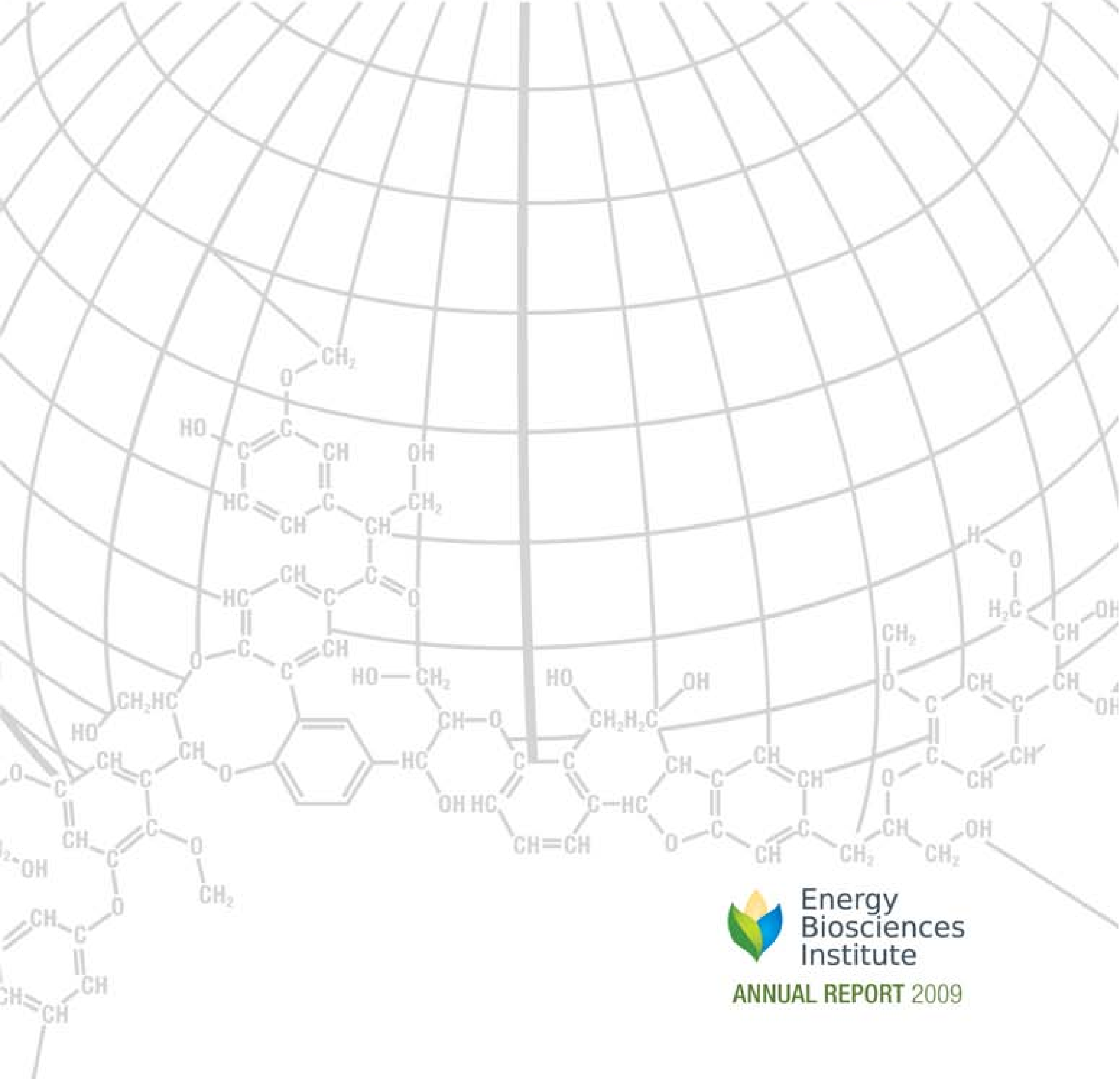
bioenergy

Exploring the Applications of Modern Biology to the Energy Sector



Energy Biosciences Institute

ANNUAL REPORT 2009

Miscanthus growing on the Energy Farm in Illinois

Message from the Director:

MULTIFACETED PROBLEMS MULTIDISCIPLINARY SOLUTIONS

November 2009 marked the second anniversary of the founding of the Energy Biosciences Institute. The following pages introduce the people who have contributed to the success of the EBI and describe the broad range of research activities supported by the EBI.

Chris Somerville

My motivation in helping to develop the EBI was concern about the consequences of continuing reliance on fossil fuels for more than 85 percent of our energy. (I should acknowledge that these are my personal views and may differ from those of my colleagues or our sponsor, the energy company BP.) The projected effects on climate of relentlessly accelerating combustion of fossil fuels are alarming to me. Additionally, the use of fossil fuels is so obviously unsustainable, and yet so hard to replace with alternatives that can reach the scale of fossil fuel consumption, that there is some urgency to explore alternatives. Although government can play an important role in establishing laws and financial incentives that support the development of alternative energy production, democracies are generally not effective at creating disruptive laws or incentives, or at production of material goods. On the other hand, I believe that major corporations can be more potent agents of change than any other sectors of society because a small number of people in a company can both make decisions to pursue new directions and also marshal large productive resources to plan and execute production of new goods and services.

As a case in point, BP was the first major energy company to acknowledge that fossil fuel combustion was causing deleterious effects on global climate, and as early as 1997 BP's then-CEO Sir John Browne committed the company to a leading role in addressing the issue (www.gsb.stanford.edu/community/bmag/sbsm0997/feature_ranks.html). He noted:

> *"As a company, our contribution is small, and our actions alone could not resolve the problem. But that does not mean we should do nothing. We have to look at both the way we use energy—to ensure we are working with maximum efficiency—and at how our products are used. It also means contributing to the wider analysis of the problem—through research and technology and through engagement in the search for the best public policy mechanisms, the actions which can produce the right solutions for the long-term common interest.*
>
> *"We have a responsibility to act, and I hope that through our actions we can contribute to the much wider process which is desirable and necessary. BP accepts that responsibility, and we're therefore taking specific steps:*
>
> - *to control our own carbon dioxide emissions*
> - *to fund continuing scientific research*
> - *to take initiatives for joint implementation*
> - *to develop alternative fuels for the long term*
> - *and to contribute to the public policy debate in search of wider global answers to the problem."*

This was not a wishy-washy statement but an announcement of resolve that has dominated the company's stance since that time. I think of it as a call to arms that is remarkably compatible with the Berkeley culture of activism. One of the outcomes of BP's commitment was the formation of the EBI, in addition to supporting other research groups pursuing complementary issues at Stanford, Princeton, MIT, and other leading universities. Thus, I view the EBI mission as providing ideas and innovations that support the company's commitment to find new, more sustainable energy technologies through research.

When we were forming the EBI, substantial thought was invested in questions about the focus of the institute. BP had requested that the EBI focus on the application of modern biology to the energy sector because other aspects of the larger problem were being explored at other partner institutions. However, within that context there are many possibilities. One hypothetical strategy might be to identify the half-dozen major technical problems and devote all of the resources to a narrow but forceful attack on those problems. Another approach might be to avoid obvious problems because of the likelihood that such problems are being studied in many other places and to only focus on radical new ideas that could not attract government research dollars.

In the end, we settled on the idea of creating an organization that would investigate all aspects of the field of energy biosciences. One reason for this approach is that many of the underlying problems are multifaceted and require multidisciplinary solutions. However, the most important reason arises from the fact that most of the research in the world is carried out in an extremely disjointed way. Publicly funded researchers typically pick problems based on individual interests rather than according to some grand plan and typically publish their results in disciplinary journals that are only read by people in the same field. We reasoned that, if the EBI could create an integrated research community, we may be able to identify research opportunities and solutions that are not obvious from a narrow research perspective. This idea acknowledges that many important discoveries will be made outside the EBI, but by having EBI investigators in all of the fields relevant to energy biosciences, there is a high probability that an EBI investigator will learn of important discoveries in their field as soon as they happen and can bring that knowledge to the mission of the institute as a whole.

Our belief is that this flux of ideas will support creative problem-solving of the kind we expect to bring to the energy problem. I have been very pleased with the many instances of interdisciplinary collaboration and cooperation that have occurred during the first two years and very much appreciate the collegiality among EBI investigators that has led to exciting advances.

The institute has completed the transition from a startup to a fully staffed organization with established processes and practices for most aspects of operation. As a result of calls for proposals in 2007, 2008, and 2009, we currently support about 60 research groups involving about 120 faculty and about 200 graduate students and postdocs. Additional solicitations are envisioned in 2010 and in subsequent years. At this juncture, the research topics encompassed by this group are mainly focused on understanding the issues associated with the proposed development of a cellulosic biofuels industry, but we also support a group that is exploring the microbial ecology of fossil fuel reservoirs. Our goal is to probe all aspects of cellulosic fuels with the highest quality academic research and to integrate knowledge from this broad investigation into a coherent understanding of the overall topic. Our goals in deep earth ecology are to understand the properties of the reservoir populations in order to evaluate whether it is possible to modulate their metabolism or population dynamics in useful ways vis-à-vis fossil fuel recovery.

In pursuit of these goals, EBI investigators are engaging in research in a wide range of academic disciplines that include agronomy, agricultural engineering, biochemistry, chemistry, chemical engineering, ecology and environmental science, economics, geography, law, microbiology, plant breeding, public policy, and systems biology. It is our hope that by supporting a broad investigation within a single framework, including common space, we can facilitate interdisciplinary research that leads to holistic understanding, innovation, and insight while simultaneously minimizing the risks of pursuing ideas that lead to dead ends.

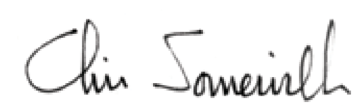
An important aspect of the research program is the co-location of a team of engineers and scientists from BP within the EBI. At present, BP rents a small amount of office space on the UC Berkeley campus where a number of BP scientists and engineers are engaged in studying energy biosciences from an industrial perspective.

> “I view the EBI mission as providing ideas and innovations that support the company's commitment to find new, more sustainable energy technologies through research.

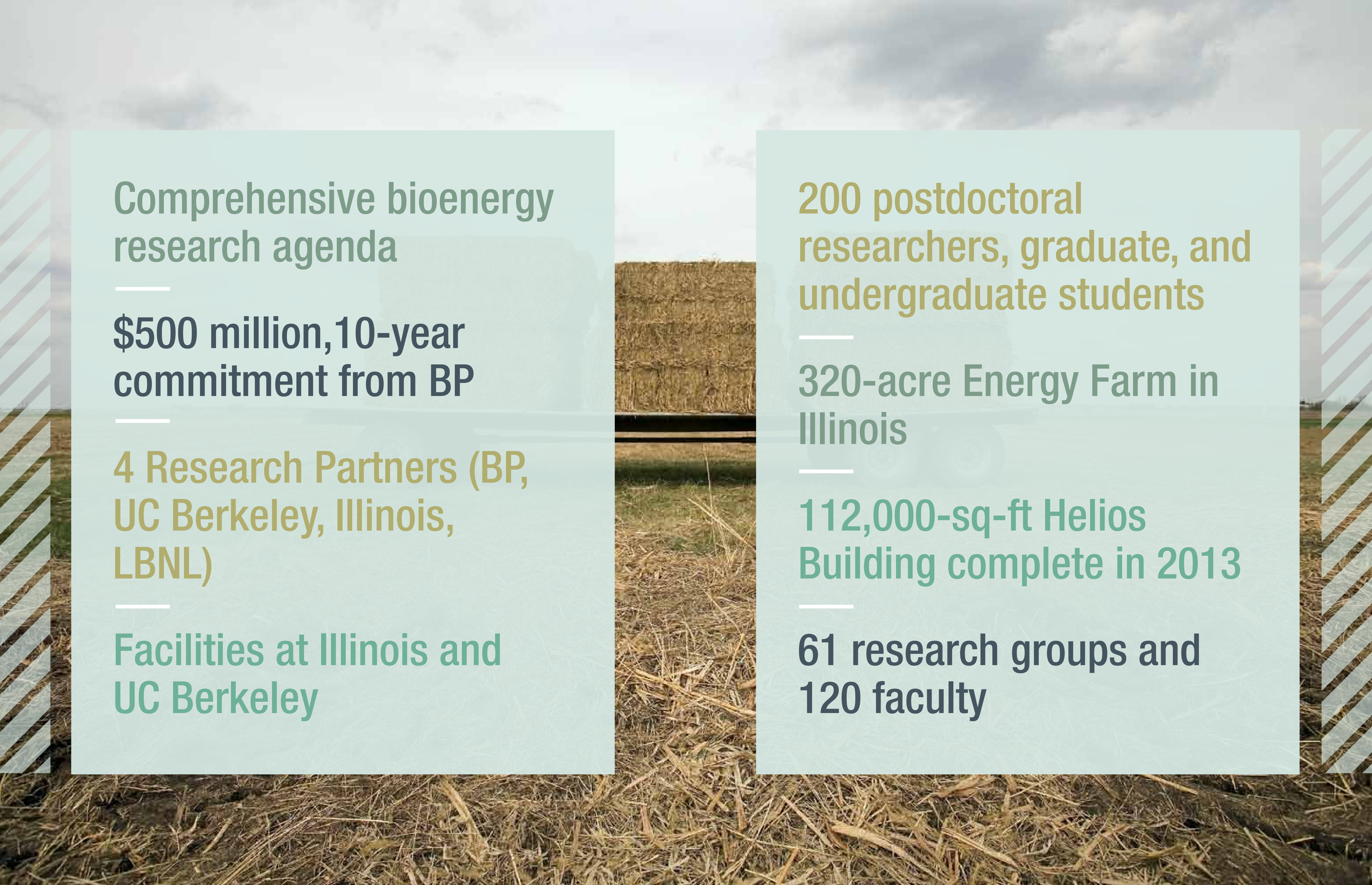
Unlike the academic members of the EBI, who are focused on fundamental academic questions, the BP fellows are engaged in envisioning how knowledge might be used in practice. The dialectic between those engaged in basic research and those charged with applied research is fertile ground for the development of new questions and a highly efficient way to translate basic research. Equally importantly, the arrangement provides a wonderful opportunity for education across both sides of the basic/applied boundary. Seminars at EBI venues and at the EBI retreat this year by BP executives Phil New and David Eyton and by many BP engineers provided exceptional opportunities to understand perspectives on important aspects of the world energy situation from people who participate directly in providing energy.

Looking forward, I expect many exciting discoveries to emerge from EBI research. The exceptional resources provided by BP have afforded a unique opportunity to explore many new areas without some of the conservative influences and financial limitations that impair much federally-funded research. Many brilliant and engaged people who had not previously worked on topics related to energy biosciences are now testing their ideas, and I expect an outpouring of innovation and creativity.

EBI's initial two years have been filled with a hopeful energy, tireless dedication of researchers and support staff, and productive first steps on our journey as reflected in these pages. Deputy Director Steve Long, Associate Director Paul Willems, and I are privileged to lead the EBI forward.



CHRIS SOMERVILLE
December 2009



Comprehensive bioenergy research agenda

$500 million, 10-year commitment from BP

4 Research Partners (BP, UC Berkeley, Illinois, LBNL)

Facilities at Illinois and UC Berkeley

200 postdoctoral researchers, graduate, and undergraduate students

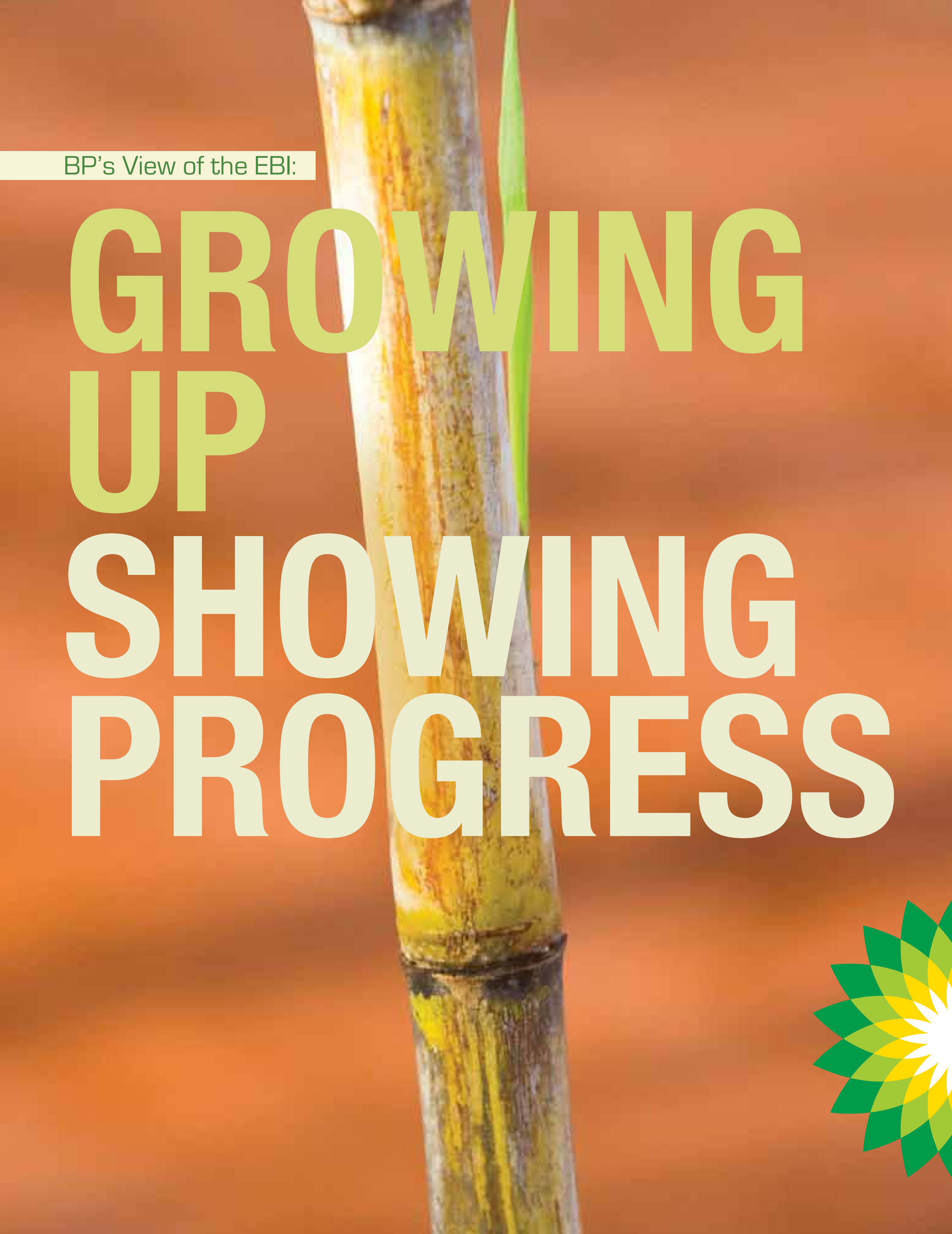
320-acre Energy Farm in Illinois

112,000-sq-ft Helios Building complete in 2013

61 research groups and 120 faculty

BP's View of the EBI:

GROWING UP SHOWING PROGRESS



The creation of the Energy Biosciences Institute—a partnership between BP, the University of California, Berkeley; the University of Illinois at Urbana-Champaign; and the Lawrence Berkeley National Laboratory—was rooted in a belief that modern biology as a science is ready to start making significant contributions to energy problems.

Paul Willems

Over the course of 2009, the EBI has transitioned from adolescence into adulthood. The look and feel of the institute has changed, the cardboard boxes are gone, people know each other, and tangible research progress is being made. In the next few paragraphs I would like to reflect on some of the aspects of our journey over the last year.

The EBI has managed to stay on its long-term intended course in 2009, despite the turmoil in the wider world. The global financial crisis, a severe economic recession, and sharp declines in energy prices have injected a dose of realism into the alternative energy space. Economic targets for sustainable energy production, which looked achievable when crude oil was $150 a barrel, look a lot more daunting at $50 or $75 a barrel. Yet this is the reality of the level of innovation that will have to be delivered to transform sustainable energy from a niche contributor to a solution implemented at scale. Without technology enabling it to be fundamentally economically competitive (i.e. without subsidy), alternative energy will not rise to the level of prominence that we all hope. BP's commitment to the need for innovation and technology development in the field is stronger than it has ever been.

BP CEO Tony Hayward, left, visited the EBI lab in Illinois in 2009.

During 2009, the management team of the EBI had the opportunity to interact deeply with the most senior leaders in BP, including a half-day session with the BP board and an additional session with our CEO, Tony Hayward. This dialogue has reinforced BP's belief about the importance of modern biology to the future of the company. It has also confirmed that partnerships like the EBI are not just vehicles for doing great academic research: They have the potential for creating distinctive value for the company.

