.” His postdoctoral researchers gathered the microbes from a cow at the

University of Illinois, where co-PI Roderick Mackie, a specialist on gut bacteria genomics, supervised a second team.

“Microbes have evolved over millions of years to efficiently degrade recalcitrant biomass,” said Rubin. “Communities of these organisms can be found in diverse ecosystems, such as in the rumen of cows, the guts of termites, in compost piles, as well as covering the forest floor. Microbes have solved this challenge, overcoming the plant’s protective armor to secure nutrients, the rich energy source that enables them and the cow to thrive.”

“The problem with second-generation biofuels is the problem of unlocking the soluble fermentable sugars that are in the plant cell wall,” said Mackie. “The cow’s been doing that for millions of years. And we want to examine the mechanisms that the cow uses to find enzymes for application in the biofuels industry.”

Rumen microbes—those found in the forestomachs of hooved animals like cows—evolved to produce molecular machines in the form of enzymes able to efficiently deconstruct plant cell wall polysaccharides such as cellulose and hemicellulose into their constituent small sugar molecules.

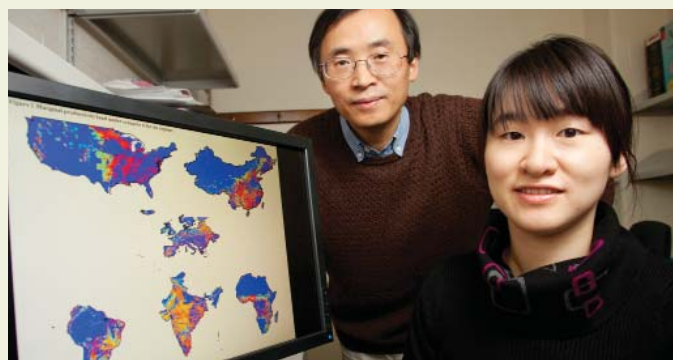


POSTDOC MATTHIAS HESS REACHES INTO FISTULATED COW AT ILLINOIS

“Industry is seeking better ways to break down biomass to use as the starting material for a new generation of renewable biofuels,” said Rubin. “Together with our collaborators, we are examining the molecular machinery used by microbes in the cow to break down plant material.”

Postdoctoral fellows Matthias Hess and Alex Sczyrba used one of the most promising large-scale bioenergy crops—switchgrass (*Panicum virgatum*)—and let the cows’ microbial symbionts perform their magic. To better control the process, Hess and

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XIMING CAI (LEFT), WITH GRADUATE STUDENT XIAO ZHANG

STUDY FINDS PLENTY OF LAND AVAILABLE FOR BIOFUEL FEEDSTOCKS

EBI investigator Ximing Cai of the University of Illinois has completed a detailed global land analysis and has found that biofuel crops cultivated on available land could produce up to half of the world’s current fuel consump-

tion—without affecting food crops or pasture land.

The civil and environmental engineer identified land around the world available to produce grass crops for biofuels, with minimal impact on agriculture or the environment. He was addressing a frequent concern about whether enough biofuel can be produced to meet demand without compromising food production.

“The questions we’re trying to address are: what kind of land could be used for biofuel crops? If we have land, where is it, and what is the current land cover?” Cai said.

The researchers collected data on soil, topography, climate and current land use from some of the best data sources available, including remote sensing maps. The critical concept of the study was that

only marginal land would be considered for biofuel crops. Marginal land refers to land with low inherent productivity, that has been abandoned or degraded, or is of low quality for agricultural uses.

In focusing on marginal land, the researchers ruled out current cropland, pasture land, and forests. They also assumed that any biofuel crops would be watered by rainfall and not irrigation, so no water would have to be diverted from agricultural land. The researchers modeled multiple scenarios for land availability—idle land and vegetation land with marginal productivity, degraded or low-quality cropland, and marginal grassland.

Factoring in low-impact high-diversity (LIHD) perennial grasses in addition to higher-yielding perennials such as *Miscanthus* and switchgrass, Cai estimated there are 1.1 billion hectares of land available globally for second-generation biofuel crops. Even after subtracting possible pasture land, the researchers estimated that this area could produce 26 to 56 percent of the world’s current liquid fuel consumption.

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EBI ANALYSTS SEE BIOENERGY CROPS INFLUENTIAL IN FARM OF THE FUTURE

Energy crops will play a significant role in the farm of the future, and the farming practices of the future will have an enormous impact on the economic viability and commercial development of next-generation bioenergy, according to analysts from the Energy Biosciences Institute.

Their report on prospective biomass supply chains appears in a special issue of the magazine *Resource*, published by the American Society of Agricultural and Biological Engineers.

Heather Youngs and Caroline Taylor, EBI Bioenergy Analysts, address farm production strategies and the risks involved in various scenarios, ranging from single-plantation feedstock sourcing to hybrid structures featuring producer contracts with multiple farms. But, they say, with limited real-scale data on the most promising new feedstocks, such as grass perennials, farmers may be reluctant to invest in the alternative crops.

“Technical barriers to economic biomass production threaten the establishment of supply networks for external procurement (by producers) of feedstocks,” the analysts state. “High perceived risk of failure during the establishment period for perennial crops negatively affects the farmer’s willingness to diversify, which is a crucial step for early adopters of new feedstocks.”

They conclude that “by directing research efforts toward identifying the best-fit feedstocks for emerging technology and eco-regional limitations, agronomists and agricultural engineers can reduce farmer risk and enable farming commu-

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WEEDING OUT THE POTENTIAL THREATS TO BIOFUEL FEEDSTOCK VITALITY

While dozens of EBI researchers in Illinois are devoting their attention to increasing the yields and biomass growing qualities of potential biofuel feedstocks such as *Miscanthus*, a team is also focusing on protecting those emerging crops against elements that could affect their survival, like weeds.

University of Illinois bioenergy expert Eric Anderson and colleagues have discovered several herbicides used on corn that also have good selectivity to *Miscanthus x giganteus* (Giant Miscanthus) and can be applied safely both before and after it emerges in the ground.

“No herbicides are currently labeled for use in Giant Miscanthus grown for biomass,” said Anderson, whose work appeared in the journal *Weed Technology*. “Our research shows that several herbicides used on corn are also safe on this rhizomatous grass.”

The team screened 16 post-herbicides and 6 pre-herbicides in a greenhouse setting. Several herbicides, particularly those with significant activity on grass species, caused plant injury ranging from 6 to 71 percent and/or reduced *Miscanthus* dry mass by 33 to 78 percent. They then narrowed these herbicides down to the safest options and evaluated them in field trials replicated over two years.

Pre-emergence herbicides and herbicides with broadleaf-specific activity generally did not produce significant injury or reduce aboveground biomass while herbicides with considerable grass activity tended to cause injury rang-

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MISCANTHUS HYBRIDS FOUND IN JAPAN EXPAND BREEDING OPTIONS

In the minds of many, *Miscanthus x giganteus* is the frontrunner in the race to find viable feedstock options for lignocellulosic bioenergy production. But relying on just one variety has its drawbacks, which is why EBI researchers are so excited about finding the first natural occurrence in several decades of *Miscanthus* hybrid plants in Japan.

“If *M. x giganteus* is the only variety available, there are certainly risks involved such as diseases or pests causing widespread establishment problems or yield losses,” said Ryan Stewart, assistant professor of horticulture in the Department of Crop Sciences at the University of Illinois. “We are trying to find *Miscanthus* hybrids to increase our options. In doing so, it’s a way to hedge our bets.”

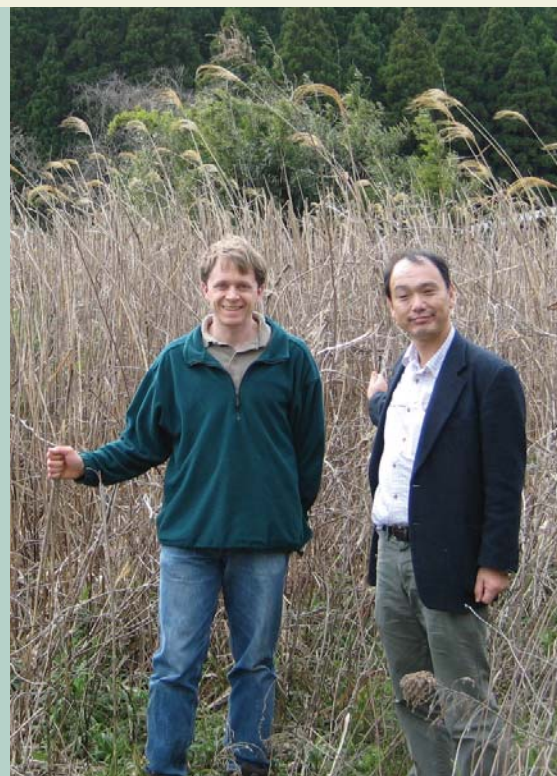
Stewart heads a team of researchers who are exploring the history of *Miscanthus*, which was originally

collected in Japan in the 1930s. The plant is revered for its hardiness, yield and relatively few nutrient requirements. But the Giant Miscanthus is sterile and can only be propagated by vegetative division, which is somewhat more difficult than growing from seed.

“Because it’s a sterile clone, it’s more or less a dead-end for plant breeders because it can’t be improved through plant breeding,” Stewart said. So he and his team investigated overlapping populations of the plant’s parents, *M. sacchariflorus* and *M. sinensis* in Japan in hopes of finding rare hybrid plants that may be similar in productivity to *M. x giganteus*.

“In Japan, even when two plant species are adjacent to one another, they may have very different flowering times, meaning the likelihood of finding a hybrid is very low,” Stewart said. Last year one of his colleagues,

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RYAN STEWART (LEFT), AYA NISHIWAKI
IN JAPANESE MISCANTHUS FIELD

EBI in the News

COW RUMEN YIELDS TREASURE TROVE OF BIOMASS-DEGRADING MICROBES

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colleagues worked with the fistulated cow model that, for scientific research purposes, allows direct access through a tube into the foregut, which can be considered as a fermentation chamber in which oxygen is absent. Instead of feeding the grasses directly to the cows, the switchgrass samples were placed in nylon bags and then inserted into the cow rumen, where they were left to be digested. After 72 hours, the bags were removed, and the DNAs from the microbes involved in digesting the material that were adherent to the switchgrass were isolated and then sequenced.

The amount of data generated for this study of rumen microbes, 270 billion letters of the DNA code, was enormous, about 100-fold greater than the number of letters in the entire human genome. Through a complicated process of data analysis, the scientists determined that a significant fraction of the 30,000 genes identified are indeed active against plant material and will be a treasure trove of novel enzymes for biofuel researchers.