The initial wave of projects in the EBI has been focused on lignocellulose-based biofuels. This is the most obvious large-scale opportunity in the field. We have augmented that this year with a collaboration focused on microbially enhanced hydrocarbon recovery (MEHR). All hydrocarbon reserves are believed to be of biological origin. In addition, microbes are believed to have played a major role in the transformation of the original biomass. Once thought to be largely devoid of life, geological formations have been shown to have a significant number of microorganisms living there. Understanding the basic biology that occurs in the deep underground may lead to new insights on how to develop these resources or how to mitigate some of the negative environmental consequences that come with their development. For instance, it might be possible to stimulate the transformation of underground heavy hydrocarbons into methane, a fuel with a much decreased greenhouse gas impact. Or it might be possible to reduce their molecular weight, enabling their production with much reduced environmental impact.

In lignocellulose-based biofuels, we have chosen to engage in a broad research portfolio, spanning the entire value chain from dedicated energy crops and their management practices to the technology used to convert them to fuels, from a variety of target fuel molecules to chemical and biological conversion pathways, etc. This

"BP's commitment to the need for innovation and technology development in the field is stronger than it has ever been.

Understanding the implications of using farmland to grow biofuels feedstocks is a hallmark of EBI research.

strategy is necessitated by the fact that these supply chains simply do not exist today. In order to achieve the long-term challenge of economic self-sufficiency, large cost reductions are needed. These will have to come from all parts of the supply chain. Working across the entire chain gives access to innovations in the individual fields and provides the opportunity to optimize across interfaces. The multidisciplinary nature of the EBI is a clear enabler here.

Our investment in socioeconomic research is one of the features that make the EBI unique: Biofuels done the right way is of crucial importance to us. Throughout 2009 the EBI has established itself as a leader in the field. We have learned that many of the potential issues concerning production of lignocellulosic biofuels need to be assessed in a big-picture context. For instance, indirect land use effects have been hotly debated this year. While most people readily understand the indirect land use concept, it has proven to be quite hard to quantitatively assess it in a scientifically sound manner. It is even harder to assess how incorporating these effects into biofuels regulation will actually lead to the desired outcomes (e.g. protecting valuable rainforests). Also, indirect land use change is not just caused by biofuels activity. Any human activity that requires land (e.g. roads, shopping malls, housing subdivisions, golf courses, etc.) has an indirect land use effect. The discussion around this important issue has led to a growing realization that land use policy and management should be addressed in its totality rather than as an indirect effect in biofuels policy.

This is just one example of how our research agenda needs to evolve as we get a better grasp of the issues.

The EBI is a public/private collaboration of unprecedented scale and scope. It is a mission-oriented, multidisciplinary, team-science-based institute. Understanding how to effectively interface and facilitate collaboration between a large corporation like BP and the EBI academic partners has been a major focus of the EBI management effort during 2009. Co-locating BP researchers at the institute has proven to be an effective first step—we now have 13 staff based at the EBI. We also have mapped out work processes for the management of Intellectual Property and for the commercialization of various types of inventions. We have identified what meetings we need, who should be in them, etc.—the mundane day-to-day aspects of a new operation.

In summary, the view from my position at the interface of BP and the EBI is that EBI has continued to make great progress in 2009, and we are excited to continue our journey in 2010.

PAUL WILLEMS
Associate Director, EBI
Technology Vice President, Energy Biosciences, BP
December, 2009

Field workers on the EBI's 320-acre Energy Farm

To address one of the 21st Century's greatest challenges—finding a technological solution to the problems associated with climate change, global warming, and the lack of sustainability of carbon-based fossil fuels—the global energy company BP assembled a unique partnership.

An Institute of

COLLABORATIONS

Exploring the Applications of Modern Biology to the Energy Sector

It combined the world-renowned scientific expertise of three public research institutions with the private business acumen of an international leader in energy production.

Thus the Energy Biosciences Institute (EBI) was born in 2007, embarking on a 10-year, $500 million quest to help the world transition from a carbon-based fuel strategy to one that incorporates sustainable, environmentally friendly fuels into a balanced portfolio of responsible, renewable energy sources.

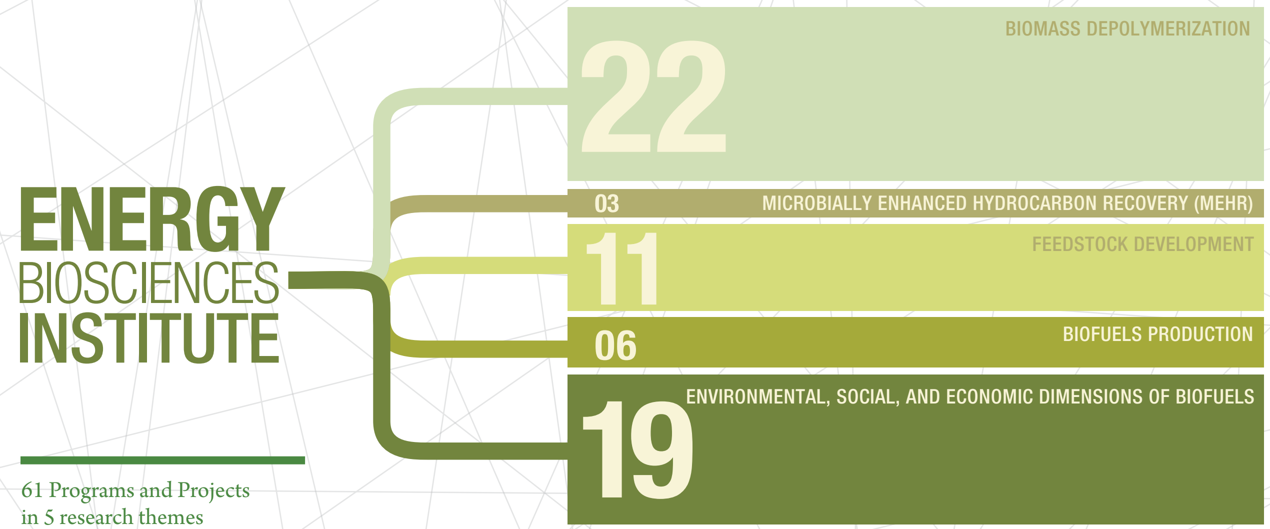
BP was the first major energy corporation to publically recognize that continued burning of fossil fuels was problematic. In 1998 in a speech at Stanford, then-BP Chief Executive Officer John Browne committed the company to comply internally with the Kyoto Protocol. "It would be unwise and potentially dangerous to ignore the mounting concern" about climate change, said Browne. As a result, BP created a renewable energy division that has grown to become a major photovoltaic and wind energy producer. More recently, Browne's successor, Tony Hayward, has called for the implementation of a cap-and-trade system in which emissions of greenhouse gasses are monetized so that the environmental costs and benefits of various energy options can be factored into the cost of energy.

The area of "energy biosciences" has been relatively unexplored and underinvested compared with the vast investment that has been made in health-related aspects of the biosciences. Thus the EBI was established, following an international competition involving about 20 major research universities. Now completing its second full year, the EBI consortium—the University of California at Berkeley, Lawrence Berkeley National Laboratory, the University of Illinois at Urbana-Champaign, and BP—is defining a new era of groundbreaking public-private partnerships.

An Integrated Approach

An overriding goal of the EBI has been to develop an integrated, holistic understanding of the research topics related to energy biosciences. Topics of interest such as cellulosic biofuels are unusually complex and involve research questions in subjects that include agronomy, microbiology, mechanical and chemical engineering, biochemistry, chemistry, geography, economics, law, and policy analysis. Because advances in one area may have important impacts in other areas, the EBI is creating dynamic intellectual bridges between the various disciplines so that information and insights flow efficiently, and new research initiatives are adopted based on a "big picture" view of the overall topic. Full-time researchers occupy common space on each campus, thus facilitating horizontal integration of disciplines.

This multidisciplinary approach will facilitate discovery and will ultimately enable optimal decision-making by the sectors in society that are responsible for implementing trade and regulatory policies and business activities. In this respect, the close association within the EBI of academics from the partner institutions and industrial managers, engineers, and scientists from BP offers a rare opportunity to accelerate the processes that are associated with conversion of academic discovery into real-world applications.



The Research Enterprise: Public and Private

Openness of the research enterprise—and the academic freedom of its faculty, graduate students, and university researchers—is paramount for the three public institutions in the EBI. Inventions made during the course of research within the EBI are owned by the academic institutions, and BP receives an automatic non-exclusive license in return for funding the research. All four partners have representation in the EBI's governing board of directors, with none having a majority or veto power, encouraging consensus in all decisions. The Executive Committee, which provides scientific direction and operational oversight, is mostly composed of professors from the academic partners.

The Licensing Executives Society, a professional organization for intellectual property specialists, chose the EBI for its 2008 "Deal of Distinction" award for being "an innovative model for collaboration between academia, government labs, and industry."

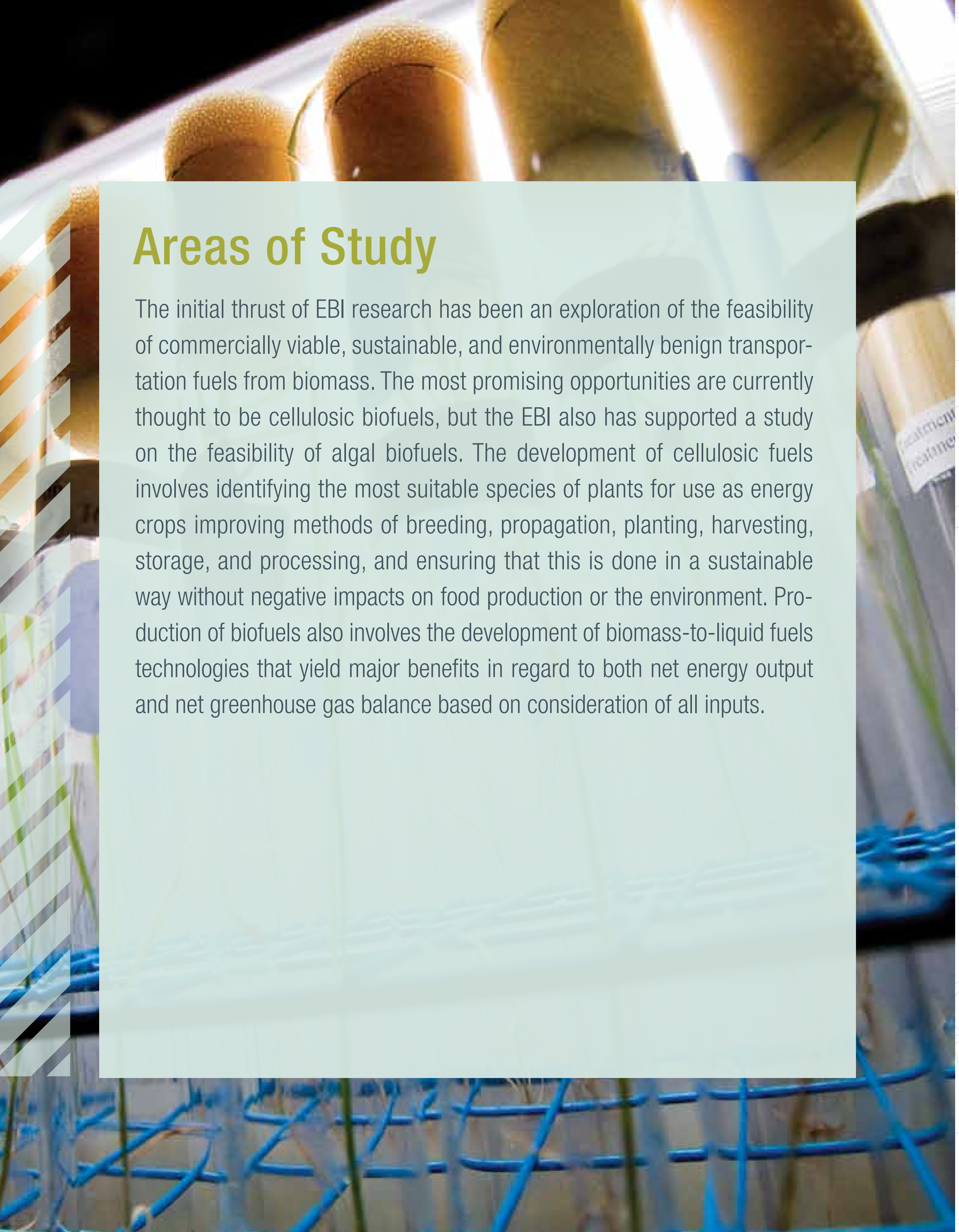
Collaborative research between universities and industry yields new ideas and more effective pathways for moving discoveries from the laboratory into commercial use. These collaborations also help prepare students for non-academic careers and address the need for real-world evaluation and implementation of solutions science.

Programs and Projects

The EBI is a mission-oriented organization. Research funds are allocated to various topics based on predefined targets. Workshops are scheduled throughout the year to share data and to help define key questions to be answered. Topical proposals that address problems defined by the EBI are solicited from faculty and scientists in the partner institutions. A peer-review process narrows proposals drawn from solicitations to a focused set of projects and programs for funding.

More than 50 high-priority research efforts received the first round of funding in 2007-08. A more narrowly focused second solicitation in the summer of 2008 and another broad call in 2009 added 11 more funded efforts. Awards are divided into two categories: programs and projects. Programs are typically large integrated multi-investigator efforts with broad goals, funded at anywhere from about $400,000 to about $1 million per year, and may continue for the 10-year life of the institute. Projects are smaller activities of two to three years in duration that are usually narrower in scope. These average about $400,000 per project.

Program research is conducted mostly within EBI space so that postdoctoral, support, and graduate student researchers from different disciplines can work side-by-side, facilitating synergy across fields. This cross-discipline model also provides a training environment and a broad appreciation of the scientific, technological, environmental, economic, and policy issues that must all be addressed to achieve the Institute's goal of environmentally sustainable bioenergy.



Areas of Study

The initial thrust of EBI research has been an exploration of the feasibility of commercially viable, sustainable, and environmentally benign transportation fuels from biomass. The most promising opportunities are currently thought to be cellulosic biofuels, but the EBI also has supported a study on the feasibility of algal biofuels. The development of cellulosic fuels involves identifying the most suitable species of plants for use as energy crops improving methods of breeding, propagation, planting, harvesting, storage, and processing, and ensuring that this is done in a sustainable way without negative impacts on food production or the environment. Production of biofuels also involves the development of biomass-to-liquid fuels technologies that yield major benefits in regard to both net energy output and net greenhouse gas balance based on consideration of all inputs.

To accomplish this, research is divided into several areas of inquiry:

Feedstock Development

Scientists seek to identify and characterize plant species that can maximize cellulosic biomass production in various regions around the world, and to learn how to grow and harvest them sustainably using minimal land, water, and energy. Because of the importance of soil carbon in the global greenhouse gas balance, the EBI is particularly interested in identifying species that can be grown on the large amounts of minimally productive land around the world. These considerations favor the use of perennial grasses and certain woody species. However, the possible utility of algal species is also being explored.

Biomass Depolymerization

The main constituents of the body of higher plants are polysaccharides and lignin. Fashioning fuel from plants requires conversion of the polysaccharides to sugars by severing the chemical bond that holds them together, among the most critical and difficult steps in the process. Today's practices are costly and inefficient. EBI scientists are investigating nature's methods of releasing these sugars to achieve an effective and less costly method of breaking down these substances. This will be key to ensuring that biofuels can be reasonably priced. The EBI is examining the processes that take place in cow rumen, termites, compost heaps, and other environments where biomass degradation takes place. In parallel, it is also exploring the development of new synthetic catalysts that can accelerate the degradation of polysaccharides and lignin.

Biofuels Production

In order to convert sugars to liquid fuels, the proportion of oxygen must be reduced. This can be accomplished by bioconversion, such as fermentation, or by chemical transformations. With no clear front-runner at the present time, the EBI is exploring several methods in parallel.

Methods used for production of biofuels today are similar to the fermentation practices used to make beer and wine, but these traditional methods are not optimized for the large-scale, energy-efficient production of cellulosic biofuels. EBI researchers are exploring ways of improving bioconversion of sugars to next-generation fuels by using the methods of systems biology to characterize new types of microbes and by testing genetic modifications of promising organisms. They are particularly interested in exploring ways of producing biofuels that will not require major changes in the transportation infrastructure. This involves researching chemical and fermentation routes to products more hydrophobic than ethanol and butanol. The EBI is also exploring alternatives to bioconversion technologies such as the use of non-biological catalysts to transform biologically-derived chemicals into fuels.

Environmental, Social, and Economic Dimensions of Cellulosic Biofuel Development

A major goal of the EBI is to understand the potential environmental, economic, and societal impacts of meeting a growing portion of the world's energy needs through cellulosic or algal biofuels. Many in the world are concerned that the demand for energy is so large that unrestrained conversion of land to biofuel production could have negative environmental effects and could further disadvantage many poor people by increasing prices for food, feed, and fiber. Therefore, the EBI is working to understand how land is used around the world and to model the impacts of growing bioenergy crops on land that is not used for food production or is providing key ecosystem services, such as carbon storage or biodiversity.

EBI investigators also are testing the environmental impacts of various bioenergy crops and developing economic models that may help to understand the feasibility of bioenergy crop production around the world. An important aspect of understanding the environmental impact of cellulosic biofuels includes development of complete life cycle models that incorporate both direct and indirect effects of biofuels.

Microbiology of Fossil Fuel Reserves

Significant populations of microorganisms are found in both coal and petroleum reservoirs deep underground. These microbial populations can contribute to the properties of the reservoirs in deleterious ways, such as through catalyzing the souring of petroleum, but they may also contribute positively by activities such as altering the porosity of the reservoirs. This could allow more efficient recovery of oil. In order to understand the effects of these microbial populations, the EBI supports studies into characterization of the organisms found in various reservoirs using the tools of modern biology such as high-throughput DNA sequencing and analysis. By understanding the genomics of the reservoir microbes, it may be possible to infer how their activities can be better controlled toward useful purposes. The collaboration with BP provides a rare opportunity for academic scientists to access deep-earth samples from reservoirs that have been geologically characterized.

The Facilities

Research in the Energy Biosciences Institute is being conducted at two primary locations—in California, at the historic Calvin Laboratory and the nearby Hildebrand Hall chemistry building at UC Berkeley, and in Illinois, at the Institute for Genomic Biology building in the heart of the campus. Just south of the Illinois campus, a 320-acre Energy Farm, the largest of its type, includes land for demonstrations, large-scale production, plant breeding, and storage.

The Berkeley center includes dedicated biotechnology laboratories and specialized facilities for high-throughput chemical synthesis and assays of many types. The Illinois program is housed in a building specifically designed for integrated research and development efforts, with a complete suite of microscopy, imaging, plant growth, microfabrication, and bioanalysis facilities and tools. In addition, individual researchers have access to the offices, technical laboratories, and user facilities of their home campuses.

The EBI is planning to move its permanent headquarters into a new five-story, 64,000-useable-square-foot facility to be built in downtown Berkeley. Construction is scheduled to begin in 2010 and conclude in 2013. The Helios Energy Research Facility will have office and laboratory space for about 250 Institute staff, including the EBI investigators, BP scientists, and laboratory personnel. The building will have amenities that will promote a collegial and collaborative research environment, including interaction spaces and conference rooms.

The Partners

THE UNIVERSITY OF CALIFORNIA, BERKELEY

Founded in 1868, the University of California, Berkeley, is the nation's top-ranked public university and the flagship of the 10-campus University of California system. It enrolls over 24,000 undergraduates, distributed among 80 degree programs, and more than 10,000 graduate students each year. The campus not only produces more Ph.D.s than any other university in the country, but a greater number of its graduates go on to earn a Ph.D. at Berkeley or elsewhere than do graduates of any other institution. The university is distinguished by its research programs, which were funded in fiscal year 2009 by $649.4 million in contract and grant awards from outside sponsors. Berkeley faculty and researchers have won 21 Nobel Prizes, six Pulitzer Prizes, 30 National Medals of Science, and 30 MacArthur "genius" Awards. Of its academic staff, more than 130 are current members of the National Academy of Sciences, and 87 belong to the National Academy of Engineering.

THE UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

The University of Illinois at Urbana-Champaign is a world-class public university whose faculty, student, and alumni honors have brought international distinction. Home of the largest public university library collection in the world, Illinois is also a leader in supercomputing designs and applications and boasts multi-disciplinary research excellence in dozens of fields. It is a world leader in agriculture, particularly in crop production. The campus contributes extensive expertise, facilities, and personnel in the breeding and processing of biomass. It has a pioneering history of sustainability research as the originator of no-till agriculture and is home of the longest running investigation of the impacts of varied crop management methods on soil quality (1876, Morrow plots) outside of Europe. The Illinois campus includes several square miles of experimental farmland, greenhouses, and controlled-environment facilities. Founded in 1867, Illinois enrolls over 29,000 undergraduates in more than 150 fields of study, and over 11,000 graduate and professional students in over 100 programs. It is among the top five universities in the United States in the number of annual doctorates awarded. Among its array of faculty honors, Illinois is one of only 11 campuses worldwide to have been awarded two separate Nobel Prizes in one year (2003).