Additional authors on the paper are Feng Chen, Rob Egan, Tao Zhang, Susannah Tringe, Axel Visel, Len Pennacchio, Tanja Woyke and Zhong Wang of the JGI; Tae-Wan Kim, Harshal Chokhawala and Douglas S. Clark of UC Berkeley; and Gary Schroth and Shujun Luo of Illumina, Inc.

—David Gilbert

STUDY FINDS PLENTY OF LAND AVAILABLE FOR BIOFUEL FEEDSTOCKS

(cont. from page 4)

Next, the team plans to study the possible effect of climate change on land use and availability. “Based on the historical data, we now have an estimation for current land use, but climate may change in the near future as a result of the increase in greenhouse gas emissions, which will have effect on the land availability,” said graduate student Xiao Zhang, a co-author of the paper. Former postdoctoral fellow Dingbao Wang, now at the University of Central Florida, also participated in the work.

“We hope this will provide a physical basis for future research,” Cai said. “For example, agricultural economists could use the dataset to do some research with the impact of institutions, community acceptance and so on, or some impact on the market. We want to provide a start so others can use our research data.”

National Science Foundation funding also supported the study, “Land Availability for Biofuel Production,” which was published in the journal *Environmental Science and Technology*.

—Liz Ahlberg

EBI ANALYSTS SEE BIOENERGY CROPS INFLUENTIAL IN FARM OF THE FUTURE

(cont. from page 5)

nities to successfully incorporate energy crops into their future portfolios.”

They discuss supply chain integration in the bioenergy industry of today and tie the direction of integration to technological difficulty and risk. Although vertical integration -- in which the energy producer supplies the feedstock in-house -- dominates today, “merging contract and hybrid structures could support a broader range of farm sizes, shifting away from the past decades’ trend of ever-greater consolidation and allowing large-scale operations to exist alongside smaller operations and family farms that share the developing networks.” Youngs and Taylor add that another scenario, in which diverse feedstocks could be pre-treated for processing at dispersed locations, would allow industry to overcome biomass transport issues and contribute value to local communities.

In the end, successful bioenergy crop development will require the complementary development of production networks and market demand, a synergy that the analysts predict will take on varying dimensions depending upon technology, ecology and the economy.

NOTE: The articles upon which the previous news stories were based are all available via the EBI web site, www.energybiosciencesinstitute.org, under the “Publications” pages.

WEEDING OUT THE POTENTIAL THREATS TO BIOFUEL FEEDSTOCK VITALITY

(cont. from page 5)

ing from 22 to 25 percent and reduce biomass by 69 to 78 percent.

Giant *Miscanthus* is sterile and predominantly grown by vegetative propagation, or planting rhizomes instead of seed. This can be a very costly investment and requires a 1- to 2-year establishment period. Anderson’s research showed that Giant *Miscanthus* does not compete well with weeds during establishment, especially early emerging weeds.

“There’s a great cost in establishing Giant *Miscanthus*,” Anderson said. “It’s important to protect this investment, especially if it goes commercial. When weeds outcompete Giant *Miscanthus*, the result is stunted growth and lack of tillering. Basically, you are risking the crop’s ability to overwinter.”

Anderson said it’s more difficult to kill a weed in a grass crop such as Giant *Miscanthus*. Identifying herbicides that don’t hurt its yield or growth and maturity also posed challenges for researchers. “I think the key is finding pre-emergence herbicides that you can get in early to take care of weed problems,” he said.

The study, “*Miscanthus x giganteus* Response to Preemergence and Postemergence Herbicides,” included contributions from EBI researchers Thomas Voigt and Germán Bollero.

—Jennifer Shike

MISCANTHUS HYBRIDS FOUND IN JAPAN EXPAND BREEDING OPTIONS

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Aya Nishiwaki of Hokkaido University, found an *M. sacchariflorus* plant, which was adjacent to some *M. sinensis* plants, with heavy seed set. Nishiwaki collected the seed, grew it out, and then used molecular analysis that confirmed the new plants were hybrids.

Stewart said he hopes these new plants will express phenotypic traits similar to that of the high-yielding *M. x giganteus* or serve as sources of genetic variation that might express resistance to recently identified diseases and pests in *Miscanthus*. They will continue searching for hybrids to build a collection of germplasm as a resource to further develop the optimal feedstock for biofuel production.

Stewart’s research was published in the *American Journal of Botany*.

—Jennifer Shike

BIG GROWTH PREDICTED FOR RENEWABLES BY 2030, BUT OIL STILL KING (cont. from page 1)

“In transport, we are starting to see diversification, driven by policy and enabled by technology, with biofuels accounting for nearly a third of energy demand growth.”

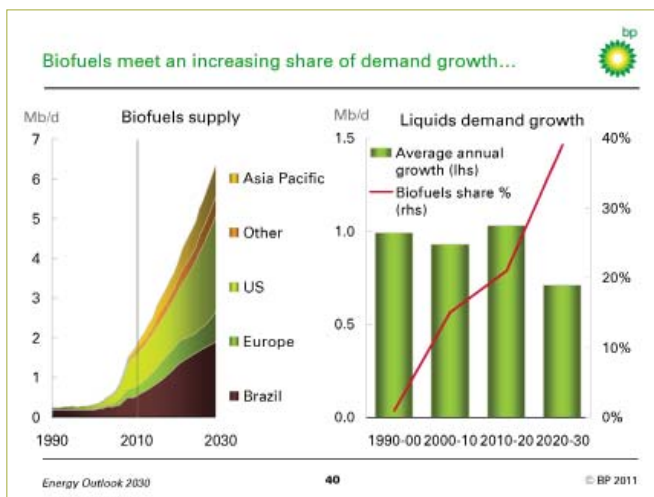
Oil is expected to be the slowest-growing fuel over the next 20 years. Global liquids demand (oil, biofuels, and other liquids) nonetheless is likely to rise by 16.5 million barrels per day (Mb/d), exceeding 102 Mb/d by 2030. Biofuels production (largely ethanol) is expected to exceed 6.5 Mb/d by 2030, up from 1.8 Mb/d in 2010—contributing 30 percent of global supply growth over the next 20 years, and all of the net growth in non-OPEC countries.