LAWRENCE BERKELEY NATIONAL LABORATORY

Lawrence Berkeley National Laboratory (Berkeley Lab) has been a leader in science and engineering research for almost 80 years. Located on a 200-acre site in the hills above UC Berkeley's campus, the Lab is the oldest of the U.S. Department of Energy's national laboratories. Managed by the University of California, it operates with an annual budget of more than $550 million and a staff of about 3,800 employees, including more than 500 students and 250 principal investigators with joint appointments at UC Berkeley. It employs a "team" concept to its research, as developed by founder Ernest Orlando Lawrence, and boasts a legacy that has yielded rich dividends in basic knowledge and applied technology, and a profusion of awards. Berkeley Lab conducts unclassified research across a wide range of scientific disciplines, with key efforts in fundamental studies of the universe, quantitative biology, nanoscience, new energy systems and environmental solutions, and the use of integrated computing as a tool for discovery. Its unique user facilities include the Advanced Light Source, the Molecular Foundry, the National Center for Electron Microscopy, and the Joint Genome Institute.

BP

One of the world's largest energy companies, BP (formerly British Petroleum) is the leading producer of oil and natural gas in the United States, and the largest investor in U.S. energy development. BP provides its customers with fuel for transportation and energy for heat and light, employing more than 100,000 people worldwide and more than 35,000 in the U.S. BP was the first major energy company to acknowledge the need for precautionary action to reduce greenhouse gas emissions, and today it continues to lead the effort to meet the world's growing demand for sustainable, environmentally responsible energy.

From left EBI Associate Director Paul Willems, EBI Director Chris Somerville, and EBI Deputy Director Steve Long

The Management Team

The EBI operates as one of several research centers within the partner institutions. The faculty and students that design and carry out the research activities of the EBI have appointments and academic responsibilities within the various academic departments of the partners. The EBI administers the financial and material resources and facilities supported by funding from BP.

The Governance Board has the responsibility to define, oversee, and review the implementation of EBI programs in the open component of research. It also appoints the EBI Director and Deputy Director. The Board has eight voting members, four from the research partners—at least one each from the Berkeley and Illinois campuses and one from Berkeley Lab—and four appointed by BP. The EBI Director, Associate Director, and Deputy Director are ex-officio members.

The EBI is managed on a day-to-day basis by a Director and a small team of colleagues and advisors from the four partners (the Executive Committee). The EBI Director, Chris Somerville, manages the conduct of research projects and EBI's public communications, education, and outreach activities. He works with the Executive Committee to develop an annual program plan with goals and milestones, and he prepares the annual budget request. The EBI's Deputy Director, Steve Long, manages research conducted at the Illinois EBI site and its integration into the Institute as a whole. The Associate Director, Paul Willems, is the BP representative on the EBI management team.

The Executive Committee is the EBI's program management body, with Director Somerville as chair. He, the deputy and associate directors, nine professors from the partner institutions (currently Adam Arkin, Alex Bell, Doug Clark, Evan DeLucia, Jody Endres, Terry Hazen, Madhu Khanna, Michael Marletta and David Zilberman), and a BP representative (Tom Campbell) comprise the committee membership. This panel proposes the annual strategic work plan, including priority research projects for institute funding, for approval by the Governance Board.

The Advisory Board, comprised of representatives from industry and academia with expertise in scientific leadership, meets annually to provide advice to the EBI Governance Board on goals, program implementation, mission, scope, operational processes, and scientific culture. In 2009 the Board included Steve Briggs, University of California-San Diego; Brent Erickson, Biotechnology Industry Organization (BIO); Steve Fales, Iowa State University; Gerald Fink and Greg Stephanopoulos, Massachusetts Institute of Technology; Barbel Hahn-Hagerdal, Lund University-Sweden; and Lynn Orr, Stanford University.

THE DIRECTORS

Chris Somerville, Director:

Dr. Somerville is a professor in the Department of Plant and Microbial Biology at UC Berkeley and a faculty scientist at the Lawrence Berkeley National Laboratory. His research focuses on the characterization of proteins implicated in plant cell wall synthesis and modification. He has published more than 200 scientific papers in plant and microbial genetics, genomics, biochemistry, and biotechnology. Dr. Somerville has served on the scientific advisory boards of many corporations, academic institutions, and private foundations in Europe and North America. He is a member of the U.S. National Academy of Sciences, the Royal Society of London, and the Royal Society of Canada.

Stephen Long, Deputy Director:

Dr. Long is the Robert Emerson Professor of Plant Biology and Crop Sciences at the University of Illinois at Urbana-Champaign. He is a researcher with UIUC's Institute for Genomic Biology and a resident scientist with the National Center for Supercomputing Applications. Much of Dr. Long's research focuses on the effects of atmospheric change on vegetation and ecosystems and on how certain perennial crops might be used as biomass energy sources. He was a contributing author and referee to the Intergovernmental Panel on Climate Change, and he has served on committees worldwide that research global climate change. He has been named one of the 250 most cited authors in animal and plant biology and one of the 25 most cited authors on global climate change. Dr. Long is a Fellow of the American Association for the Advancement of Science (AAAS).

Paul Willems, Associate Director:

As Technology Vice President for Energy Biosciences at BP, Dr. Willems is responsible for integrating biotechnology into BP's business activity. His duties include leading the development and execution of an integrated technology strategy which incorporates all of BP's bio-related activity and is fully integrated with BP's company-wide business strategies. He has held a variety of technical, manufacturing, and commercial leadership roles throughout his 20-year career, including business technology manager for BP's global PTA (purified terephthalic acid, a polyester raw material) business, and technology vice president for acetyls and aromatics. Willems earned his Ph.D. in Chemical Engineering from the University of Ghent in Belgium.

The Team Behind the Science

In any successful organization, behind every good product there is an infrastructure of capable people who ensure that the work gets done, that policies are followed, and that barriers to an accomplished mission are removed. The EBI is no exception. In the support staff at Berkeley and Illinois, one finds an abundance of skills and expertise that enable researchers to accomplish their goals at maximum capacity with minimum inconvenience.

As the lead administrator for research and operations, EBI Assistant Director **Susan Jenkins** is key point person for short and long-term strategic planning. In addition to overseeing the ongoing activities of the institute, she is responsible for significant outreach activities conducted by the EBI.

Assistant Deputy Director **Jenny Kokini** is the point of contact for the Institute's Illinois operations. She serves the needs of 10 program leads, their co-PIs, and 15 different projects in everything from supplies and equipment, to recruitment and hiring, to budget control.

Tim Mies is Director of Operations for the 320-acre EBI Energy Farm in Illinois. His expertise in large-scale agricultural research implementation is crucial to the success of programs and projects at Illinois and Berkeley.

Mitchell Altschuler is EBI's Intellectual Property Manager. He assists researchers with their inventions, disclosures, patent applications and related research product policies. In addition, he provides strategic advice to the EBI management and researchers and coordinates activities with outside counsel.

Bioenergy analysts **Caroline Taylor** and **Heather Youngs** are on the Berkeley staff, assisting scientists by reviewing existing research results, assessing status and prospects of various biofuels programs, and developing feedstock scenarios and processing options. Ecologist **Sarah Davis** of Illinois, the third member of the team, provides expertise in feedstocks and land use modeling.

Stefan Bauer directs the EBI's Analytical Biochemistry Group, which provides well-characterized materials for research projects. In addition to conducting independent research to further our understanding of lignocellulose, Bauer supervises a team of four technicians in Berkeley: **Sandra Villa**, **Ana Ibanez Zamora**, **Mark Toews**, and intern **Mark Schnepper**.

The EBI's Information Technology Manager is **Adam Cohen**, who is based in Berkeley and supports researchers with computing expertise and guides the EBI's electronic future, including expansion of database support, enhancing the EBI wiki environment, and building new applications for future implementation.

Calvin Laboratory building manager and EH&S specialist **Zack Phillips** is the key facilities manager and onsite health and safety expert. He is the main liaison with campus safety and facilities programs and outside contractors and clients.

The Institute's laboratory managers—**Mara Bryan** at Berkeley and **Jing Dong** at Illinois—are scientific resources to the researchers, assisting with instrument acquisition, providing guidance on lab protocols, and conducting data analysis. Dong took over in 2009 for Rachel Shekar, Illinois' first lab manager, who left to become Assistant to the Deputy Director at the host Institute for Genomic Biology.

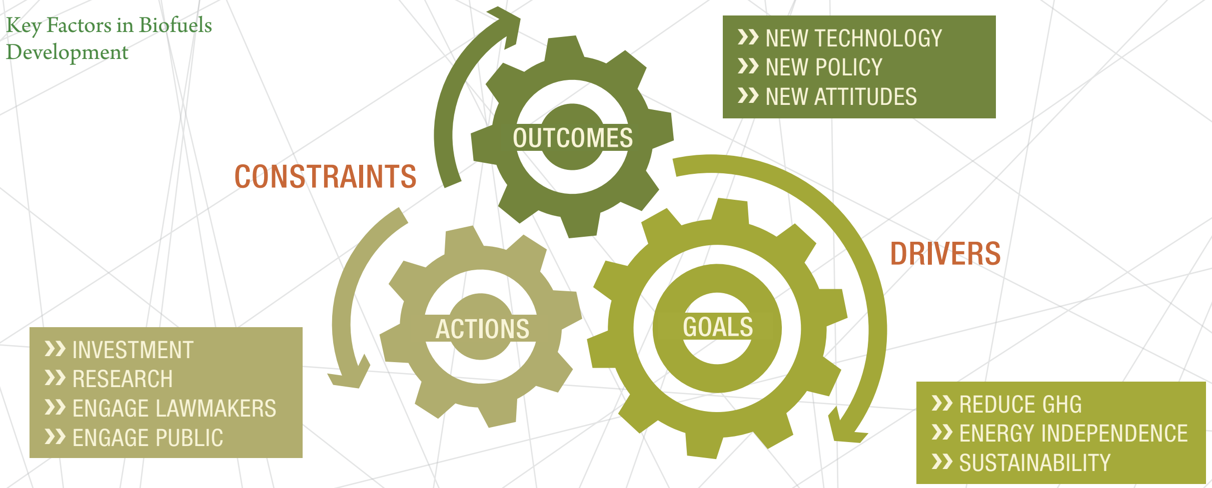
Mark Shaw is the EBI's Operations Manager, engaging in resource analysis, planning and oversight, non-research budget development and management, and protocol and procedure development and implementation.

Research administrator **Anne Krysiak** manages program and project budgets and coordinates the research proposal process, including the calls, evaluations and external reviews.

Ron Kolb, the EBI's Communications Manager, provides content for the EBI web site and produces news releases, the EBI's external (The Bulletin) and internal (EBInsider) newsletters, and the EBI Annual Report. His Illinois colleagues—**Melissa Edwards**, **Haley Ahlers**, and **Kyeon Heo**—design and produce the EBI's publications.

Berkeley Administrative Services Manager Trisha Togonon left for nursing school in 2009 and was replaced by **Shelley Brozenick**, who manages human resources for the Berkeley staff and also assists with program development and intellectual property activities. **Susie Leblovic** is Administrative Services Specialist and plays a key role in managing the support services at Berkeley, while **Becky Heid** is the administrative services counterpart in Illinois.

Key Factors in Biofuels Development



The EBI Support Centers

BIOENERGY ANALYSIS TEAM

The Bioenergy Analysis Team (BAT) provides a bird's-eye view of the entire EBI research portfolio. BAT projects involve integrating and forecasting emerging knowledge in biofuels and bioenergy science, technology, environmental impacts and policy.

During their productive first year in 2009, the analysts tackled a range of assessment projects, including the creation of scenarios describing many of the technological and agronomic aspects of biofuels production. Guided by emerging research and incorporating a balance of constraints and drivers, these scenarios provide the EBI community with a picture of how biofuels production may develop and with consistent baselines against which alternate pathways and futures can be compared. Similar to other staff and researchers in the EBI, the analysts represent the EBI on relevant external committees, providing insight and perspective to a range of renewable energy and climate stakeholders.

ANALYTICAL CHEMISTRY GROUP

A team of analytical chemists and engineers help EBI researchers to understand the complex biomaterials that lay at the heart of biofuels technology development. They provide and characterize lignocellulose, defined oligosaccharides, enzymes free of major contaminants, etc., for researchers at both Berkeley and Illinois. Staff members are specialists in mass spectrometry and the separation of small molecules from biological matrices, environmental chemistry, and protein chemistry. It's a one-stop shop for the researcher who needs basic biological and chemical ingredients, produced and interpreted.

EBI ENERGY FARM

Approximately 100 different species and varieties of feedstocks are being grown and monitored in a 320-acre outdoor laboratory known as the EBI Energy Farm. A one-half-square-mile living library of potential biofuel feedstock adjacent to the University of Illinois campus at Urbana-Champaign, the farm is a test site for plant yield, planting and harvesting equipment, and agronomy techniques. It also serves as a classroom for visiting schools, extension groups and service organizations. And as the crops grow, EBI researchers maintain a constant surveillance above and below ground. A new permanent building at the Energy Farm was constructed in 2009 to host a feedstock preparation lab, equipment storage, sample archives, and staff offices.

The newly constructed farm building at the EBI Energy Farm

Converting plant sugars to fuel. Converting to a biofuel-based economy. Discovering new enzymes. Discovering better ways to plant Miscanthus rhizomes. EBI research spans the entirety of the biofuels spectrum.

ASSESSING BIOENERGY ON ALL FRONTS

MISSION:
The EBI will empower outstanding academic researchers and interdisciplinary teams to envision and enable new approaches to the production of energy.

VISION:
Exploring the application of advanced knowledge of biological processes, materials, and mechanisms to the energy sector.

DEVELOPING EFFICIENT BIOFUEL FEEDSTOCKS

FEEDSTOCKS: AGRONOMY, ENGINEERING, AND ENVIRONMENT

BY STEVE LONG

Feedstocks research has so far centered on perennial grasses for their sustainability, productivity, and ability to yield on land unsuited or marginal for food crop production. Initially we have focused on Miscanthus for the temperate zone, which has now extended to its close relatives, energy cane and sugarcane, for tropical and subtropical regions. Miscanthus currently holds the highest yields so far recorded for the temperate zone, while sugarcane is among the most productive tropical species known. For perennials, however, a minimum of two to three years is required before mature crops can be obtained. This, however, has not stopped very significant progress and discovery over 2009. Over this period the EBI has established itself as the global leader for understanding Miscanthus from genomics and agronomy to pests and diseases, while also making significant advances with prairie grasses, including switchgrass, and laying the foundations for leading R&D on energy cane, sugarcane, and miscane.

The EBI has taken advantage of field trials of Miscanthus and switchgrass that were established at seven sites across Illinois between 2001 and 2004 and continues to maintain them. Annual harvests taken, now in the eighth year of some of these trials, show that yields are maintained at between 30 and 45 dry tons per hectare (t/ha), and more than double that of switchgrass. Remarkably, the highest yields continue to be observed in the south of Illinois, where soils are generally the poorest in the state and considered marginal or unsuited for grain crop production. Equally remarkable, these high yields are obtained without any addition of fertilizer or other chemicals. Only at two of the seven sites has any yield improvement resulted from the addition of nitrogen to subplots. At two of the three sites established in 2001, yields remain just as high on plots to which no nitrogen has been added as compared to plots receiving nitrogen, even though these sites consistently yield over 40 t/ha each year. Although our analyses have shown the crop is very effective at recycling nitrogen back to the roots and soil, each fall before harvest some nitrogen is still removed in harvest. The crop appears to be obtaining nitrogen from another source, and the discovery of nitrogen fixing bacteria in Miscanthus by the **Endophytic Bacteria in Biofuel Crops Project** might provide the answer.

How will Miscanthus perform outside of Illinois? The EBI has taken two approaches to this question.

1) **The Feedstock Production Modeling Project** has built a mechanistic model of growth and production of Miscanthus which mimics the underlying physiological and physical processes. Calibrated on sites in Britain, the model was tested against data for sites across Europe and provided remarkably accurate predictions of both growth across the year and final yield, from geographically gridded records of climate and soil. The model also provided excellent predictions of yields obtained in Illinois and their year-to-year variation with weather. It has been used to project both yield and yield stability (i.e. year-to-year variation) across the 48 contiguous states.

Cochliobolus blight of *Miscanthus x giganteus* in Georgia

It projects viable yields (>20 t/ha) at most locations in the states abutting the Mississippi, and at all points to their east. Many sites where stable yields above 40 t/ha can be obtained also are indicated. The model has been adapted to switchgrass and is currently being adapted to include energy cane.

2) The **Feedstock Agronomy Program** established trials of Miscanthus, switchgrass and other feedstocks in nine additional states in 2009, extending from Florida and Louisiana to Wisconsin and South Dakota. These use an identical planting design to that used in the Illinois trials to ensure comparability. At each site, local experts were asked to include the species and cultivars they considered most productive in their region for comparison to Miscanthus and switchgrass. The trials in the southern states therefore include energy canes. These trials will of course be a key test of the model, which will allow extrapolation beyond these test sites.

While several pests and diseases are known to affect energy cane, Miscanthus and switchgrass have generally been considered largely pest- and disease-free. The **Biotic Stress Program** has quickly established itself among the world leaders in understanding pests and diseases of these crops and has delivered good news and bad news in 2009. The bad news: a wealth of potential pests and pathogens can be found on both Miscanthus and switchgrass. While pests and diseases of corn were expected to be the likely culprits, analyses have in fact shown that sugarcane aphids and viruses are among the most common to affect Miscanthus. A rare fungal disease was shown to seriously affect the establishment of the Feedstock Agronomy Program's Miscanthus in Kentucky. The Biotic Stress Program is now examining which of the viruses, fungi, and pests found on these crops is actually affecting yield. An early finding here is that nematode worms are significantly affecting the root system of both Miscanthus and switchgrass.

So what is the good news? First, many of these diseases or pests could be controlled chemically, if they are shown to take a significant toll on yield. There is also likely to be significant variation in disease and pest tolerance within these crops, which can be exploited in breeding. Secondly, if Miscanthus can achieve these remarkably high yields with a significant pest and disease burden, even higher yields will be possible when these are controlled.

The Feedstock Agronomy Program has dealt with two other notable concerns from growers in 2009: how to plant Miscanthus more rapidly and how to get rid of it. Using newly modified equipment, the program has moved from being able to plant 0.25 ha/day to 14 ha/day. It also has shown that the crop can be eradicated with two applications of Roundup, with the second following planting of Roundup-ready corn or soybean. Additionally, the program has established a mixed-grass prairie, together with large areas of Miscanthus and switchgrass on the EBI Energy Farm at Illinois. These large plots provide a key and common facility for all of the feedstock programs from engineering to environmental services. Smaller scale trials of other feedstocks from fiber sorghums and cordgrasses to multiple switchgrass lines have been established as well.

In 2008, understanding the huge genomes of sugarcane and Miscanthus appeared beyond reach. However, being at the cusp of new genomic technologies, the **Feedstock Genomics Program** has moved our knowledge of the Miscanthus DNA sequence from less than 1 percent in 2008 to about 10 percent today. It has also provided a wealth of DNA markers that will improve breeding of both

Advances in machinery used to grow biofuels feedstocks will make raising energy crops more feasible.

Biomass plot combine on the EBI's Energy Farm

Agronomist K.C. Ting:

Tracking Biomass From Field to Fuel

K. C. Ting is directing one of the broadest and most ambitious of the Energy Biosciences Institute research programs. Twenty researchers at the University of Illinois—faculty, postdocs, graduate students, visiting scholars and technical specialists—in five task areas are tracking the biofuel production process holistically.

"We are studying the issues and logistics involved in getting biomass from field production to the gate of the biorefinery," said Ting, who heads the Agricultural and Biological Engineering Department. "When the truck delivers the biomass to the refinery, that's when our job as agricultural engineers is largely finished."

A lot has to happen to a plant from the time it first captures sunlight in a field to being dispensed as fuel at the pump. For corn-to-ethanol, that path is fairly predictable, but for energy crops such as Miscanthus or switchgrass, the journey is still through somewhat uncharted territory.

"There's not as much information on energy crops as we have on corn and soybeans and wheat and cotton," Ting said. "So we have to build on those past successes and learn. What we are doing is using the same methodology and modifying it to conduct new experiments on energy crops. For example, there's a lot of information on how to store corn and hay. You have to deal with sugarcane within 72 hours. There's a lot of science on how to study the preservation of crops, so we start with that."

Ting said energy crops need special treatment. "Energy crops like Miscanthus cut differently; a corn harvester cannot be used to harvest energy crops. Maybe the closest comparison is hay, but that's not a perfect comparison, either," he said.