“Energy used for transport will continue to be dominated by oil, but should see its share of global energy use decline as other sectors grow more rapidly,” according to the analysis. “Growth is expected to slow over the next 20 years to average 1.1 percent annually vs. 1.8 percent from 1990-2010.” The growth of oil in transport slows largely because of displacement of oil by biofuels and is likely to plateau in the mid-2020s. Currently, biofuels contribute 3 percent on an energy basis and this is forecast to rise to 9 percent at the expense of oil’s share.

The report notes that continued policy support, high oil prices in recent years, and technological innovations all contribute to the rapid expansion of renewables.

It is predicted that the U.S. and Brazil will continue to dominate ethanol production; together they will account for 68 percent of total output in 2030 (from 76 percent in 2010). First-generation biofuels are expected to account for most of the growth. After 2020, roughly 40 percent of global liquids demand growth will be met by biofuels—up from 13 percent in 2010—with the U.S. and Europe leading consumption growth. By 2030, this figure approaches 60 percent.

The contribution of fossil fuels to primary global energy growth is projected to fall from 83 percent (1990-2010) to 64 percent (2010-2030). The contribution of renewables to energy growth increases from 5 percent (1990-2010) to 18 percent (2010-2030).



Even with the predicted growth in lower-emission fuel sources, the BP outlook expects global CO₂ emissions to continue rising, along with import dependence in many key consuming regions. This does not mean BP downplays the importance of climate change or the role of energy security in international relations. Rather, “it reflects a ‘to the best of our knowledge’ assessment of the world’s likely path from today’s vantage point,” according to the report.

Bob Dudley, BP Group Chief Executive, adds, “To me personally, it is a wake-up call, not something any of us would like to see happening.”

The world will still get most of its fuel from fossil sources, the report states. By 2030, oil, natural gas and coal will each account for around 26 to 27 percent of the world’s energy diet while hydroelectric

power, nuclear power and renewables will account for around 6 or 7 percent each.

Other predictions:

- China, India, Russia and Brazil will dominate energy demand as non-fossil fuel consumption grows rapidly and energy output from coal almost matches that of oil.
- Non-OECD Asia will account for nearly two-thirds of non-OECD consumption growth and more than three-quarters of the net global increase, rising by nearly 13 million barrels a day.
- Primary energy use will grow by nearly 40 percent over the next 20 years; China will displace the U.S. to become the largest oil consumer.
- Natural gas will be the fastest growing fossil fuel at 2.1 percent per year. Coal will increase 1.2 percent a year and is likely by 2030 to provide virtually as much energy as oil, excluding biofuels.
- By 2030, gas will account for 41 percent of fossil fuel-created electricity in the U.S. Worldwide, gas’s share of total electricity will grow from 20.5 percent to 22 percent and its share of fossil fuel-created power will jump from 30 to 37 percent.
- Emissions will grow by 10 percent in OECD countries and 53 percent in non-OECD nations. Emission levels will stabilize but not enough to get below 450 parts per million.

BP VOWS TO CONTINUE INVESTMENTS IN RENEWABLE ENERGY IN 2011

BP plans to invest \$1 billion in 2011 in its renewable energy business, roughly the same amount it invested last year.

During the company’s fourth-quarter earnings call, BP CEO Bob Dudley said that a bulk of the company’s green capital expenditures would support biofuel and wind projects.

Since 2005 BP has invested about \$5 billion in its renewable energy business.

In addition to its investments in the Energy Biosciences Institute, BP will be constructing a biofuel refinery in Highlands County, FL under the Vercipia cellulosic ethanol program. BP also acquired Verenum Corp.’s cellulosic biofuels business last year.

In Brazil, BP holds a 50 percent stake in Tropical BioEnergia and plans to invest \$1 billion to operate two ethanol refineries there.

8TH FEEDSTOCK SYMPOSIUM SHOWCASES LATEST IN BIOMASS RESEARCH

By Sarah Williams



Nearly 200 researchers, academics, farmers, and industry leaders shared ideas and information at the eighth annual University of Illinois Bioenergy Feedstocks Symposium at the I-Hotel Jan. 11 and 12 in Champaign, IL, sponsored in part by the Energy Biosciences Institute.

Attendees learned about the latest from both Illinois researchers and various external institutions and companies that focus on the use of grasses, forbs, and woody plants as potential renewable energy sources and profitable alternative crops. Presenters also shared insights about the environmental impacts of bioenergy crops, the variety of biomass crops available for commercial use, an overview of renewable energy development at Illinois, and the

process of taking feedstocks from farm use to commercial sales.