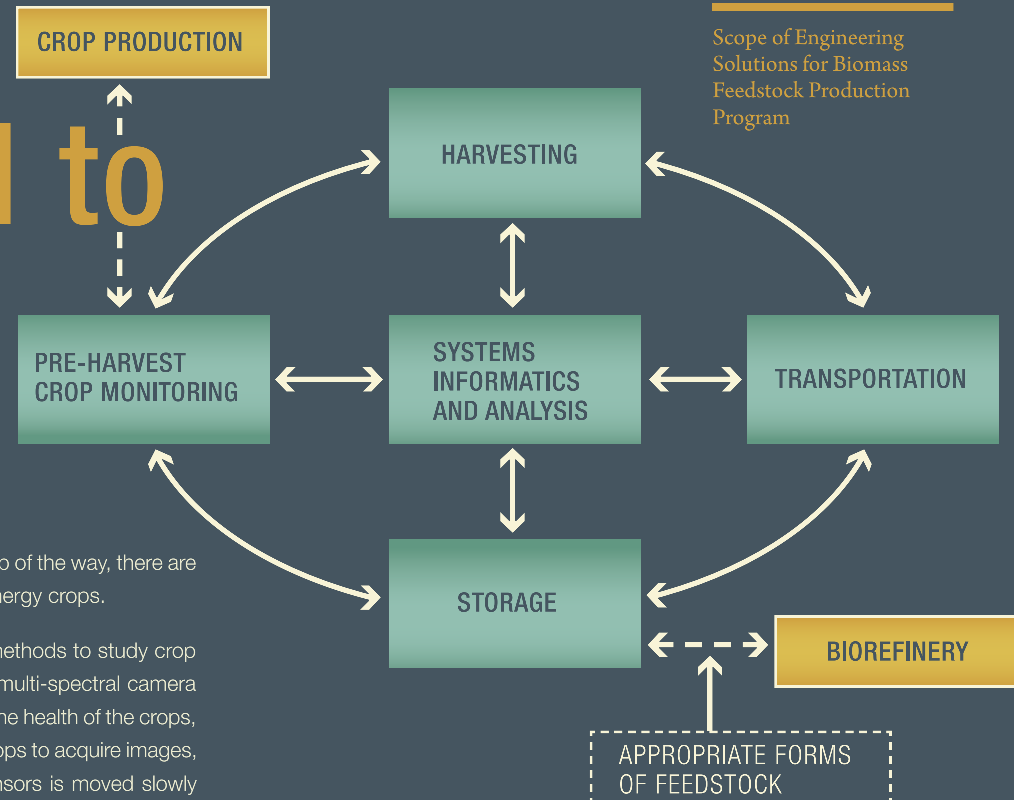
The EBI program breaks down the journey into five tasks—pre-harvest crop monitoring, harvesting, transportation, storage, and the overall analysis of information. Every step of the way, there are new challenges and questions unique to energy crops.

Ting's program team is using a variety of methods to study crop activity. A tower over 100 feet high with a multi-spectral camera watches over four nine-acre plots to study the health of the crops, a small unmanned helicopter can fly over crops to acquire images, and a cube-shaped robotic frame with sensors is moved slowly across the crops. "Using these precision agriculture methods, we can help growers monitor crop growth, detect problem areas, and suggest what they need to do. With cotton, if you take an image, you can tell whether it is suffering from drought or insect or disease. But energy crops are so new, there's minimum data," said Ting.

More information is needed at the refinery end of the chain, too. Can processors just accept the whole bale? Unload it at the gate? Or use a different biomass configuration, like pellets or particles?

Like passing a baton in a foot race, Ting explained the need for a seamless interface between the "runners" in the energy crop value chain. The program is examining those interfaces that occur along the way, gathering data that will help find solutions and improve the overall process. "You can have the best harvesting, storage, and transportation, but how do you link them? Global optimization is as important as local optimization. Our program looks at the whole system."

Ting used the analogy of constructing a building. "You have lighting, you have air conditioning, you have carpeting. Someone has to come up with an overall blueprint and then resolve the differences. And to enable interfaces to happen seamlessly, you need to identify the information you need. Without information, no one can do anything."



Scope of Engineering Solutions for Biomass Feedstock Production Program

Ting said that technologically, biomass-to-fuel is doable today. "But it's very expensive and non-optimal. The challenge is, how can we do it with the least cost, labor, energy consumption, and greenhouse gases while delivering the highest-quality biomass?"

Ting, a native of Taiwan from a family with an agricultural background, said his career was largely decided when officials there evaluated his college entrance examination and placed him in the agricultural engineering track at National Taiwan University. Upon graduation, he ventured to the United States, earning his Ph.D. at the University of Illinois. It was another 24 years—through university service at Houston, Rutgers and Ohio State—before he returned to his alma mater in 2004 to take over the Illinois program.

Ting's leadership skills earned validation again this year. For the fourth year in a row, his department's undergraduate program has been ranked the best in the nation by U.S. News and World Report. The 2010 edition of "America's Best Colleges" placed Illinois in the top spot for agricultural engineering instruction.

Debra Levey Larson of the University of Illinois contributed to this story.

An Unmanned Aerial Vehicle (UAV) remotely monitors crops from the air at the EBI Energy Farm

Miscanthus and sugar/energy canes. It has now developed a strategy for producing a first complete genome sequence of Miscanthus by the end of 2010. This should provide a template for elucidating the sequence of the even larger genome of its close relative, sugarcane. New technologies have also been the hallmark of the **Feedstock Engineering Program**, which has perfected a tower and air-borne-mounted sensor for rapid estimation of the amount of crop across wide areas. Coupled with their new web-based decision support systems software, this facilitates efficient harvest scheduling, storage, and transportation to ensure a regular and continuous supply to the processing plant. By investigating the biophysical properties of Miscanthus, the engineering program has also improved cutters for harvesting, and optimal size reduction and compaction methods from transportation and subsequent processing. These techniques will be transferable to other feedstocks.

Steve Long is Deputy Director of the Energy Biosciences Institute and Professor of Plant Biology and Crop Sciences at the University of Illinois at Urbana-Champaign.

Remarkably, the highest yields continue to be observed in southern Illinois, where soils are generally the poorest and considered marginal or unsuited for grain crop production.

Miscanthus rhizomes at the Energy Farm in Illinois

ECOSYSTEM SERVICES: SEEKING SUSTAINABILITY

ENVIRONMENTAL AND LIFE CYCLE ANALYSIS

The Environment Program evaluates the ecological impacts of cultivating bioenergy feedstocks. To do this, researchers contrast alternative biofuel agroecosystems against current land use practices in the Midwestern U.S. University of Illinois Professor Evan Delucia and his team are measuring the biogeochemical cycles and biodiversity of crops that include *Miscanthus* x *giganteus*, *Panicum virgatum L.* (switchgrass), *Zea mays L.* (maize), and a mixed prairie community. Preliminary data has already been collected and used to parameterize ecosystem process models, allowing the group to synthesize environmental impacts and develop hypotheses about key characteristics of agroecosystems that contribute to sustainable bioenergy feedstock production.

So far, it is hypothesized that (1) the efficient carbon assimilation and nitrogen fixation of Miscanthus will result in lower greenhouse gas emissions relative to other crop options and (2) both soil microbial communities and insect communities will change significantly with feedstock crop choices. Ongoing data collection will test these hypotheses and provide mechanistic explanations for differences between feedstocks.

These studies will also be vital to gaining informed Life Cycle Assessments (LCAs), an activity that is the focus of research by Arpad Horvath and Thomas McKone at Berkeley. The specific aim of this program is to develop and apply LCA methods to understand the overall health, environmental, resource, and economic impacts and performance of the various pathways from biomass to fuels. The long-term goal of this effort is to enable the appropriate use of biofuels based on scientific insights, metrics, and tools to understand the conditions that make biofuels sustainable. The program team has now compiled in a single framework the life cycle external costs, carbon footprint, ecological damage, and human health burdens that accrue from each of the biofuel life stages: feedstock production, biofuel production, transportation and storage, and fuel use.

In the next steps, life cycle impacts will be compared to a range of alternative fuels (such as petroleum gasoline, oil-sands gasoline, etc.) and an Illinois-Indiana case study will be expanded to the full U.S.

The Feedstock Programs on Environment, Agronomy, and Engineering in Illinois are working to measure greenhouse gas and energy balances and to define the most sustainable farm operations and opportunities for land conversion that would maximize opportunities for sequestering carbon from the atmosphere, as opposed to releasing carbon. A complementary project led by Ryan Stewart at Illinois is focused on studying the effects on soil quality of Miscanthus stands in Japan that are more than 1,000 years old.

Plants transpire enormous amounts of water during growth, and such water emissions can affect soil moisture and the amount of water runoff that may support streams and lakes. The potential effects of cellulosic crops on water are being modeled using climate and soil data in a collaboration between Carl Bernacchi at Illinois and Tracy Twine at the University of Minnesota. Early reports indicate that the production of Miscanthus is likely to increase evapotranspiration due to increased water use, and that the increased water use will decrease water drainage or subsurface runoff.

In addition, a detailed analysis of the potential effects on the well-characterized Lake Bloomington (IL) watershed is being carried out by Ximing Cai, John Braden, Wayland Eheart and George Czapar at Illinois. The team has developed a decision analysis model to analyze producers' behavior regarding crop choices and biofuel technology adoption. Two surveys of farmers and crop advisors have been completed.

Tom McKone and Arpad Horvath

Modeling a Multifaceted Biofueled World

Although the idea of Life Cycle Assessment (LCA) was conceived in the late 1960s and the LCA process was defined by the Environmental Protection Agency in the mid-1990s, when it comes to developing an LCA for biofuels, it's almost like starting from scratch.

That's been the challenge for Berkeley civil and environmental engineer Arpad Horvath and co-principal investigator Tom McKone, an expert in health and environmental risk assessment who splits his time between UC Berkeley and Lawrence Berkeley National Lab. Their Energy Biosciences Institute program is designed to guide decision-making for biofuels by analyzing issues confronting the industry from every conceivable pathway, biomass to fuel, inputs to impacts (direct and indirect).

"Think of the (biofuel) economy like a huge airline route map," says Horvath, whose expertise is in environmental assessment of infrastructure. "Every little dot is connected. They all interact and affect each other. It's not just a fuel issue. It's about how the product overlaps with all other sectors of society."

Their program is dedicated to analyzing the complete biofuels life cycle—from the initial plantings in the field, through the production, distribution, and use in vehicles and industry. Applying and building modeling tools of their trade, plus analyzing data previously developed from other studies, the diverse team of 17— engineers, economists, environmental health scientists, biomass production specialists, and energy and fuel analysts—are trying to make sense out of a constantly changing and still uncertain industry.

"Biofuels problems are very different today than what they were two years ago," Horvath says, "and they will be different again in two years." McKone adds, "We're making models that are dynamic, that can react to the marketplace, to regulations, people's habits, and vehicle technologies. We're taking multiple pathways to the problem."

They've been helped in their search by the development of a series of scenarios for biofuel production by EBI bioenergy analysts Carolyn Taylor and Heather Youngs. These scenarios allow the team to define specific prioritized bioethanol pathways from which assessments can be made for the overall human health and environmental impacts of biomass production; converting and processing this biomass into fuel; storing, transporting, and distributing that fuel; and the actual combustion and use of the biofuel.

We're making models that are dynamic, that can react to the marketplace, to regulations, people's habits, and vehicle technologies. We're taking multiple pathways to the problem.

The overall metric, according to Horvath, is the cost—both the revealed and the hidden costs of the ecosystem services and environmental impacts. They will be calculating that under a variety of circumstances.

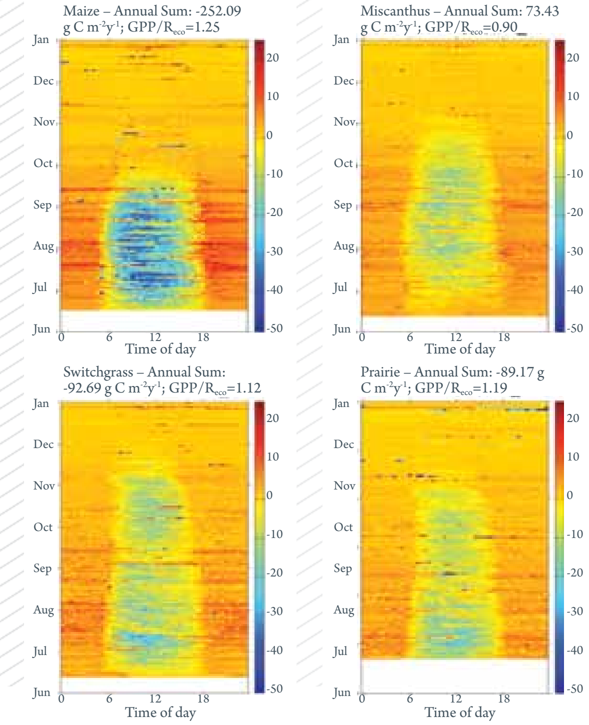
"We are building capacity to answer the questions," he says, noting that after two years they are comfortable with the extraordinary amount of data they have gathered, within the limitations of model variations and the dearth of existing information in certain areas. "We need finer spatial resolution about the impacts and more data on emissions factors, even for gasoline, to remove some of the key uncertainties about how fuel switching plays out," says McKone. "We use models as exploratory tools, and we set them up to be transparent, refutable, and flexible."

Just don't expect them to give you a bottom-line conclusion on the best fuel. "We want to get away from the notion that all biofuels are better or worse than all conventional fuel," adds McKone. "We want to avoid making grand comparisons. So we are adding the spatial and temporal specificity that will define and distinguish our conclusions. We've plugged most of the (data) holes, but we will demand better data as we go along."

"Our major realization is the interconnectedness in the energy sector," Horvath says. "It's like pulling levers. You don't know how a system will trickle through, so we are learning all about leverage points. The more you learn, the better your models can be. Given what we know so far, we should have robust answers in two years."

Those answers should prove to be invaluable to the landowners considering biofuel plants as possible crops, to manufacturers who need to realize solid return on investment, to legislators and regulators in whose charge a healthy environment rests, and to consumers who want their transportation affordable and sustainable.

Pictured above, from left to right, Tom McKone and Arpad Horvath



Carbon fingerprints for the establishment year of various feedstocks

Concerns about water and land use have stimulated interest in the possibility of using algae to produce biofuels. Preliminary analyses of the costs of fuels produced from algae indicate that there are challenges associated with bringing the costs of production in line with alternatives. A feasibility study by Berkeley Lab scientist Nigel Quinn and colleagues has produced an integrated assessment of the technological, engineering, energy balance, and environmental aspects of algae-based biofuel to determine the prospects for large-scale cultivation and harvesting. Engineering, resource, economic, and regulatory issues were also reviewed, and a final report is expected in 2010.

Berkeley Lab climatologist Norman Miller's project focuses on the use of ecosystem model-based and data-driven analyses to optimize bioenergy production on prioritized land. His team has advanced model descriptions for C4 plants and perennial grass and has developed a generalized gridding system for evaluating and simulating feedstock growth at a wide range of spatial scales. Multi-year simulations and analyses for three Illinois sites were completed for switchgrass and begun for Miscanthus. They also developed a land analysis prioritization scheme that incorporates the last 10 years of satellite and Geographic Information System (GIS) imagery and land cover products to identify the potential conversion of different vegetation types to specific biofuel crops.

Combining regional feedstock simulations driven by climate data with economic, ecological, and sociopolitical data will transform the GIS into a spatially explicit tool for determining land suitability for particular crops.

Chaetomium thermophilum, a heat-loving filamentous fungi being studied for potential depolymerizing enzymes

ENGINEERING PLANT DEPOLYMERIZATION

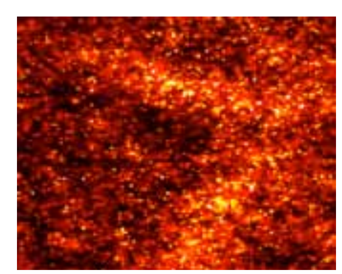
BIOCONVERSION: ATTACKING CELLULOSIC DEGRADATION ON SEVERAL FRONTS

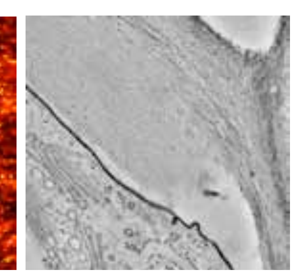
BY DOUG CLARK

Bioconversion research at the Energy Biosciences Institute is addressing several of the major bottlenecks impeding the practical production of biofuels, such as ethanol and butanol, from lignocellulosic feedstocks. These programs have several interrelated components—the discovery and characterization of fungi and thermophiles that produce new enzymes for lignin and cellulose deconstruction, protein engineering and kinetic modeling of improved cellulases, new organism discovery and cellular engineering for enhanced biofuel production and improved tolerance of the biofuel product, and bioprocess engineering to optimize fermentation.

Visualizing Lignocellulose

Lignocellulose is a composite material made up of a variety of polymers that are tightly bound to one another. Because the polymers are too small to be seen by most types of microscopy, the exact molecular structure of the cell walls that comprise plant biomass is not known. Berkeley Lab scientist Manfred Auer and colleagues are using new methods of electron microscopy to visualize biomass at nanometer resolution. His colleagues Paul Adams and Jim Schuck are building a novel Raman microscope that they expect will provide spatially resolved chemical information about cell walls that will be complementary to other types of imaging. Used together, these types of imaging provide insights into how various treatments affect the structure of biomass and facilitate improvements in the overall conversion process.



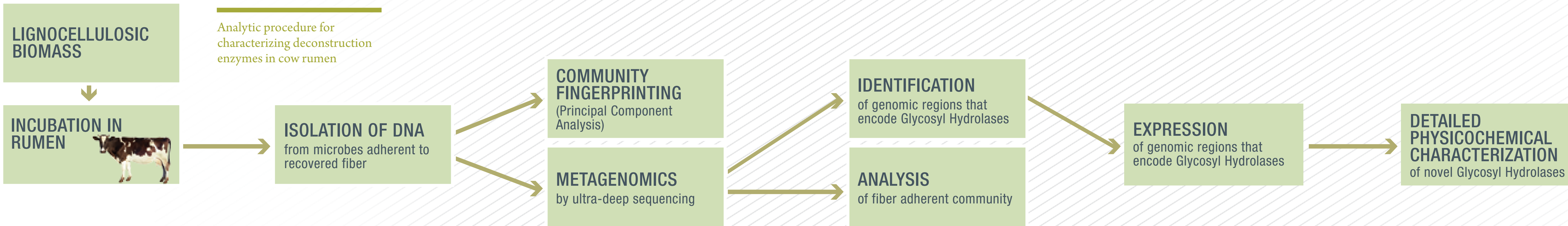


Plant cell wall microscopy images show a fusion protein bound to cellulose fibers (left) and primary and secondary cell walls (right)

Bioprospecting and Discovery of Novel Organisms and Enzymes for Biomass Depolymerization and Conversion to Biofuels

The ability to carry out biomass processing or fermentation at relatively high temperatures has several advantages. In addition to lower risk of microbial contamination, a higher temperature accelerates enzyme-catalyzed reactions and would reduce cooling costs and facilitate ethanol (or butanol) removal and recovery. To enable translation of these advantages to practice, EBI investigators are seeking to isolate and characterize enzymes from several extremely thermophilic bacterial strains specifically adapted for cellulose and hemicellulose degradation.

For example, Doug Clark, Harvey Blanch, and co-workers at UC Berkeley, in collaboration with the Frank Robb laboratory at the University of Maryland, are cultivating communities of organisms from hot springs with cellulosic substrates with the aim of enriching for, and isolating, novel thermophiles that produce biofuels and/or thermostable cellulolytic enzymes. Bioprospecting for cellulose/hemicellulose degradation systems is assisted by whole genome sequencing of novel isolates. So far this approach has produced several cultures that degrade cellulose at temperatures



Analytic procedure for characterizing deconstruction enzymes in cow rumen

ranging from 70° to 90°C, and the isolation and characterization of thermophilic cellulases from these sources is in progress.

Hot Spring (top) at Surprise Valley, CA, sampled for thermophyllic bacteria in 2008; fluorescent micrograph of cells (bottom) shows organisms present in sediment sample in medium containing Miscanthus

Efforts to exploit the advantages of thermophilic microorganisms for biofuels production also are underway at the University of Illinois in a program led by Isaac Cann, Rob Mackie, and co-workers. Research in their laboratory has resulted in the discovery of myriad genes encoding promising enzymes from a variety of sources, including mesophilic and thermophilic bacteria. Several of these enzymes have been expressed, purified, and characterized for their hydrolytic activity.

They also are turning to ruminant animals as a promising source of cellulolytic microorganisms that function efficiently at more moderate temperatures. Ruminant animals are specialized in the utilization of grasses as a source of feed. Both switchgrass and Miscanthus, in mesh bags, have been placed in the rumen of fistulated cattle at the University of Illinois. These have then been recovered and the attached microbes investigated by Eddy Rubin and co-workers at Berkeley Lab's DOE-Joint Genome Institute to identify possible sources of robust cellulolytic enzymes for efficient conversion of cellulosic biomass into fermentable sugars. These two complementary programs aim to identify enzymes produced by the abundant microbes responsible for degradation of plant cell wall polymers in the rumen of forage-feeding animals that can potentially be co-opted for cellulosic biomass conversion. A related goal is to develop tailor-made enzyme "cocktails" optimized for

Matthias Hess collects samples from the rumen of a fistulated cow

saccharification of specific bioenergy crops with their subsequent conversion to alcohol fuels.