“We envision the University of Illinois’ involvement in bioenergy research, education, and outreach will offer a means to diversify farming, generate economic growth, create employment opportunities, and allow the U.S. to achieve energy independence and security,” said the EBI’s Tom Voigt, a member of the symposium organizing committee and associate professor and extension specialist for the Department of Crop Sciences at Illinois.

The increased need for research and discussion about bioenergy crops stems in part from the 2007 Energy Independence and Security Act that requires

the use of 36 billion gallons of renewable transportation fuels in the U.S. by 2022. Of that amount, 16 billion gallons must be cellulosic biofuels, while ethanol from corn is capped at 15 billion gallons. The legislation also requires that these advanced biofuels must achieve at least a 50 percent reduction in life-cycle greenhouse gas emissions.

To help achieve that energy independence, speakers suggested the use of bioenergy feedstocks such as Miscanthus, switchgrass, and prairie cordgrass.

EBI Deputy Director Steve Long, the Gutgsell Endowed Professor of Crop Sciences and of Plant Biology at the University of Illinois, reflected on the start of bioenergy crop research at Illinois in 2002. He compared that with current research, which encompasses a 320-acre Energy Farm (the largest bioenergy crop research farm in the United States) as well as large-scale trials across the country that include more than 35 species and 1,000 genotypes. He and more than 60 faculty feedstock experts are conducting research in various bioenergy feedstock areas.

One of those researchers is the EBI’s Evan DeLucia, a Professor of Plant Biology at the University of Illinois, who discussed the ecological impact of perennial feedstocks on land use. His research has expanded to consider the ecological consequences of deploying biofuel crops on the landscape. He urged attendees to think carefully about how to balance food and fuel production within ecosystems.

(cont. on page 10)

EBI CROP SCIENTISTS AT ILLINOIS EVALUATING WOODY FEEDSTOCKS

EBI researchers at the University of Illinois are studying novel woody plants as short rotation crops for biomass production to help diversify and expand bioenergy research efforts.

Gary Kling, an associate professor in the Department of Crop Sciences who spoke at the 2011 Bioenergy Feedstocks Symposium, said woody plants offer many advantages as a feedstock for biofuel production.

“Woody plants typically have a lower ash content when burned as compared to grasses, thus reducing the amount of waste generated,” he said. “In addition, grasses usually have a higher chlorine content than woody plants, which can be damaging to boilers. The wide range of woody plants will likely be adaptable to a wider range of environments than grasses alone.”

Because most of the woody plants being evaluated are natives, they represent less of a threat to the environment than introduced or hybrid grasses, Kling added. Woody plants can also provide year-round wildlife cover to a much better extent.

The plants chosen for the study include red maple, silver maple, thinleaf alder, river birch, hybrid chestnut, northern catalpa, common hackberry, bloodtwig dogwood, American filbert, American smoketree, possumhaw, American sweetgum, tulip-tree, Osage-orange, sycamore, eastern cottonwood, black cherry, scarlet oak, flameleaf sumac, black locust, and Sherburne willow.

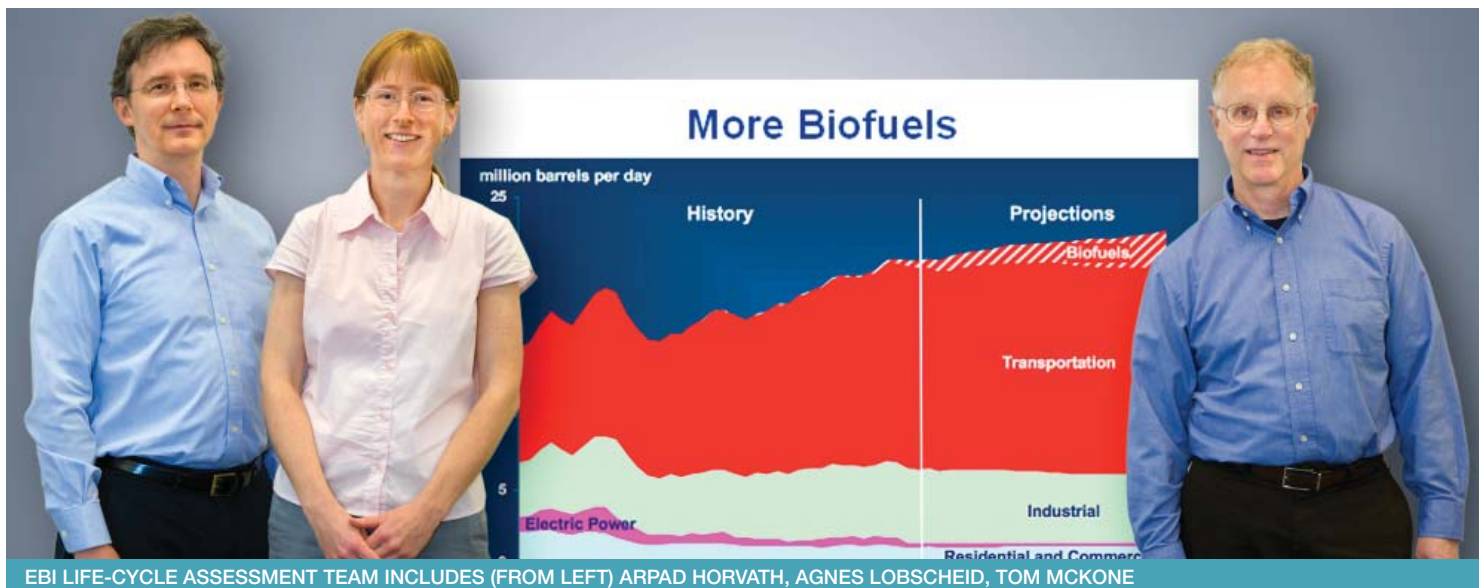
Woody plants provide a viable supplement to other feedstock options being pursued as sources of biomass, according to Kling. “Diversification of your plant materials for biomass production is sound

from an ecological standpoint – a greater diversity of species minimizes the risk from serious disease or insect outbreaks that could threaten a large percentage of production when only a few species are utilized,” he said.

Two-year-old seedlings were planted in the spring of 2010 and will be grown for 1 to 2 seasons before cutting back to induce coppicing, Kling said. Then, they will be grown for a 3- to 5-year harvest cycle. Researchers will be collecting growth and environmental data along the way.

Researchers collaborating in this study include Kling, Evan DeLucia, Michael Dietze, Stephen Long, Ryan Stewart, Tom Voigt, Anthony Bratsch, Sarah Davis, Ziaohui Feng, David LeBauer and Dan Wang of the EBI.

—Jennifer Shike



CHALLENGES FOR BIOFUELS—NEW LIFE-CYCLE ASSESSMENT REPORT FROM EBI

By Lynn Yarris

A team of Energy Biosciences Institute experts has identified seven “grand challenges” that must be confronted to enable life-cycle assessments that effectively evaluate the environmental footprint of biofuel alternatives. The new report, “Grand Challenges for Life-Cycle Assessment of Biofuels,” appears in this month’s issue of *Environmental Science and Technology*, a publication of the American Chemical Society.

“These challenges include constraints imposed by economics and markets, resource limitations, health risks, climate forcing, nutrient cycle disruption, water demand, and land use,” says Tom McKone, an expert on health risk assessments who holds a joint appointment with the Lawrence Berkeley National Laboratory and UC Berkeley and was the lead author on the report.

McKone and UC Berkeley professor Arpad Horvath are co-principal investigators for an EBI program on “Life-Cycle Environmental and Economic Decision-Making for Alternative Biofuels.” They are studying the potential social, economic, environmental and health impacts, from cradle to grave, of various renewable energy sources. McKone says the new paper doesn’t outline grand challenges for Life-Cycle Assessment (LCA) “as hurdles to be cleared, but rather as opportunities for the practitioner to focus attention on making LCA more useful to decision makers.”

The areas of challenge include:

- *Understanding farmers, feedstock options, and land use:* Biomass production for biofuels could displace existing products from land currently used for food, forage and fiber, which could increase

the price of these goods in global markets. It could also induce deforestation that would exacerbate global climate change.

- *Predicting biofuel production technologies and practices:* Many options exist for biofuel production processes and final products. Much of the variability among LCA results for biofuels arises from lack of knowledge about how these different possible production and operation processes will evolve.
- *Characterizing tailpipe emissions and their health consequences:* Credible and reliable impact estimates for biofuel combustion are needed, but few studies of the health impacts from transportation fuel use have extended beyond air pollutants. Those that included an explicit metric for health damages emphasized mortality rather than morbidity and the overall disease burden.
- *Incorporating spatial heterogeneity in inventories and assessments:* The health consequences of pollutant emissions vary depending upon where the pollutant is released, with factors such as proximity to large populations looming large. Geographical variability also influences other factors, including soil carbon impacts and water demand consequences.
- *Accounting for time in impact assessments:* Air emission impacts from tailpipes and production facilities accrue within years and can be allocated to the year of emissions without discounting. Greenhouse gas emission impacts are distributed over decades and even centuries using integrated assessment models, and are often discounted. Decisions about discounting can strongly influence

the outcome of impact assessments, yet there is no rational basis for making these decisions.

- *Assessing transitions as well as end states:* In addressing transitions, emerging technologies could profoundly change the assumptions that underlie biofuel LCAs. For example, changes in protein production and consumption patterns or in urban land use policies could open up substantial agricultural land for biofuel production, an action that would fundamentally change a biofuel LCA.
- *Confronting uncertainty and variability:* Addressing uncertainty is among the greatest of LCA challenges, not only for biofuels, but for other LCA efforts as well. To confront uncertainty and variability, the “doable” and “knowable” must be separated from assumptions that are conditional components of the LCA.

The authors say confronting these challenges requires a good balance between the needs of technology momentum and adaptive decision-making, something that has not always been well articulated among practitioners of LCA. “We must recognize that LCA is not a product but an ongoing process for organizing information and prioritizing information needs,” McKone says. “LCAs should be viewed as tools for building scenarios from which one can learn, rather than truth-generating machines.”

Other authors in the report include Horvath, William Nazaroff, Peter Berck, Maximilian Auffhammer, Tim Lipman, Margaret Torn, Eric Masanet, Agnes Lobscheid, Nicholas Santero, Umakant Mishra, Audrey Barrett, Matt Bomberg, Kevin Fingerman, Corinne Scown, and Bret Strogon. It can be read via a link on the EBI web site in the “Publications” section at www.energybiosciencesinstitute.org.

NEW YEAST DEVELOPMENT SHOWS HOW EBI COLLABORATIONS WORK

(cont. from page 1)



ILLINOIS YEAST TEAM (FROM LEFT): SUK-JIN HA, YONG-SU JIN, SOO RIN KIM

pathway for yeast to ferment cellobiose, a dimer of glucose, which is a compound made of two glucose molecules linked together. Cellobiose is not part of yeast's diet – not unlike yeast's typical reaction to xylose.