Another natural bioconverter that efficiently breaks down and transforms plant biomass is the termite hindgut. In a program aimed at discovering novel enzymes capable of degrading wood lignocellulose, Phil Hugenholtz and co-workers at the DOE-Joint Genome Institute have performed metagenomic and bioinformatic analyses of several species of wood-feeding and grass-feeding termites. These studies are being complemented by functional screening of selected enzymes and characterization of the plant cell wall polymers present in the food sources, hindgut segments, and fecal pellets of grass-feeding termites. Thus today's wood-chomping pests may prove to be important players in tomorrow's biofuel-producing technology.

Today's wood-chomping pests may prove to be important players in tomorrow's biofuel-producing technology.

In addition to cellulose, a particularly problematic component of plant biomass is lignin. Lignin is a complex, decay-resistant, highly cross-linked aromatic polymer. Almost all research on lignin and lignocellulose biological degradation has so far focused on fungi that decay wood. However, grass cell walls are very different from the walls of conifers, and in nature, none of the wood-decaying fungi are known to decompose grasses. To find the fungal enzymes best adapted to deconstruction of grass cell walls, the Berkeley team headed by John Taylor, N. Louise Glass, and Tom Bruns has used modern high-throughput culture methods developed by the pharmaceutical industry to bring into cultivation 900 isolates of fungi that decompose the litter that has been accumulated at Illinois under the feedstock of choice—*Miscanthus* x *giganteus*, a C4 grass closely related to sugarcane—and that has accumulated in Louisiana under sugarcane itself.

Using modern methods of molecular identification, these 900 cultures have been placed in 95 species of fungi and representatives of each species are being assayed for deconstruction of Miscanthus biomass and relevant enzyme activity. Results of these analyses will inform the Taylor-Bruns-Glass team about which species to subject to transcriptional profiling and protein characterization to identify enzymes that will improve industrial lignocellulolytic bioconversion.

In a collaborative project with the Jamie Cate and Michael Marletta labs, members of the Glass lab have been elucidating the regulatory network associated with plant cell wall degradation in the model filamentous fungus, *Neurospora crassa*. A research paper describing genes and proteins induced by growth of *N. crassa* on the preferred biofuel crop, Miscanthus, has recently been accepted in the Proceedings of the National Academy of Sciences. By screening about 350 gene deletion mutants, about 40 *N. crassa* mutants have been identified that affect cellulase or xylanase activity, nine of which encode transcriptional regulatory factors, 16 of which encode carbohydrate utilization enzymes, and 15 of which encode proteins of unknown function. *N. crassa* also is being developed into a platform for gene expression and enzyme secretion. About 200 gene constructs have been made and heterologous expression of various enzyme implicated biomass depolymerization are currently being characterized.

Berkeley chemist Michelle Chang is taking a different approach—she is exploring bacterial enzymes that are able carry out the chemical transformations associated with lignin breakdown. This work also involves enzyme characterization and the development of high-throughput assays of ligninase activity, along with development of a heterologous expression platform for ligninases.

Protein Engineering of Improved Cellulases

Protein engineering has proven to be a powerful tool in creating enzymes with new and improved properties. However, designing and employing methods to screen or select cellulase mutants using solid cellulosic substrates remains a largely unmet challenge. Research

Microbiologist Louise Glass:

Applying the Lessons of Nature

Induction of fungal enzymes and pathways increases the efficiency and utilization of lignocellulosic depolymerization products

Unfortunately, the GiantMicrobes.com Web site didn't have a *Neurospora crassa* fungus in its stuffed toy collection, so microbiologist N. Louise Glass had to settle for *Trichophyton montagrophytes* (athlete's foot), the only orange one available. It sits on her desk in her UC Berkeley laboratory, a silent mascot for the team that is trying to translate the fungus' plant-digesting qualities for possible application to biofuel production.

"Out in nature, Neurospora grows on sugarcane," Glass says of the organism she's been studying for two decades. "Many people think of Neurospora simply as a laboratory model for genetic research, not for what it's doing in nature, which is eating plant cell walls."

> If we can tailor enzymes for plant cell wall deconstruction to a fermentation-only organism, we might get higher yields of biofuels.

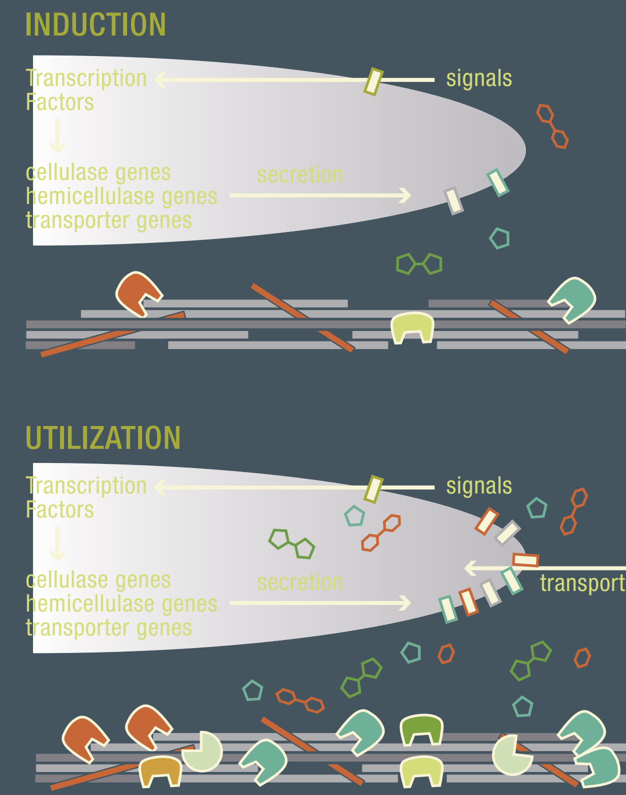
Glass, in collaboration with the John Taylor/Tom Bruns Berkeley team, is working in the Energy Biosciences Institute program to investigate fungi's deconstruction of lignin and other components in the cell walls of the energy feedstock Miscanthus. Neurospora has also become a hot property with biochemist Jamie Cate's team studying cellulose-decomposing enzymes. Together, the Glass and Cate groups are seeking knowledge about Neurospora enzymes and genes that are responsible for the breakdown of plant components into sugars for potential use in bioprocessing.

Glass explains that Neurospora was originally isolated as an orange bread mold in French bakeries. Quickly reproducing, easy to culture, and with a well-characterized genome, Neurospora has proved useful as a model organism in research on cell fusion, circadian rhythms, genetic regulation and metabolism. Every two years, Glass is among the hundreds who now attend an international Neurospora research conference at Asilomar in California.

"Neurospora is a discovery-oriented organism where we can identify heretofore unknown processes," she says. "It has a very good gene expression system, and with the tools we have, we could provide many reagents for scientists who want to understand the enzymology of plant cell wall deconstruction and perhaps lead to more rationally-designed enzymes. Right now, commercially available enzyme mixtures for plant cell wall degradation are very concentrated, with a large number of proteins in the mix that may not be needed. If we can tailor enzymes for plant cell wall deconstruction to a fermentation-only organism, we might get a higher yield of biofuels."

And in biofuel production, higher yield at lower cost is the coin of the realm.

Growing up in Colorado, Glass said she gained an appreciation for nature's wonders at an early age. In her second year of biology study at Colorado State University, she found herself fascinated by the life cycles of fungi, which most students hated, and focused on mycology with professor Brent Reeves. That began a career path that included a stint as a mycologist at the American Type Culture Collection in Rockville, MD, the worldwide repository for microbes and cell lines.



After receiving her doctorate from the University of California, Davis, Glass worked as a postdoc with Bob Metzenberg at the University of Wisconsin, one of the world's leading authorities on Neurospora. "Often at lab meetings, Bob would come in and propose experiments with Neurospora that would address a biological phenomenon that was not being explored in other eukaryotic systems. Bob thought deeply about how organisms worked (including Neurospora) and was fascinated by broad biological questions," she says.

The girl who had her own Drosophila lab in high school now runs her own laboratory in Berkeley's Plant and Microbial Biology Department, asking questions about microbes just like her mentor. In this case, they have to do with the problems of biofuels and how a common bread fungus might unlock the mysteries of deconstruction and production.

The EBI, she says, brings a distinct advantage. "It's interdisciplinary, unique. Here you can have collaborations with investigators, post docs and students with complementary skill sets. It's exciting to see. The expertise and perspectives are so different, but we are all working together on the same goal."

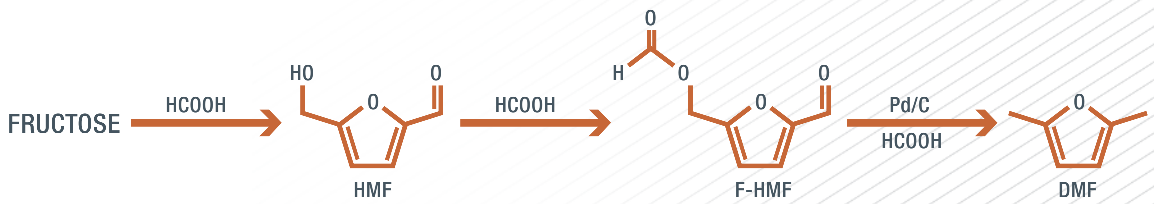
by Clark, Blanch, and co-workers aims to overcome this challenge and that of developing more cost-effective cellulases, by developing high-throughput solid substrate assays and applying them in the directed evolution of thermophilic cellulases and of cellulases with high activity in ionic liquids. The methodology developed will be applicable to the generation and study of improved cellulases that can be used in various process configurations for the production of biofuels from cellulosic biomass.

Enzyme and metabolic pathway engineering are also among the tools being used by the Illinois group led by principal investigators John Gerlt and John Cronan in a program directed toward overcoming biomass recalcitrance as a key obstacle in biofuel production. The objectives of their program are to identify and characterize degradation pathways for lignin, characterize the enzymes that are involved in those pathways, engineer these enzymes so that they will have enhanced catalytic properties, and design new metabolic pathways in organisms so that biofuel production can be enhanced.

In addition to soluble cellulases, some organisms degrade cellulose using cellulosomes, which are comprised of cellulases organized in a complex assembly of enzymes and scaffolding on the bacterial cell wall. The goal of research carried out by Cate, Marletta, and co-workers at UC Berkeley is to develop new experimental systems to study cellulosome degradation of cellulosic biomass. The Cate and Marletta team aims to develop model systems that will enable them to study the enzymatic properties of cellulosomes at a fundamental level. In particular, they have been studying cellulosomes from the mesophilic bacterium *Clostridium payrosolvens* C7 (CpC7). In addition, the team is comparing the cellulosomal system from CpC7 to the suite of lignocellulolytic enzymes secreted by the mesophilic fungus *Neurospora crassa*. Understanding the molecular mechanisms may provide the key insights needed to reconstitute "designer cellulosomes" optimized for depolymerization of plant biomass.

Modeling for Optimal Cellulase Design and Cellulose Hydrolysis

Accurate kinetic models of cellulose hydrolysis by cellulases are of critical importance for evaluating cellulase-component compositions and for designing and optimizing processes for cellulose conversion to biofuels. Such models will also aid in the development and characterization of improved cellulolytic systems generated by protein engineering and synthetic biology.



SCHEME 1: Synthesis of DMF from fructose

Projects under way at UC Berkeley headed by Clark, Blanch, and Clayton Radke are developing a comprehensive model of cellulose hydrolysis that can be used to predict cellulase performance, guide cellulase design, and optimize the hydrolysis of various cellulosic substrates, including those obtained from EBI investigators.

As an important first step toward modeling cellulose hydrolysis and engineering improved cellulases, Clark, Blanch, and co-workers have isolated the individual components of the cellulase mixture secreted by the fungus *Trichoderma reesei*. Determining the kinetics of these enzymes toward various recalcitrant substrates, with and without pretreatment, including cellulosic substrates dissolved in ionic liquids, will provide a useful baseline against which the activities of newly discovered and developed cellulases can be compared.

Alleviating Product Toxicity in Biofuel Production

The development of new microbes with greater tolerance toward the final fuel product, such as butanol, could lead to substantial improvements in the cost-effectiveness of producing biofuels from cellulosic biomass. To this end, the Clark/Blanch program is working to engineer enhanced tolerance toward existing and potential biofuels into bacteria, yeast, and Clostridia. The group also is exploring the design of extractive fermentation systems that may be used to minimize inhibition of cultures by the products of fermentation. The use of additives that reduce toxicity of fuels during fermentation is being explored by Illinois engineer Hao Feng. He is also researching novel biomass pretreatment methods that reduce production of toxic byproducts.

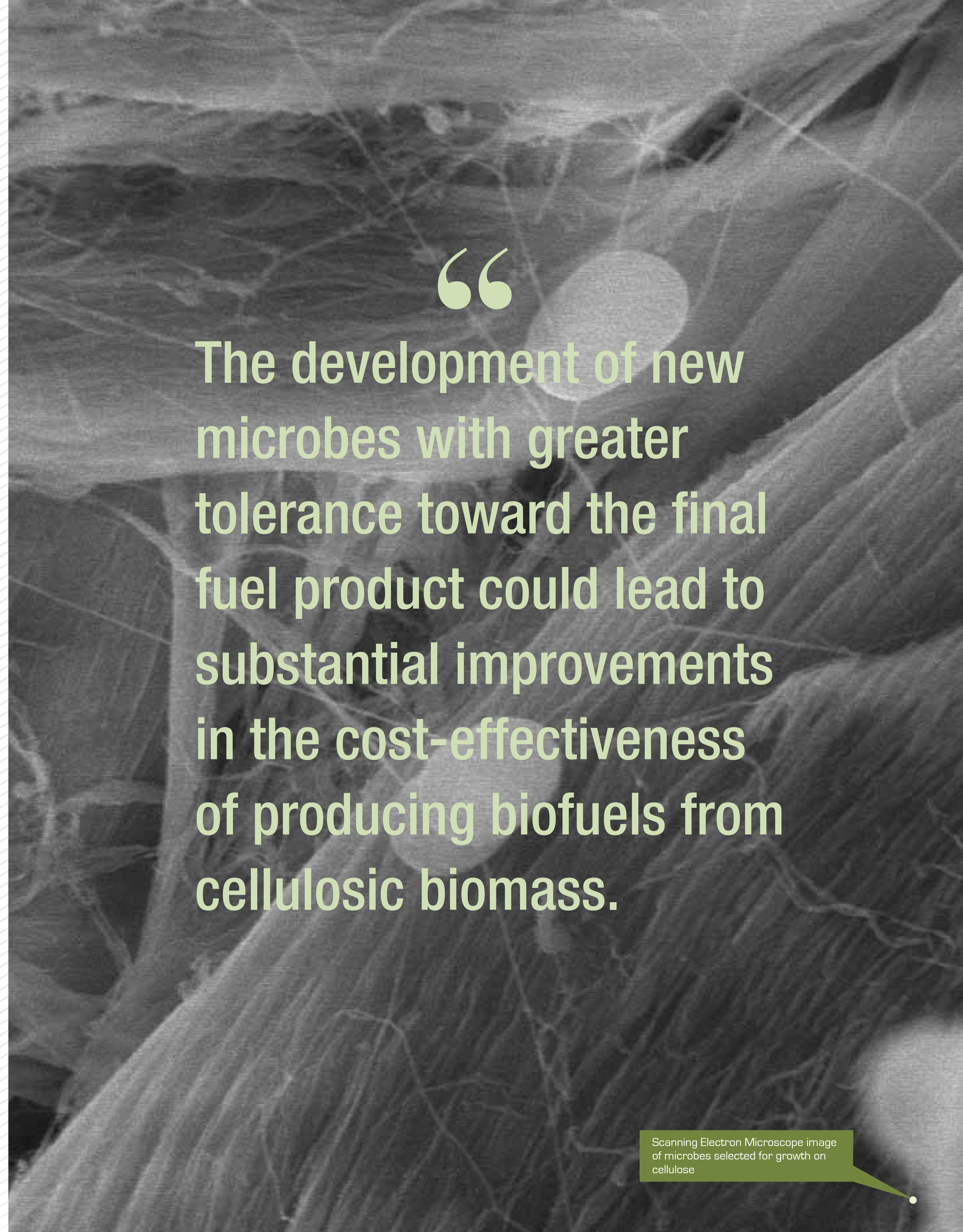
Another component of the proposed effort is the development of a simultaneous saccharification and fermentation process that can operate near the boiling point of ethanol. Ethanol production during saccharification of cellulose/hemicellulose at 75°C will increase process efficiency, minimize contamination, and facilitate removal of the fuel product.

One of the disadvantages of low-mass alcohols like ethanol is that they are miscible with water. This feature contributes to the toxicity of such fuels to the producing organisms and also imposes energy costs in dehydrating the fuels by distillation. Berkeley engineer Nitash Balsara is exploring the development of advanced membranes that can selectively separate fuels from the aqueous phase. The successful development of membrane separation technologies could facilitate the implementation of much more efficient processes than are currently possible.

Chemical Transformations—Alternatives to Bioconversion

An alternative approach to bioconversion of biomass to fuels is to use synthetic chemical catalysts to either hydrolyze biomass or convert hydrolysis products to fuels, or both. This relatively undeveloped area is being explored on several fronts. Berkeley chemical engineers Blanch, Clark, and John Prausnitz are investigating the use of ionic liquids to pretreat and solubilize biomass with the objective of removing and decrystallizing the cellulose and solubilizing the lignin component. Alexis Bell and Berend Smit are studying the hydrolysis of cellulose in ionic liquids and are examining the possibility of using chemical catalysts to convert the glucose released from cellulose into several possible fuel products. Berkeley chemist Dean Toste is exploring the development of novel synthetic homogeneous catalysts for polysaccharide hydrolysis, and Berkeley chemical engineer Alex Katz is studying the use of heterogeneous catalysts. Lignin cleavage by synthetic catalysts is also being researched by Berkeley chemists Jonathan Ellman and Robert Bergman and Illinois chemists Tom Rauchfuss and John Hartwig. The promise of this new line of research is that entirely novel ways of transforming biomass to fuels may be discovered. This work also opens up the possibility of finding routes to types of fuels that cannot be produced by bioconversions.

Douglas S. Clark is Executive Associate Dean of the College of Chemical Engineering at UC Berkeley, and he is a member of the EBI Executive Committee.



Scanning Electron Microscope image of microbes selected for growth on cellulose

Biofuel fermenters in the EBI lab

BIOFUEL PRODUCTION: CONVERTING POLYSACCHARIDES TO FUEL

TRANSFORMING FEEDSTOCK TO FUEL WITH MICROBES—OR BUGS TO BANG

BY ADAM ARKIN

A number of molecules of biological origin can serve as fuels. Ethanol is perhaps the most industrially successful, biologically produced fuel. Other molecules—ranging from more complex branched-chain alcohols to fatty acids and hydrocarbons—have a variety of properties that make them more or less attractive as targets for production. Additionally, they can be made in plants and microbes. Some burn cleaner than others, while some have higher energy density, possess different octane ratings, or can be physically transported or produced more cost-effectively and reliably with less energy input. Those closer in form to what is found in gasoline, diesel, and jet fuel may prove superior.

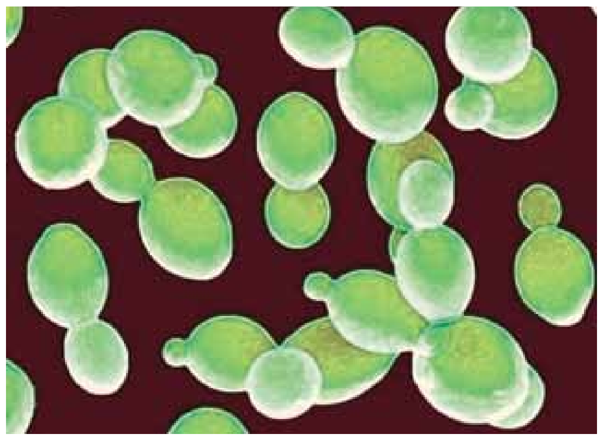
The routes to biological production of fuel molecules are numerous and include sources such as plant oils or algae. However, one of the most effective routes is through microbial fermentation and synthesis. Deconstruction of feedstocks leads to hydrolysates rich in 5- and 6-carbon sugars such as glucose, xylose, and arabinose along with other compounds that can be toxic. Natural or engineered yeast and bacteria can metabolize the sugars into different variants of the possible fuel molecules, but are inhibited to various extents by toxins and the fuel products themselves.

Nonetheless, the relative success of this approach derives from a number of factors. Microbes express an amazing array of natural abilities to consume simple feed molecules and create complex organic chemicals. The revolutions in molecular biology and genomics have enabled scientists to discover the genes responsible for these capabilities and to transplant their function into industrially robust host microbes. Advances in quantitative and genome-scale measurement of cellular physiology down to the single-cell level give unprecedented insight into the factors that restrict optimal production by limiting metabolic flux and microbial growth. These tools have only just begun to be applied systematically to improve microbial fuel production and, despite some early successes, there remains much room for improvement.

The Energy Biosciences Institute has initiated a cutting-edge microbial engineering program combining synthetic biology, protein engineering, and metabolic engineering approaches to create new routes to fuels in bacteria and fungi as well as systems biology and genomics approaches to measure and diagnose function.

The Microbial Characterization Program

Researchers at UC Berkeley and Lawrence Berkeley National Laboratory have established a high-throughput genetics and genomics capability and are developing an experimental and computational program to determine the genetic mediators of optimal fuel production in microbes. Berkeley bioengineer Adam Arkin leads a collaborative team, with environmental microbiologist Terry Hazen and computational biologist Inna Dubchak, that employs the ethanol-producing bacterium *Zymomonas mobilis* as a model system and will screen large-scale genetic knockout libraries and generate high-resolution whole-genome gene expression compen-



Improved fermentation is the goal of research on the yeast *Saccharomyces cerevisiae*

dia of the bacterium exposed to different feedstock hydrolysates, their purified inhibitors and sugars, and the various possible fuel products. This data will provide deep annotation and metabolic pathway reconstruction for the organism and allow dissection of the mechanisms that impact the ability of Zymomonas to grow and metabolize sugars into fuel and suggest routes for engineering more efficient production. The computational framework and experimental facility created as part of this program will ultimately scale to aid the engineering of resistance and fuel molecule synthesis in this organism and the others being pursued at the EBI.

Engineering Yeast for Sugar Utilization

Two research groups at the University of Illinois seek to improve the utilization of the different sugars present in hydrolysates. Most microbes used for fuel production cannot effectively use both the 5- (like xylose and arabinose) and 6-carbon sugars (such as mannose and glucose) that are the most abundant products from the feedstock deconstruction. Complete fermentation of all these sugars to biofuels is necessary to maximize yield and minimize waste in the production process. Investigators, led by chemical engineer Huimin Zhao and microbial geneticist Yong-Su Jin, will identify genes that transport the sugars into the cell and metabolize them into common precursors for fuel synthesis in a number of different fungi. These will be transplanted into industrially important host cells starting with the ethanol-producing yeast *Saccharomyces cerevisiae*. Advanced approaches for metabolic analysis and engineering will be employed to optimize yield and rate of production.

Elucidating the Impact of Regulation and Heterogeneity on Sugar Metabolism in Bacteria

Engineered pathways are usually dependent upon the normal metabolism of the host. The endogenous regulatory machinery often hampers fuel yields. Metabolic regulation is evolved to sense environmental conditions and deploy the right pathways to allow organisms to survive in an uncertain and competitive world. However, they may be triggered by the particular conditions found in industrial bioreactors and lead to poor sugar utilization and production. These effects are further complicated in large-scale fermentations where cells experience fluctuating nutrient condi-

Research Associate Jordan Mar records data from EBI Investigator Adam Arkin's Lab in Berkeley, charting bacterial growth in petri dishes.

There are many routes to the biological production of fuel, but one of the most effective is microbial fermentation and synthesis

Biochemical Engineer Chris Rao:

Giving Bacteria a Sugar Rush

Yellow – arabinose metabolizing cells
Red – xylose metabolizing cells
Left – cells grown in arabinose
Right – cells grown in xylose yield mixture of both metabolic types

Homogeneous metabolism

Heterogeneous metabolism

For a guy who admittedly "hated" biology class in college, Christopher Rao sure has become immersed in the field.

The young chemical engineer at the University of Illinois finds himself in the middle of the Energy Biosciences Institute's search for ways to coax microbes into deconstructing lignocellulose for sugar fermentation into biofuels. "EBI is very problem-focused and has a defined objective over a short time horizon," said the New York City-born Rao. "That's an intriguing aspect for me, (the prospect of) lots of solutions but just one problem."

It wasn't always like that. Rao, like many of his colleagues in cellular engineering and computational biology, relies on funding from the National Institutes of Health to do very basic laboratory research. What are cells' fundamental mechanisms? How do cells respond to the environment? But the NIH is not keen on funding metabolism studies of organisms, which lean toward research applied to specific problems.

"NIH expects you to do half the work before they fund it," Rao said. "EBI gives you a chance to do something different, to get into a new field. (Director) Chris (Somerville) says, 'Think outside the box' and 'Come up with new ideas.' This was my opportunity to do a more applied project." He added, "It's a lot easier explaining my work to relatives."

His focus for the institute is the *Escherichia coli* bacteria and its ability to natively metabolize all three sugars from the hydrolysis of lignocellulose—glucose, arabinose, and xylose. Before E. coli can be a competitive candidate organism for sugar conversion in commercial cellulosic ethanol production, scientists will have to overcome the bacteria's penchant to preferentially consume just one sugar while repressing the metabolism in the others. Such a limiting characteristic can drastically impair fermentative productivity.

"We initially thought the problem was due to transporters (proteins that move molecules across a biological membrane)," he recalled. "But (selective sugar utilization) is actually a result of genetic regulation."

More specifically, he and his team are studying metabolic gene regulation. Genes are encoded on DNA, he explained, and like a tickertape, they line up on linear polymers, directing function within an organism. "But upstream are promoter regions," Rao said, "where regulatory proteins bind to activate or repress genes. Not all genes are active at the same time cells express different proteins depending upon what they see in their environment. We want to change that regulatory sequence."

> EBI is very problem-focused and has a defined objective over a short time horizon. That's an intriguing aspect for me, lots of solutions but just one problem.

They have been able to crack the hierarchy of sugar metabolism, glucose-to-arabinose-to-xylose. Next, they plan to engineer cells that will consume all three sugars at once.

Engineering was always Rao's preferred career path, though his mother wanted him to be a doctor. Taking a class in chemical engineering as a medical school prerequisite at Carnegie-Mellon University in Pittsburgh convinced him (along with that "boring" biology class) that his future lay in the laboratory. He pursued systems engineering, including automation systems for chemical processes, for his graduate work at the University of Wisconsin.

In 2000, he took his Ph.D. to UC Berkeley for a four-year postdoc assignment with microbial bioengineer Adam Arkin, where his journey passed through comparative genomics, pathway engineering, and bacterial pathogenesis. Today, both men are principal investigators of EBI projects. Arkin's Microbial Characterization Facility is designed to develop an experimental and computational pipeline for discovering and engineering the stress response and metabolic pathways that affect the ability of microbes to make biofuels. Then an organism, perhaps a mutant form of an ethanol-producing bacterium like *Zymomonas mobilis*, might be optimized for commercial biofuel production.

Rao said he hopes his work "will culminate in the construction of a quantitative model describing the regulation of multiple sugar utilization. This model will, in turn, facilitate the design of (microbial) strains capable of simultaneously and efficiently metabolizing all three sugars."

It's not medical school, Mom, but the potential application of Chris Rao's work, and his contribution to global need, will be no less important.

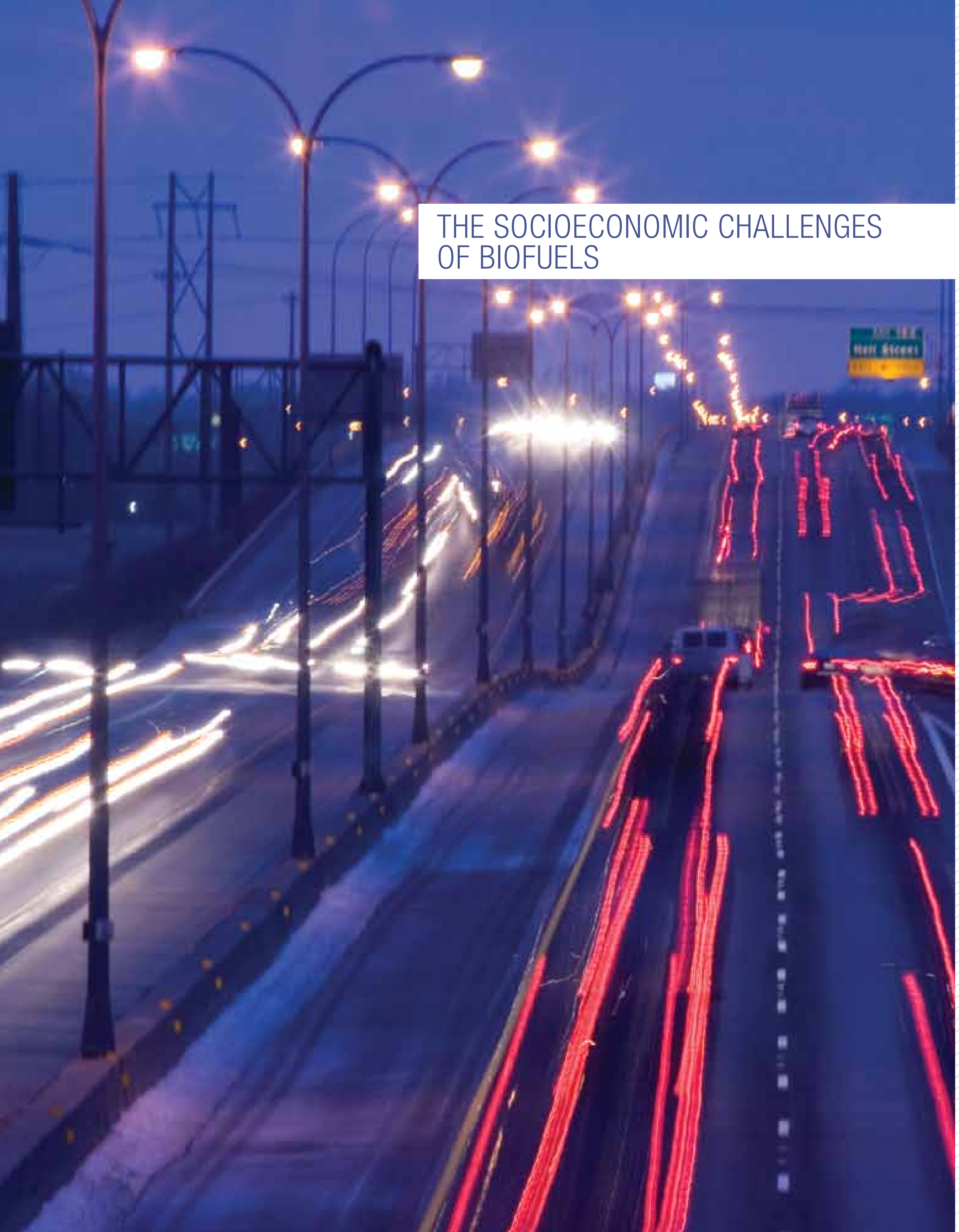
tions due to imperfect mixing. The populations will be physiologically heterogeneous, and thus not all individuals will be producing optimally. EBI scientists in Illinois under biochemical engineer Christopher Rao and physicist Ido Golding seek to overcome this problem in *Escherichia coli*. They will dissect the molecular factors to implement the unwanted regulation and aim to create a quantitative model of the system that will facilitate the design of strains capable of homogeneously, simultaneously, and efficiently metabolizing the arabinose, xylose, and glucose.

Producing Diesel Substitutes in Bacteria

While ethanol production is an initial target for the EBI, other fuel molecules may prove to have superior properties. Biodiesel is an alternative fuel, widely used in the alternative energy economy. It is usually composed mostly of fatty acid alkyl esters (FAAE). These molecules are often produced by a catalyzed reaction of methanol with fatty acids from plants, tallow, and used cooking oils. Current estimates predict that production costs for this fuel will remain substantially higher than petroleum-based products. Berkeley Lab scientists led by Athanasios Lykidis are applying biochemical and metabolic engineering approaches, similar to those above, augmented with experimental evolution to generate bacteria with enhanced production of free fatty acids, triacylglycerols, or FAAE.

Together these programs and projects are creating an integrated engineering framework for designing microbes for transformation of feedstocks into fuels. In the next few years, the EBI will add synergetic programs to the Biofuels Production effort that will move the frontiers even farther.

Adam P. Arkin is Assistant Professor of Bioengineering and Chemistry at UC Berkeley, senior scientist at Lawrence Berkeley National Laboratory, and a member of the EBI Executive Committee.



THE SOCIOECONOMIC CHALLENGES OF BIOFUELS

IMPLICATIONS FOR LAND USE, MARKETS, AND THE ENVIRONMENT

BY MADHU KHANNA

The socioeconomic program in the EBI is developing modeling frameworks and applying them using extensive economic and biophysical data to investigate the socioeconomic, environmental, and intellectual property issues associated with the introduction of the next generation of biofuels. Researchers are using both a microeconomic (bottom-up) approach to assess impacts on land use, food production, and carbon emissions at the local, regional, and national levels, and a macroeconomic (top-down) approach that examines inter-relationships between the prices of biofuels, food and fuel, welfare of consumers and producers, and the environment. In particular, teams are conducting research in the following areas:

Economics of Cellulosic Biofuel Production and Land Use Impacts

Madhu Khanna's program is developing an integrated, interdisciplinary framework to investigate the effects of large-scale production of cellulosic biofuels in the U.S. on land use, crop production, farm income, and carbon mitigation over a 15-year period. Her group has developed a national scale economic model with crop, livestock, and fuel sectors to determine the impact of biofuel production under various policy scenarios on allocation of land among various uses. Demand for fuel and biofuels is linked to demand for vehicle miles travelled. The model uses simulated yields of energy crops, Miscanthus and switchgrass, obtained from a crop growth model; county level estimates of the costs of production of these and other crops and national demands for various crops and livestock to determine food, feed and fuel production, and prices. Historical data on county level changes in crop-specific acreages, crop yields, prices, and weather are used to determine and predict changes in crop acreage and yields in the future in response to changes in crop prices due to biofuels.

Estimates of the costs of production of energy crops show that breakeven costs of bioenergy crops are critically dependent on the yields of bioenergy crops and the yields of the annual crops they are likely to displace. High yields of bioenergy crops reduce the per ton opportunity cost of land. The latter is particularly high in locations where the yields of corn and soybeans are high and a land owner would need to forgo considerable returns by switching to an energy crop. Moreover, low fertilizer application rate, low replanting probability, and low harvest loss also play a significant role in reducing the break-even costs of bioenergy crop production. The spatial differences in costs of production of energy crops, corn, and crop residues (corn stover and wheat straw) suggest that a mix of cellulosic feedstocks is likely to be more economically viable than a single feedstock, and this mix is expected to evolve from greater reliance on crop residues initially, then to reliance on energy crops, like Miscanthus, over time.

The outcomes of the land use and biofuel production model are then used to examine the optimal capacity, timing of construction, and location of biorefineries as a function of the regional distribution of bioenergy feedstocks, the costs of transportation of feedstocks, biofuels, and co-products (DDGS) and the demand centers for biofuels and DDGS. The analysis shows that it would be cost-effective to locate refineries within(on average) 50 miles of the biomass and corn producing areas and to transport feedstock by truck.

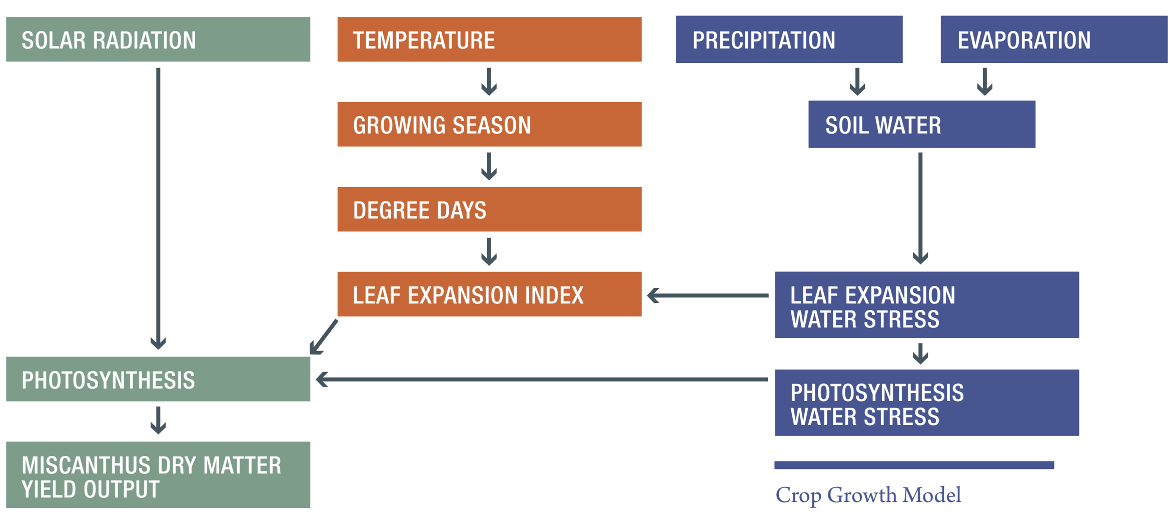
Food and Fuel Market Impacts

David Zilberman's team has developed quantitative models and measures to assess the relationships between ethanol, corn, gasoline, and oil prices using threshold vector error correction models. They use historical data on these prices to examine conditions under which ethanol prices are affected by fluctuations in the price of corn and fluctuations in the price of oil as well as the extent to which ethanol prices affect the price of oil and corn. Their analysis shows that when ethanol markets are in disequilibrium, an ethanol price shock will lead to higher corn prices and to higher gasoline prices, with both prices peaking after seven months. They also find that when markets are close to being in equilibrium, an increase in gasoline prices lowers the price of ethanol, but a positive shock to ethanol prices raises the price of gasoline. Their analysis suggests that gasoline producers view ethanol as a substitute while ethanol producers view gasoline as a complement.

Zilberman's group is continuing to assess the impact of biofuel on the energy market dominated by non-competitive entities like OPEC. They have found using econometric analysis that OPEC nations subsidize their motorists but use their monopolistic power to establish oil prices for the rest of the world. The introduction of biofuels may lead to a reduction in oil prices, some reduction in OPEC exports (to counter some of the price reduction), and reduction in greenhouse emissions.

Zilberman's group also compares the effects of a Renewable Fuel Standard (RFS) with those of a national Low Carbon Fuel Standard (LCFS) on fuel prices, fuel consumption, and greenhouse gas emissions. They find that while an RFS is likely to promote greater biofuel production and lower fuel prices relative to an LCFS, it is also likely to result in higher greenhouse gas emissions.

Khanna's group examines the implications of a carbon tax policy for food and fuel prices and for biofuel production. They show that a carbon tax raises land rents and affects food prices and real wages and that the optimal carbon tax must consider these effects on the food and labor markets. Unlike a cap-and-trade policy (in which carbon permits are provided free to firms), a carbon tax provides revenues that can be recycled to reduce other distortionary taxes



Crop Growth Model

in the economy, such as the labor income tax. They show the conditions under which this has a potential to generate a net gain in social welfare for the economy.

Hadi Esfahani, Clifford Singer, and John Vasquez use historical data on energy use to extrapolate rate of biofuel use in the "Cane" producing and "Grain" producing regions of the world. They find that extent of future market penetration by biofuels is sensitive to biofuel production costs, oil prices, and land costs. Using global scale models, they also analyze the patterns of energy and agricultural flows, how these patterns are changing with shifts in energy sources, and their impact on energy prices.

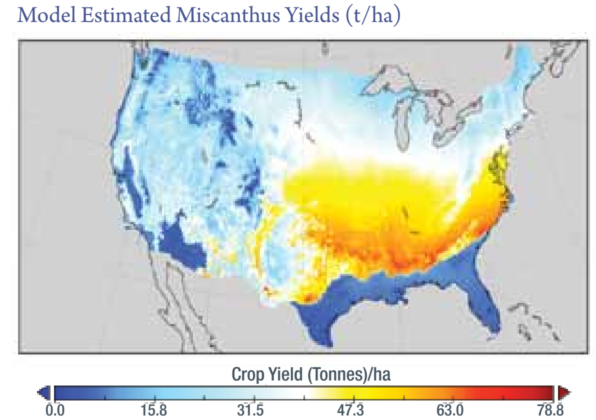
Brian Wright and his team are examining the effects of inventories and biofuel demand on food prices and assessing the role of public inventory management. They find that when commodity stocks are low, prices become very sensitive to small, unanticipated shocks in demand or supply. To achieve greater food security, their analysis implies a need for governments to increase commodity storage and target feed programs to the most vulnerable sections of the population when commodity prices increase. They also suggest that a more cost-effective alternative to increasing storage would be for the government to buy call options from domestic biofuel producers or animal feeders to divert grain from biofuels to food and feed during a crisis. Work also is proceeding on improved estimation procedures for markets for storable commodities, and a paper on sugar is well under way. Work on trend estimation is being pursued for application to major grain markets as well.