Cate and his team took genes from grass-eating fungi and incorporated them into yeast, creating strains that produce alcohol from cellulose that normal yeast can't digest. *Neurospora crassa*, a common fungus whose preferred diet is fire-damaged plants, can digest cellulose, though it doesn't produce alcohol. *N. crassa* has been studied in the laboratory for more than 100 years, so the Cate team did a genome-wide systems analysis and turned up a family of genes which produces proteins that transport sugars into the *Neurospora* cell to be used as fuel.

8TH FEEDSTOCK SYMPOSIUM SHOWCASES LATEST IN BIOMASS RESEARCH

(cont. from page 8)

Matt Maughan, a Ph.D. candidate at the University of Illinois, spoke about his research with *Miscanthus x giganteus*. He has been working with other researchers at Illinois as well as at locations throughout the United States to determine ideal growing conditions for *Miscanthus*. Sites besides the Illinois energy farm include the University of Nebraska at Lincoln, the University of Kentucky, and Rutgers University in New Jersey.

Breeding bioenergy crops such as *Miscanthus*, prairie cordgrass, big bluestem, and perennial sorghum was the focus of Erik Sacks' presentation. Sacks, an assistant professor of crop sciences and one of the newest EBI researchers, and his team are looking at breeding opportunities for these crops that will increase yield. Since most of these plants are still wild, breeding is important to domesticate them. He also reviewed key traits that the team wants to breed

The researchers suspected that some of these transporters would allow *Neurospora* to import short-chain sugars called cellobextrins, and that these could be further broken down into glucose molecules by the enzyme beta-glucosidase. Only then could yeast work its magic and turn the glucose into alcohol.

"Most sugar transporters let one sugar in at a time," said Berkeley graduate student Jon Galazka, lead author of the article that appeared in *Science*. "The sugar transporters we found in *Neurospora* actually let in an entire chain of sugars." He and his colleagues speculated that the inability of *S. cerevisiae* to utilize cellobextrins is due to the lack of a functional transport system.

Apparently, Galazka said, while normal yeast cells can't import cellobextrins or digest them once they're inside the cell, if a yeast cell is given a *Neurospora* transporter and a beta-glucosidase from the fungus that stays inside the cell, it's able to do both. One strain produced 60 percent more alcohol than normal yeast when grown on the two-glucose molecule, cellobiose.

Idea of Integration

Illinois' Jin recalls that BP scientist Xiaomin Yang, who knew of Jin's work and that of Cate, thought, "Wouldn't it be a great idea to integrate this research into one microorganism?" So, with Yang as a catalyst, Jin and Cate worked together and did just that. They determined that yeast ferments

into bioenergy crops: strong yield, late flowering, winter hardiness, and seed shattering.

Other speakers included Bill Deen from the University of Guelph in Canada, who discussed the ecological sustainability of biofuel crops and climate services of ecosystems; Gary Letterly from the University of Illinois Extension Office, who spoke about biomass heat and power in Illinois; Udaya Kalluri from Oak Ridge National Laboratory, who shared information on how to improve feedstocks for more sustainable biofuel production; and Heather Youngs, a bioenergy analyst at the EBI in Berkeley, who spoke about bioenergy feedstock scenarios for policymakers, industry producers, farmers, and consumers.

Besides discussing feedstocks such as *Miscanthus*, switchgrass, and corn, Anna Hale, a research geneticist with the U.S. Department of Agriculture, shared information about the sugarcane breeding program that she oversees in Louisiana. She discussed the process, successes, and challenges of crossing commercial sugarcane with its wild

xylose and cellobiose at the same rate, with no preference between the two compounds. It was an ingenious solution: instead of making xylose more appealing, the researchers instead made glucose less appealing to the yeast. The researchers found that by co-fermenting xylose and cellobiose, the new yeast more than doubled the amount of ethanol achieved if each reaction occurred separately.

It took Jin more than 10 years to engineer yeast to metabolize xylose. But when he and Cate collaborated, the experiments to engineer yeast to ferment both xylose and cellobiose took a mere six months.

"This is one of the most important discoveries in the biofuels industry," says Jin. "It was a big success due to the active collaborations in EBI. That's the main beauty of having the EBI. Under its umbrella we collaborate with no barrier even between labs or the two campuses. We don't have to worry about conflict of interest, we just collaborate for one clear mission—to create a microorganism for better biofuel production."

Other authors of the paper, which appeared in *The Proceedings of the National Academy of Sciences*, were Suk-Jin Ha, Soo Rin Kim, Jin-Ho Choi, Jin-Ho Seo, and N. Louise Glass. It is posted online, and the link can be found in the "Publications" section of the EBI web site at www.energybiosciencesinstitute.org.

Diana Yates and Deb Aronson contributed to this story.



relatives and also noted the research they have been conducting to use "energy cane" as a biofuel.

Charles Abbas from Archer Daniels Midland Company and Mike Edgerton from Monsanto both spoke about progress with corn stover harvesting, transportation, and storage. They hope that research will lead to an easier way to process high-quality stover at a low cost.

The symposium concluded with Paul Carver, CEO of New Energy Farms, discussing how his company is working with stakeholders, agricultural land owners, end users, and breeders to provide perennial energy crops, with an emphasis on *Miscanthus*, to produce renewable biomass feedstock for heat, electricity, and liquid transportation fuels.