Role of Innovation and Intellectual Property Rights

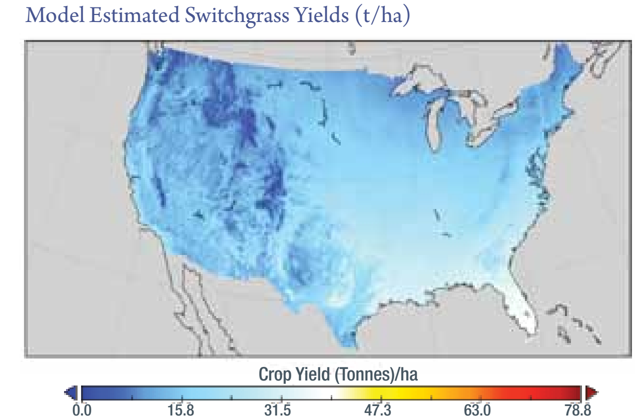
Wright is assessing the intellectual property issues relevant to biofuels research beyond current ethanol production. An initial assessment of the landscape indicates that patent applications are now increasing rapidly in this area. His analysis shows that the public sector is highly active in biofuels research, and in particular dominates fermentation research in this area. Scandinavian firms dominate enzyme research. It is therefore critical to understand public/private relationships in this area. He is conducting a survey to assess the evolving effects of intellectual property protection of inputs and outputs of research and of some relevant regulatory constraints from the viewpoint of EBI scientists involved in activities relevant to the initiative and those of a control group of scientists.

Work is proceeding on a study of patenting and licensing at the UC Office of Technology Transfer. In particular the work investigates the relationship between sponsorship and intellectual property

Model Estimated Miscanthus Yields (t/ha)

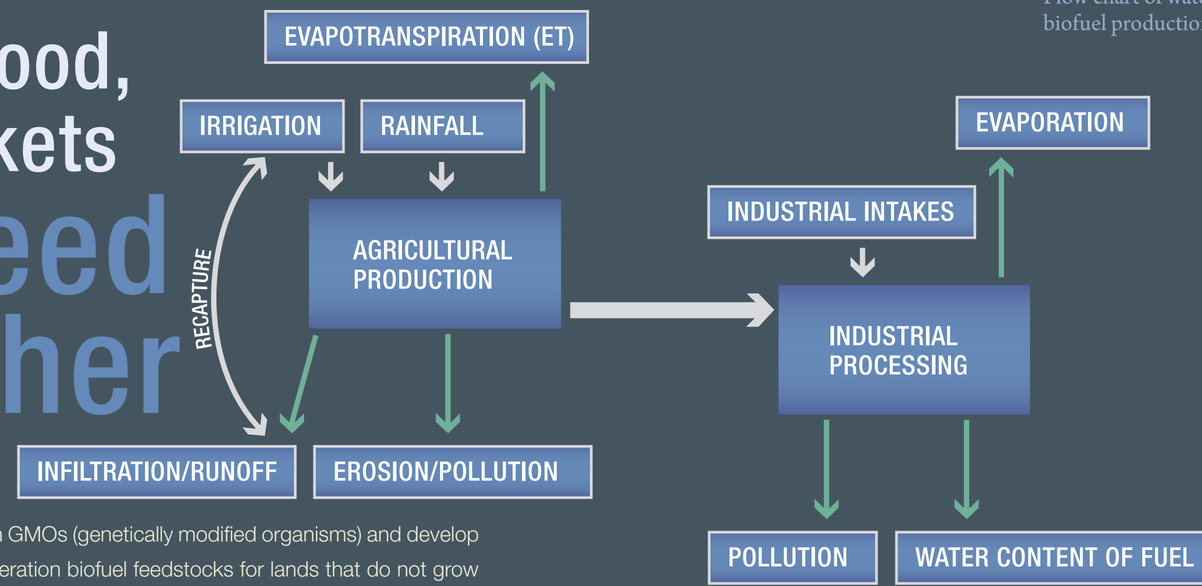


Model Estimated Switchgrass Yields (t/ha)



Economist David Zilberman:

Making Food, Fuel Markets Succeed Together



Flow chart of water use in biofuel production.

David Zilberman has come a long way since he started his UC Berkeley professorial career by becoming the premier authority on the economics of manure.

That was in 1973, after growing up in Israel, serving in the army, living and working on a kibbutz, doing computer programming for a big corporation, and graduating from Tel Aviv University with a degree in economics and statistics. He realized that to succeed in Israel "you need to be a general or a professor," so he chose the latter.

This decision brought him to graduate school in Berkeley and his research on animal waste. Following his Ph.D., "I was hired to teach agricultural policy, and I knew nothing about it," Zilberman recalls. "So I learned by teaching, and acquired knowledge on risk management and the political economy of agriculture. Then I developed a theory of technology adoption and applied it to different agricultural problems." And from the manure grew a mature and distinguished career.

Today he is one of the world's authorities on agricultural and nutritional policy, and the economics of technological change, natural resources, and micro-economic theory. He is applying his expertise to issues surrounding the Energy Biosciences Institute's interest in a new biofuels industry. His work is directly in the crosshairs of the food vs. fuel controversy.

"To address the issue, we need to increase the supply of traditional agricultural crops," he said, reflecting on the opinion of many that biofuel production has exacerbated the global hunger situation by replacing food crops. "Unless we improve agriculture with GMOs (genetically modified organisms) and develop second-generation biofuel feedstocks for lands that do not grow food, we will have problems."

Therein lies one of the big controversies—the development via biotechnology of genetically-altered strains of crops. Zilberman quotes statistics that show how such adaptations have enhanced farm productivity in America and India and have increased worker safety in China, and he cannot comprehend the restrictions on GMO use in Europe and underdeveloped countries. His stance has not made him popular with those who still consider biotech manipulation to be irresponsible.

"In my life, I have been criticized by environmentalists and by industry," he said. "But I feel if you use rigorous science to address relevant issues, you can make a difference. Economics is an integrating discipline. We have to combine logical thinking, sound values, and scientific knowledge to produce good decision rules that lead to sensible changes that help people live better lives while sustaining the environment."

> We cannot save the planet by saving the status quo.

This isn't the first time Zilberman found himself in the midst of political conflict. Some years ago, a California ballot proposition called "Big Green" proposed banning all pesticides from use on food crops due to environmental and health risks. The economist noted pesticides' virtues in preventing plant diseases and dramatically increasing food supply. He also pointed out that banning pesticides would mostly impact the poor. The proposition eventually failed.

Another study challenged the perception that GMOs do not increase crop yields by documenting immense increases in yields in India, where GMO cotton varieties controlled diseases that were not affected by chemical pesticides. "I got calls from all over, even India in the middle of the night, when the results were published," Zilberman recalls. "They said I was going against God. When you take a position, you have to deal with the consequences."

The feisty investigator doesn't shy away from a fight, but he is also realistic, especially when it comes to biofuels. "Biofuels should be supported as long as they save greenhouse gas emissions compared to alternatives and they do not compromise the availability of foods," he said. "We try to look at biofuels within the context of international trade and trade policy, to compare and quantify the merits of alternative regulations of biofuels, to assess how financial and technological risk considerations will affect investment in and adoption of biofuel. We recognize that addressing food, fuel, and greenhouse gas concerns requires understanding tradeoffs and taking bold actions. We cannot save the planet by saving the status quo."

He also feels the EBI is in the perfect position to affect positive change through sponsorship of dialogue-rich conferences and the diverse research portfolio it brings to bear on the problems. Zilberman is a basketball fan, who calls his colleagues "intellectual athletes" and views Chris Somerville, the EBI Director, as a superstar. "Getting him from Stanford was like the (Los Angeles) Lakers getting Pau Gasol, and I hope it will make us champs, too."

protection. A separate study addresses the effects of licensing on patent citations. This work has generated interest and an offer of modest support at the National Academies. His group also is preparing papers on public/private partnerships relevant to bioenergy and on relevant legal issues.

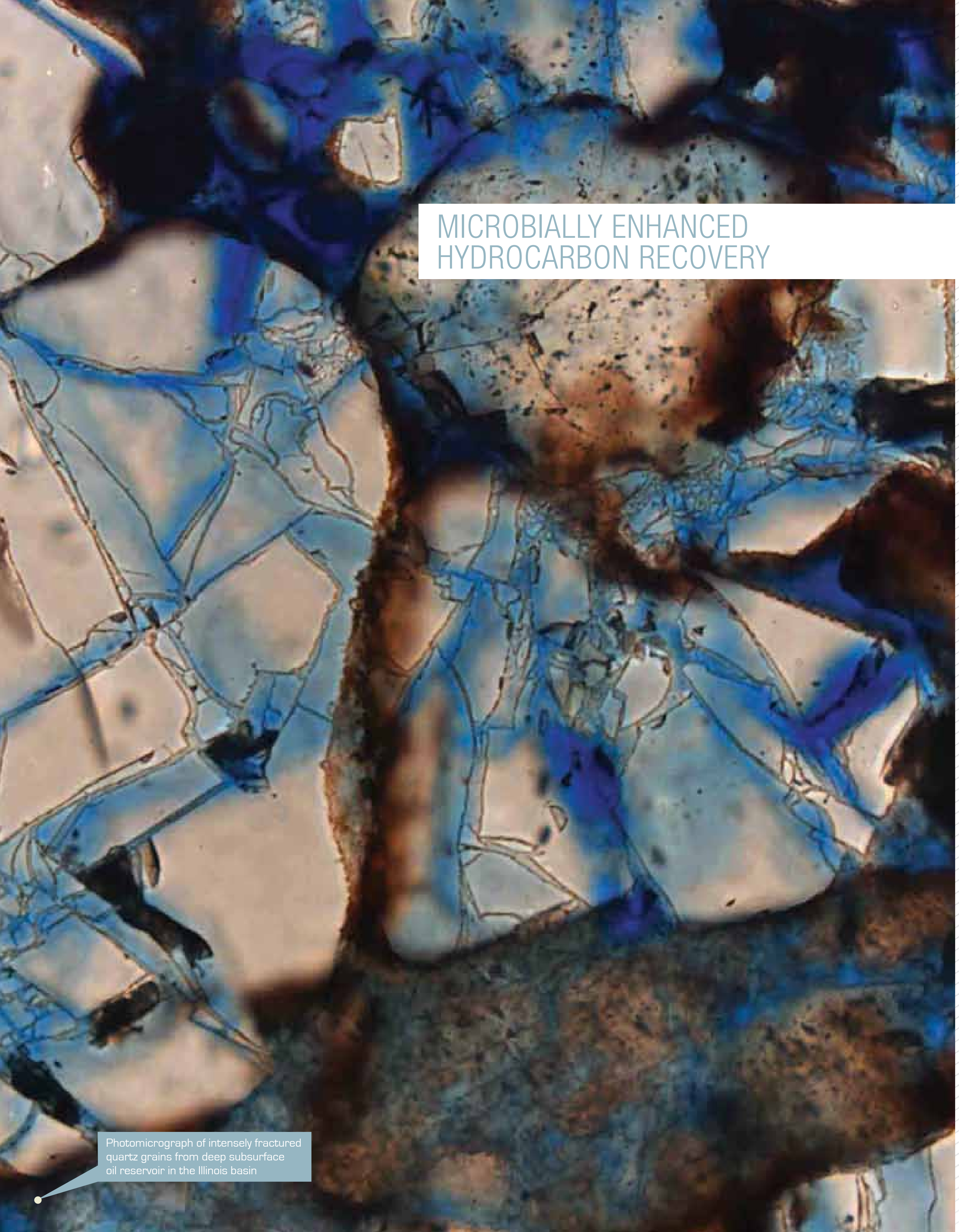
Environmental Impacts of Biofuels

Several research teams within the socio-economic program are assessing various aspects of the environmental impacts of biofuels. Khanna's team uses life cycle greenhouse gas emissions analysis with detailed spatial data on production methods and land use to determine the potential for biofuel-driven land use changes and displacement of gasoline to mitigate carbon and the costs of achieving mitigation. An assessment of the emissions from biofuels from Miscanthus, switchgrass, corn stover, and sugarcane (in Brazil) shows their potential on average to reduce greenhouse gas intensity relative to gasoline of 95%, 62%, 91%, and 73% respectively. John Braden's group analyzes the trade-offs between reduced greenhouse gas emissions due to biofuels and water quality degradation due to greater fertilizer use for some feedstocks, such as corn. Zilberman's group compares the greenhouse gas implications of a RFS and a national LCFS. Using different modeling approaches these groups converge to similar findings that show that biofuel mandates and tax credits are likely to result in negligible benefits in the form of greenhouse gas emissions reduction. Moreover, they are likely to significantly increase nitrogen fertilizer use due to additional corn production. A policy that prices carbon (such as a carbon tax or cap and trade policy) is critical for achieving desired reductions in greenhouse gas emissions. Additional policy instruments such as a nitrogen fertilizer tax are needed to control nitrogen use and resulting damages to water quality.

Madhu Khanna is Professor of Agricultural and Consumer Economics at the University of Illinois at Urbana-Champaign and a member of the EBI Executive Committee.

There won't be a single solution to the global energy challenge. But there will be solutions.

Sugarcane at a processing plant in Brazil



Photomicrograph of intensely fractured quartz grains from deep subsurface oil reservoir in the Illinois basin

MICROBIALLY ENHANCED HYDROCARBON RECOVERY

ENLISTING BACTERIA TO GET THE MOST OUT OF WELLS—RESPONSIBLY

BY TERRY HAZEN

Microbes have had a profound effect on the quality of petroleum found in reservoirs and conversion of coal to methane. Typically only 33 percent of oil available from any well is recoverable. It has become clear that it may be possible to biotransform oil and coal to make it more recoverable and/or to do *in situ* refining, thereby eliminating contamination of other environments. Ecogenomics, when combined with new methods for monitoring biogeochemistry, allows a systems biology approach that will enable new strategies for microbial enhanced hydrocarbon recovery (MEHR).

MEHR techniques involve the introduction of microorganisms, nutrients, and oxygen into the reservoir to produce metabolic events that lead, by a variety of mechanisms, to enhanced oil recovery. Several challenges prohibit the implementation of MEHR. To overcome these challenges, it is imperative to 1) develop an understanding of native reservoir bacteria and their potential to enhance oil recovery, through alteration of crude oil molecular structure or associated flowpaths; 2) engineer microbial strains to promote traits that facilitate recovery; 3) predict microbial growth and reactivity within petroleum reservoirs with enough accuracy to guide MEHR treatments; 4) develop *in situ* procedures to implement MEHR effectively and over large spatial scales, and 5) monitor treatments and associated products in real-time and over field-relevant scales.

An understanding of oil reservoir microbial physiological processes using both model organisms and mixed population assemblies harvested from active reservoirs is a prerequisite to developing microbial technologies to enhance the quantity and quality of energy recovered. MEHR strategies can be one or a mixture of processes which alter the lithology of the reservoir matrix, the flow and viscosity of flood waters, the viscosity and chemical (molecular) characteristics of the oil, or the *in situ* hydrostatic pressure.

MEHR mechanisms are expected to be impacted by processes and properties that occur over a wide range of length scales, ranging from surface interactions and microbial metabolism at the submicron scale, to changes in wettability and pore geometry at the pore scale, to geological heterogeneities at the petroleum reservoir scale. To eventually ensure successful implementation of MEHR models under field conditions, it is necessary to develop approaches that can remotely monitor and accurately predict the complex microbially facilitated transformations that are expected to occur during MEHR treatments in reservoirs, such as the evolution of redox profiles, viscosity modification, and the microbial effects on reservoir matrix porosity and permeability.

The Monitoring and Modeling Crosscutting Core of the MEHR program focuses on developing novel approaches for monitoring and predicting MEHR processes using synchrotron, isotope, geophysical, and reactive transport modeling methods, and on investigating how the molecular to pore-scale processes are manifested at the larger reservoir scale.

The EBI's research core has worked closely with the Illinois State Geological Survey (ISGS) during the last 10 months of 2009 to sample pristine formation water and reservoir rock from a carbon sequestration injection well that reached a depth of 2.1 km within the Illinois Basin. The well is located in Decatur, IL, and was drilled as part of a carbon capture, transport, and storage (CCS) research project by the Midwest Geological Sequestration Consortium.

Analyses of the formation water, reservoir rock, and the indigenous subsurface microbial communities are underway. The goal is to determine the conditions of the Mt. Simon sandstones as a quantitative context for understanding the distribution and metabolic activity of microbial communities inhabiting the deep subsurface.

Integrated molecular analyses are being completed to identify metabolic signatures (biomarkers) composed of community-level suites of microbial metabolic genes that will be an indicator of subsurface microbial biosphere ecology.

Terry C. Hazen is Senior Scientist and Head of the Ecology Department at Lawrence Berkeley National Laboratory, and is a member of the EBI Executive Committee.

Briefings, conferences, and workshops throughout 2009 provided tangible evidence that the Energy Biosciences Institute's reach is international, and the EBI's educational mission is central to its collaborative core.

OUTREACH AND EDUCATION

EBI leaders and principal investigators traveled extensively to share the Institute's vision and to forge connections with similar programs. EBI Director Chris Somerville alone fulfilled requests both local and global, speaking at more than 50 public and scientific forums.

Outreach efforts in 2009 advanced the EBI's global mission of developing alternative forms of bioenergy in sustainable and economical ways.

Global Renewables Research Society

The EBI was central in the formation of two international scientific organizations dedicated to the promotion of science-based knowledge in the continuing development of biofuel technology and policy. The Global Renewables Research Society, comprised of leading scientists from eight countries—including the EBI in the United States—was launched in May in Portugal to provide balanced views on the bioeconomy debate for policymakers throughout the world. The founding partners of the society are scientists from leading international biorenewables centers: the EBI; B-Basic, Kluyver Centre, Copernicus Institute of Utrecht University (Netherlands); Institute for Biotechnology and Bioengineering (Portugal); Institute of Advanced Studies of the University of São Paulo (Brazil); University of Queensland (Australia); Imperial College, Cambridge University, the University of York (United Kingdom); Catholic University of Valparaíso (Chile); and Indian Institute of Technology-Kharagpur (India).

Council of Energy Research and Education Leaders (CEREL)

EBI Assistant Director Susan Jenkins, representing the UC Berkeley campus, was named president-elect of the newly formed Council of Energy Research and Education Leaders (CEREL), a consortium of more than 30 universities committed to advancing the role of higher education in the energy field to improve education, decision-making, and societal well-being.

India Institute of Techology (IIT)

The EBI's affiliation with the India Institute of Techology (IIT) at Kharagpur, India's premier research and education institute, continued for the second year with the hosting of seven summer exchange students and two faculty representatives who visited in February to discuss their programs and to begin forging ties with the Institute's investigators. Assistant professor of chemical engineering Saikat Chakraborty made the case for why India is urgently

Three of the EBI's seven 2009 summer students from the Indian Institute of Technology in Kharagpur, from left, Shailabh Kumar, host faculty member Manfred Auer, Dolonchampa Maji, and Gourab Chatterjee.

interested in alternative fuels. Assistant Professor of Biotechnology Ramkrishna Sen of IIT-Kharagpur and business consultant Kunal Sinha, an IIT graduate now with ZS Associates in New York, also spoke to EBI leaders about their plans to launch a biofuel center in 2010. EBI Director Chris Somerville has been appointed to the advisory board of the new institute.

31st Symposium on Biotechnology for Fuels and Chemicals

The EBI was a sponsor and several researchers made presentations at the premier American scientific conference on biofuels, the 31st Symposium on Biotechnology for Fuels and Chemicals, sponsored by the Society of Industrial Microbiology (SIM), in San Francisco in May. The symposium served as a forum for experts from around the world to discuss the latest research breakthroughs and results in sustainable fuels and chemical production. The scientific program facilitated exchange of new information and technical progress among attendees from industrial, academic, and government sectors. "We're beginning to see the first real commercialization of the field," EBI Director Chris Somerville said at a symposium press briefing. "Six companies represented at the meeting say they are in small-scale production or soon will be. Another 20 have declared their intent. This is a very important change in the sector. It's coming to a stage in industry where, after years of modeling and step-by-step improvements, we'll find out how it all really works."

First Annual Biofuels Law and Regulation Conference

The world's first comprehensive conference on biofuels law and regulation, sponsored in part by the EBI at the University of Illinois in April, featured more than 90 industry, research, and trade association representatives reflecting on regulations that have the potential to encourage or discourage biofuels production. By gathering together experts from several countries, the group was able to share information about successes and struggles within different cultural, economic, and legal frameworks.

Transatlantic Dialogue on Biofuels

The Director, Deputy Director, Associate Director, and a renowned agricultural engineer from the EBI were invited by the German Ministry of Food, Agriculture and Consumer Protection and the Agency for Renewable Resources to engage in a "transatlantic dialogue" on biofuels in October. Chris Somerville, Steve Long, Paul Willems, and K. C. Ting made the trip to Berlin to share their views and the EBI's mission with their German counterparts, focusing in particular on sustainability criteria for biomass production. Also participating were Deutsche BP, the energy company's program in Germany, and the Deutsche BiomassForschungsZentrum (DBFZ), the German biomass research center.