The symposium was sponsored by the University of Illinois College of Agricultural, Consumer and Environmental Sciences (ACES); LI-COR Biosciences; New Energy Farms; and the EBI.



PUBLIC FASCINATION WITH BIOFUELS IS HIGHLIGHT OF CAL SCIENCE FESTIVAL



What was conceived as a satellite event to a 2010 national celebration of science became a local 2011 tribute to UC Berkeley's science and engineering programs, with the Energy Biosciences Institute front-and-center at the festival.

The event, designed to educate and excite communities—in particular the country's youth—about the possibilities that science brings to life and career, was originally scheduled for the weekend of Oct. 23. A signature program on the Washington D.C. mall was the celebration's centerpiece. Unfortunately, rain washed out Cal's participation in Berkeley.

The holidays passed, and event coordinator Rachel Winheld and her planning team rescheduled the festival indoors for Sunday, Jan. 23, which ironically dawned bright and sunny. The delay certainly didn't dissuade hundreds of curious families from descending upon Sutardja Dai Hall on campus to investigate the wonders of some 17 campus and community science and engineering programs, which mounted displays and demonstrated their technologies.

In the thick of it, occupying 400 square feet of space on the second floor of the building, was the EBI exhibit, promoted as "turning cellulose into energy." Under the leadership of postdoc Corinne Hausmann, an intrepid band of volunteers spent the day ushering waves of young scientist hopefuls and their parents through the stages of lignocellulosic biofuel production – biomass, deconstruction, and fermentation. Before supplies ran out, lucky visitors received safety glasses with the EBI logo, M&M's with "EBI" on one side, EBI stickers, and genomic playing cards courtesy of the DOE Joint Genome Institute and its bovine mascot, a papier-mache fistulated cow named "Betty."

Hosting and educating the guests were Hausmann and teammates Brad Dotson, Jon Galazka, Yongquin Li, Bret Strogon, Mike Fisher, and Padma Gunda. EBI Managing Director Susan Jenkins worked full-time answering questions at the welcome table, while her young daughters Morgan and Taylor made sure giveaways were restocked and fielded questions on what role a cow plays in biofuels. Exhibit developers who could not attend were Charlie Anderson, Mika Shiramizu and Matthias Hess.

As the photos at left demonstrate, making biofuels was a high-energy attraction at the festival.



FROM TOP, BRET STROGON EDUCATES A VISITOR; TAYLOR JOHNSON TENDS TO BETTY THE COW AMID MISCANTHUS PLANTS; EBI'S MIKE FISHER AND JON GALAZKA TALK ABOUT FERMENTATION; A YOUNG SCIENTIST LEARNS ABOUT BIOFUELS.

'BIOENERGY CONNECTION' MAGAZINE DEBUTS; A RESOURCE FOR ALL

BIOENERGY CONNECTION

A new resource designed to inform and advance the public dialogue about the future of bioenergy, with an emphasis on renewable transportation fuels, has been launched by the Energy Biosciences Institute under the title "Bioenergy Connection." The magazine, initially publishing two issues per year, will debut this month.

The goals of the publication are to introduce the questions that drive current research, to spotlight people who are moving the bioenergy field forward, and to provide explanations to a range of issues in terms that will broaden knowledge and understanding by specialists and non-specialists alike.

"As informed participants in the academic side of the bioenergy field, my colleagues and I at the Energy Biosciences Institute are frequently approached by individuals from government, the media, educational institutions, and industry seeking to understand at a deeper level the complex issues facing our new field," writes EBI Director Chris Somerville in his introductory letter in the first issue.

"We welcome these inquiries and are always grateful for the opportunity to provide analysis and understanding when we can and to learn from the issues raised and the dialog that ensues. In response, we have launched 'Bioenergy Connection' in the

hope that this new magazine can become a useful summary of contemporary research, emerging policies, and trends in the general field of energy biosciences."

The inaugural articles include a cover story on current trends in biofuel research, development and use; an analysis of the path to commercialization of cellulosic biofuels written by EBI analyst Heather Youngs; and a special "briefing" pull-out section on sustainability and renewable fuels developed by Jody Endres, EBI senior regulatory associate from the University of Illinois. In addition, the issue includes a look at careers in bioenergy and a feature on the life-cycle analysis of biofuels, including an interview with LCA pioneer Michael Wang.

A complementary web site will be developed to provide supplemental information on relevant subjects and to enable direct interaction with readers, according to the magazine's editor, Marie Felde. A former journalist and a communications manager at UC Berkeley for more than 17 years, Felde says EBI's expertise, paired with an editorial advisory board of bioenergy leaders from the U.S. and abroad, are vital to setting the magazine's content and direction.

She is assisted in the publication's design by art director Haley Ahlers and associate editor Melissa Edwards, from the University of Illinois' Institute for Genomic Biology, the site of EBI-Illinois. Ron Kolb, EBI communications manager in Berkeley, is doing double-duty as an associate editor of the magazine.

Comments and suggestions about the magazine are encouraged and can be provided, in addition to subscription requests, via e-mail to bioenergyconnection@berkeley.edu.

COMING SOON:

2010 EBI ANNUAL REPORT



- SUMMARIES OF ALL EBI PROGRAMS AND PROJECTS
- FEATURE PROFILES ON LEADING BIOENERGY RESEARCHERS
- PERSPECTIVES ON THE STATE OF THE BIOFUELS FIELD

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EBI BULLETIN

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