Brazil-U.S. Higher Education Council

Two EBI graduate students from UC Berkeley were among 30 chosen to participate in the first Brazil-U.S. Higher Education Council short course on biofuels technology. Their classroom experience was funded by the Fulbright Commission for Educational Exchange between the two countries to promote improved com-

Visiting a processing plant in Brazil at summer biofuels course were (from left) Chris Phillips, Abigail Martin, and Anand Gopal.

munications between scientific institutions in the area of biofuels. Chris Phillips, who works in Michael Marletta's lab, and Abigail Martin, who works on Dick Norgaard's team, said their 11-day immersion into the Brazilian biofuel culture allowed them to build connections with potential colleagues, compare notes on common issues, and talk candidly and informally with leading researchers in the field.

Expedition Antarctica 2009

Norm Miller, Berkeley Lab staff scientist and adjunct professor in UC Berkeley's Geography Department, was one of eight academic experts invited to join the BP-sponsored "Expedition Antarctica 2009" to see the planet's iciest continent and observe how the warming climate is changing it. Miller was asked by noted polar explorer and environmental pioneer Robert Swan to be among the cruise' instructors, teaching 60 students chosen from 30 universities in 20 countries about real-time energy issues while encouraging their discussion of energy challenges. An EBI principal investigator, Miller led sessions on climate change phenomena while the ship negotiated glacial seas being impacted by global warming. BP, the EBI's sponsor, arranged the voyage.

PROGRAMS & PROJECTS OF THE EBI

Feedstock Development:

Programs

Assessing the Potential Impact of Insect Pests and Plant Pathogens on Biomass Production of *Miscanthus x giganteus* and Switchgrass (*Panicum virgatum*)

Principal Investigator: Michael E. Gray, U. of Illinois

Co-PIs: Carl Bradley, Terry Niblack, Kevin Steffey

Postdocs: Monday Ahonsi, Bright Agindotan, Jeff Bradshaw, Tesfa Mekete, Jarrad Prasifka, Tesfamariam Mengistu

Visiting Researcher: Kimberly Reynolds

Researchers: Omar Ali, Niteesh Chitturu, Bret Hash, Teresa Tufte, Karla Ruiz, Ekta Rajpal, Lauren Diebold, Rommel Morales, Blessing Sokoya

Undergraduate Students: Sarah Heald, Aubrey Hinrichs

Genomics-Enabled Improvement of Andropogoneae Grasses as Feedstocks for Enhanced Biofuel Production

Principal Investigator: Stephen Moose, U. of Illinois

Co-PIs: Matt Hudson and Ray Ming, U. of Illinois, and Dan Rokhsar, UC Berkeley

Postdocs: Magdy Alabady, Cuixia Chen, Megan Hall, Kankshita Swaminathan

Research Associates: Jayanand Boddu, Brandon Smith

Graduate Students: Adam Barling, Brandon James, Liang Xie, Won Byon Chae, Dedeepya Vaka

Undergraduate Students: Sharon Lee, Ornella Ngamboma, Arthur Rudolph

Subcontractor: Hawaiian Agricultural Research Center

Engineering Solutions for Biomass Feedstock Production

Principal Investigator: K.C. Ting, U. of Illinois

Co-PIs: Alan Hansen, Qin Zhang, Tony Grift, Lei Tian, Steven Eckhoff, Luis Rodriguez

Postdocs: Konstantinos Domdouzis, Hala Chaoui, Qingting Liu, Yogendra Shastri, Tofael Ahamed, Ming-Che Hu

Research Associate: Zewei Miao

Graduate Students: Yung-Chen Liao, Candace Godbolt, Bernardo Vidal, Esha Khullar, Yanshui Jiang, Phillip Johnson

Undergraduate Students: Jude Holscher, Jordan Tate, Kevin Luke, Ethan Ford, Michael Valentine, Jesse Woodruff

Faculty Collaborator: Vijay Singh

Research Technicians: Dennis Mohr, Kent Valentine

Administration: Ronda Sullivan

Visiting scholars: Francisco Pinto, Yonghua Xiong, Yan Jiang, Yuliang Zhang

Feedstock Production/Agronomy Program

Principal Investigator: Tom Voigt, U. of Illinois

Co-PIs: German Bollero, D.K. Lee, Stephen Long, Michael Dietze

Researchers: Drew Schlumpf, Emily Thomas, Xinguang Zhu, Melissa Kocek

Horticulturalist: Anthony Brasch

Visiting Researcher: Richard Pyter

Graduate Students: Eric Anderson, Rebecca Arundale, Bosola Oladeinde, Andy Wycislo

Undergraduate Students: Ruth Johnson, Erica Schwer, Chee-Kei Chan, Adrienne Perkins, Matt Senft, Neal Makela, Ben Lewis, Ariel Ranieri, Noel Piatek

Projects

Reproductive Barriers in *Miscanthus sinensis* and Other Biofuel Plants

Principal Investigator: Sheila McCormick, UC Berkeley

Postdoc: Yuefeng Guan

Model Development to Predict Feedstock Production of Miscanthus and Switchgrass as Affected by Climate, Soils, and Nitrogen Management

Principal Investigator: German Bollero, U. of Illinois

Co-PIs: Fabian Fernandez, Stephen Long

Postdoc: Fernando Miguez

Germplasm Collection, Nutrient Cycling, Cold Hardiness, Photosynthetic Capacity, and Flowering Phenology of *Miscanthus sacchariflorus*, *Miscanthus sinensis*, and Their Natural Hybrids in Native Stands Ranging from Central to Northern Japan

Principal Investigator: Ryan Stewart, U. of Illinois

Co-PIs: Fabián Fernández, Germán Bollero

Faculty Researchers: Toshihiko Yamada, Aya Nishiwaki

Postdocs: Yo Toma, Lauren Quinn

Gradute Students (Hokkaido and Miyazaki Universities): Hiroya Matuura, Sachi Yamaguchi, Shotaro Kuwabara, Shohei Sato, Yahuhisha Kajihara, Natsumi Iizuka, Fuyuko Hazama, Yuuta Nabeoka, Ayano Kishimoto, Miya Okada, Akane Kitagawa

Consultant: Theresa Culley

Collaborators: Adam Davis, David Matlaga

Improvement of Bioenergy Crops via Transformation

Principal Investigator: Jack M. Widholm, U. of Illinois

Co-PI: John A. Juvik

Researchers: Vera Lozovaya, Olga Zernova, Qiang Liu, Nadeza Kholina, Li Zhang, Xiaoguang Chen

Graduate Student: Hyoung Seok Kim

Regional Biofuels Modeling

Principal Investigator: Michael Dietze, U. of Illinois

Postdocs: Dan Wang, David LeBauer

Graduate Students: Xiaohui Feng, Matt Locus

Undergraduate Student: Kevin Shah

Developing Prairie Cordgrass as a Cellulosic Bioenergy Crop

Principal Investigator: D.K. Lee, U. of Illinois

Postdoc: Chang Hoon Lee

Undergraduate Student: Allen Parrish

Understanding Low-Temperature Limits of Miscanthus and Its Variation and Basis

Principal Investigator: Stephen Long, U. of Illinois

Graduate Students: William Hay, Ashley Spence

Biomass Depolymerization:

Programs

Chemical Imaging of Plant Biomass with Micro- and Nano-Raman Spectroscopy

Principal Investigator: Paul Adams, Lawrence Berkeley National Laboratory

Co-PI: Jim Schuck

Postdocs: Martin Schmidt, Pradeep Perera

Cell Wall 3D Architecture at Nanometer Resolution Using Correlative Raman and Electron Tomography Imaging

Principal Investigator: Manfred Auer, Lawrence Berkeley National Laboratory

Co-PIs: Kenneth H. Downing, Jan Liphardt, Bahram Parvin

Postdocs: Purbasha Sakar, Philip Jess

Researchers: Elena Bosneaga, Ju Han

Undergraduate Students: Gourab Chatterjee, Shailabh Kumar, Dolonchampa Maji

Mechanism of Plant Cell Wall Modification

Principal Investigator: Chris Somerville, UC Berkeley

Co-PI: Shauna Somerville

Postdocs: Clarisa Bejar, Heidi Szemenyei, Charlie Anderson

Researchers: Shaolin Chen, Ian Wallace, Shivani Khanna

Biomass Pretreatment & Chemical Synthesis of Transportation Fuels

Principal Investigator: Alexis Bell, UC Berkeley

Co-PI: Harvey Blanch

Postdocs: Ting Chen, Sasisankar Padmanabahn, Mandan Chidambaram, Dusan Bratko

Graduate Students: Sean Dee, Katherine Pfeiffer, Kierston Shill, Paul Wolski, Kristopher Enslow, Eric Sacia

Undergraduate Student: Shriharsha Jayanti

Faculty Collaborators: John M. Prausnitz, Berend Smit

Visiting Students: Manuel Hoerl, Qin Xin, Michael Kim, Hongxue Xie

Visiting Scholar: Stefan Farnik

Cellulosomes Optimized for Biofuel Production

Principal Investigator: Jamie H. D. Cate, UC Berkeley

Co-PI: Michael A. Marletta

Researchers: Daniel A. Fletcher, Evan R. Williams

Postdocs: Haw Yang, Corinne Hausmann, Tai-De Li, Hu Cang, Charlie Chen

Research Associate: Bruno Martinez

Graduate Students: William Beeson, Meadow Anderson, Jonathan Galazka, Padma Gunda, Chris Phillips, Veronica Zepeda, Elizabeth Znameroski, Kai Zhang

Specialist: Tony Iavarone

Visiting Scholar: Raphael Lamed

Enhanced Conversion of Lignocellulose to Biofuels: Bioprocess Optimization from Cellulose Hydrolysis to Product Fermentation

Principal Investigator: Douglas S. Clark, UC Berkeley

Co-PIs: Harvey W. Blanch, UC Berkeley, and Frank T. Robb, U. of Maryland

Postdocs: Harshal Chokhawala, Melinda Clark, Cong Trinh, Tae-Won Kim, Seth Levine, Ross Eppler, Joel Graham

Graduate Students: Craig Dana, Dana Nadler, Jerome Fox, Sarah Huffer, Priya Jayachandran, Katherine Pfeiffer, Kierston Shill, Paul Wolski, Zachary Baer, Christine Roche

Research Associate: Kyong Heon Kim

Visiting Scholars: In Jung Kim, Stefan Farnik

Discovery and Characterization of Hydrolytic Enzymes to Improve Biocatalysis and Conversion of Plant Cell Wall Polysaccharides to Biofuels

Principal Investigator: Isaac Cann, U. of Illinois

Co-PIs: Roderick Mackie, Satish Nair

Faculty Investigator: Charles Schroeder

Research Scientist: Ashley Spies

Postdocs: Yejun Han, Shinichi Kiyonari, Shosuke Yoshida, Young Hwan Moon, Xiaoyun Su

Graduate Students: Dylan Dodd, Allie Sexton

Undergraduate Students: Robert Beverly, Jason Kim, Brad Jelinek, Charles Hespen, Humera Hazari

Researchers: Mikhil Ranka, Hiroshi Miyagi

Biochemistry, Structure and Engineering of Enzymes and Metabolic Pathways to Overcome Biomass Recalcitrance

Principal Investigator: John A. Gerlt, U. of Illinois

Co-PIs: John E. Cronan, Satish K. Nair, Yi Lu, James Bristow, Lawrence Berkeley National Laboratory (DOE Joint Genome Institute)

Postdocs: Sudipta Majumdar, Jose Solbiati, Ahmed M. Abdel-Hamid

Graduate Student: Salehe Ghasempur

Undergraduate Students: Adam Kolakowski, Logan Traylor

Researchers: Jose Solbiati, Stephen Lowry, Konstantinos Mavrommatis

Enzyme-Inspired Catalysts for Enhancing Biofuels Production

Principal Investigator: Alexander Katz, UC Berkeley

Postdocs: Andrew Solovyov, Tatiana Luts, Oz Gazit

Graduate Students: Ted Amundsen, Alexander Charmot

Development of Novel Catalysts

Principal Investigator: Dean Toste, UC Berkeley

Postdocs: Hyun-Ji Song, Sunghee Son, William Brenzovich, Ann Gonzalez, Matthew Campbell, Heather Burks

Graduate Student: Mika Shiramizu

Undergraduate Student: Diana Zheng

Visiting Researcher: Kun Wang

Projects

Fungi and Deconstruction of Lignin and Other Components of Miscanthus Cell Walls

Principal Investigator: John Taylor, UC Berkeley

Co-PIs: Louise Glass, Tom Bruns

Postdoc: Chaoguang Tian, Prachand Shrethsa, Trevor Starr

Research Associates: Spencer Diamond, Henry Han, Maria Isobel Santos

Research Technician: Tim Szaro

Biomass to Transportation Fuel via Hydrodeoxygenation

Principal Investigator: Jonathan Ellman, UC Berkeley

Co-PI: Robert Bergman

Postdocs: Jason Nichols, Jerome Volkman, Jiayi Xu

Graduate Student: Lee Bishop

Enzyme Discovery in Grass-Feeding Termites for the Depolymerization of Lignocellulosic Biomass

Principal Investigator: Philip Hugenholtz, Lawrence Berkeley National Laboratory (DOE Joint Genome Institute)

Researchers: Falk Warnecke, Natalia Ivanova, Nikos Kyrpides

Surface Kinetic Mechanisms of Enzymatic Cellulose Deconstruction

Principal Investigator: Clayton J. Radke, UC Berkeley

Graduate Student: Sam Maurer

Analysis of Bovine Rumen Microbiota Under Different Dietary Regimens for Identification of Feedstock-Targeted Cellulolytic Genes

Principal Investigator: Edward Rubin, Lawrence Berkeley National Laboratory (DOE Joint Genome Institute)

Co-PI: Rod Mackie, U. of Illinois

Researchers: Matthias Hess, Tao Zhang, Suzannah Tringe-Green

Detoxification of Miscanthus Hydrolysates with a New Phase Separation Method

Principal Investigator: Hao Feng, U. of Illinois

Graduate Students: Pradip B. Dhamole, Jose de Frias, Bin Zhou

Visiting Scholars: Prafulia Mahajan, Yuanqin Liu

Visiting Researcher: Bin Wang

Researcher: Hong Yang

Fractionating Recalcitrant Miscanthus by a Two-Stage Treatment Under Mild Reaction Conditions

Principal Investigator: Hao Feng, U. of Illinois

Postdocs: Pradip Dhamole, Zhilong Wong

Graduate Student: Jose de Frias

Visiting Scholars: Pablo Cesar Torres Aguilar, Xiao Juan Wang

Ecology and Exploitation of Endophytic Diazotropic Bacteria in Biofuel Crops

Principal Investigator: Angela Kent, U. of Illinois

Postdoc: Daniel Keymer

Graduate Students: Dongfang Li, Ariane Peralta

Organometallic Chemistry Relevant to Delignification

Principal Investigator: Thomas B. Rauchfuss, U. of Illinois

Postdocs: Todsapon Thananatthanachon, C. Matthew Whaley, Didier Morvan

Graduate Students: Alexey Sergeev, Aaron Royer, Zachariah Heiden

New Approaches to Lignin Depolymerization

Principal Investigator: Michelle Chang, UC Berkeley

Graduate Student: Margaret Brown

Research Technician: Stacey Hess

Development of *Neurospora crassa* as an Expression Platform and Model Organism to Explore Functional Analysis of Cellulolytic Enzymes

Principal Investigator: N. Louise Glass, UC Berkeley

Postdocs: Jianping Sun, Terry Shock, Trevor Starr

Research Technician: Spencer Diamond

Nanostructured Polymer Membranes for Selective Alcohol Transport

Principal Investigator: Nitash Balsara, Lawrence Berkeley National Laboratory

Postdocs: Ashish Jha, Leung Chen

Biofuels Production:

Program

Microbial Characterization Facility: Engineering Microbes for Fuel and Hydrolysate Tolerance

Principal Investigator: Adam Arkin, Lawrence Berkeley National Laboratory and UC Berkeley

Co-PIs: Terry Hazen, Inna Dubchak

Researchers: Adam Deutschbauer, Jeffrey Skerker, John Prausnitz, Michael Samoilov

Postdocs: Cindy Wu, Anna Gerasimova, Sharon Aviran

Student Research Associates: Jordan Mar, Kelly Wetmore

Faculty Collaborator: Doug Clark

Visiting Scholar: Chris Roberge

Administration: Gwyneth Terry

Projects

Bioengineering and Selection for Biodiesel Production in Bacteria

Principal Investigator: Athanasios Lykidis, Lawrence Berkeley National Laboratory (DOE Joint Genome Institute)

Co-PI: Nikos Kyrpides

Researchers: Maria Pilar Francino, Maria Billini, Parwez Nawabi

Improvement of Xylose Fermentation by Recombinant Saccharomyces cerevisiae Through Systematic and Combinatorial Approaches

Principal Investigator: Yong-Su Jin, U. of Illinois

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Engineering a Yeast Strain that Efficiently Utilizes C5/C6 Sugars

Principal Investigator: Huimin Zhao, U. of Illinois

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Robustness to Environmental Heterogeneity: Engineering Strains Optimized for Large-Scale Fermentation

Principal Investigator: Christopher Rao, U. of Illinois

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Characterization and Optimization of Efflux Pumps for Biofuel Secretion

Principal Investigator: Danielle Tullman-Ercek, UC Berkeley

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Environmental, Social, and Economic Dimensions of Biofuels:

Programs

Life-Cycle Environmental and Economic Decision-Making for Alternative Biofuels

Principal Investigator: Arpad Horvath, UC Berkeley

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Economics of Biofuel Adoption and Impacts

Principal Investigator: David Zilberman, UC Berkeley

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Environmental Impact and Sustainability of Feedstock Production Program

Principal Investigator: Evan DeLucia, U. of Illinois

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Undergraduate Students: Anthony Duong, Samirah Ali, Keara Fanning, Michael Bolouri, Morgan Davis, Robert Orpet, Micah Sweeney, Christopher Novotney, Andrew Groll, Allen Lawrence, Alan Yanahan, Jaclyn Marganski, Gevan Behnke, Heather Grames, Matthew Rundquist, Lauren Segal, James Lee, Owen Cofie, Robert Lane

Faculty Collaborators: Arthur Zangerl, Robert Darmody

Subcontractor: William Parton

Biofuel Law and Regulation Program

Principal Investigator: Jay P. Kesan, U. of Illinois

Co-PI: Bryan Endres

Postdoc: Atsushi Oyama

Research Associate: Jody Endres

Graduate Students: Molly Novy, Christopher Miller, Dallas Bartz, Lucas Holl, Timothy Slating

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Economic and Environmental Impacts of Biofuels: Implications for Land Use and Policy

Principal Investigator: Madhu Khanna, U. of Illinois

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Postdocs: Seungmo Kang, Miaoling Liang

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Researchers: Ashley LaVela, Andre Mbassa

Programmer: Matthew Erickson

Projects

Development of Biofuel Productivity Potentials for Economic Analysis Under Changing Climate, Land Use, and Societal Demands

Principal Investigator: Norman L. Miller, Lawrence Berkeley National Laboratory

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Postdoc: Alan DiVittorio

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Undergraduate Student: Jonah Lipsitt

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Explaining, Contextualizing, and Comparing Bioenergy Life Cycles in Brazil

Principal Investigator: Richard Norgaard, UC Berkeley

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Graduate Students: Avery Cohn, Abigail Martin, Marcelo Bessa, Andrew Miccolis, Marcia Ribeiro

A Realistic Technology and Engineering Assessment of Algae Biofuel Production

Principal Investigator: Nigel W.T. Quinn, Lawrence Berkeley National Laboratory

Co-PIs: Tryg J. Lundquist, John R. Benemann, Gary Anderson, Robert Dibble

Research Associates: Ian Woertz, Mike Podevin

Researcher: Yvette Piceno

Intellectual Property Protection and Contractual Relations for Biofuels Innovations

Principal Investigator: Brian Wright, UC Berkeley

Researchers: Kyle Jensen, UC Davis, and Gregory Graff, Colorado State University

Research Specialist: Josephine Mutugu

Postdoc: Zhen Lei

Graduate Students: Kryiakos Drivas, Di Zeng, Gina Waterfield

Assistants: Astrid Sky, Wendy Kan

Consultant: Carol Nottenburg, Cougar Patent Law

Food Security Management in an Era of Biofuels

Principal Investigator: Brian Wright, UC Berkeley

Visiting Scholar: Carlo Cafiero

Graduate Students: Di Zeng, Fei Han, Kryiakos Drivas

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Regional Environmental Impacts of Biofuel Feedstock Production—Scaling Biogeochemical Cycles in Space and Time

Principal Investigator: Carl J. Bernacchi, U. of Illinois

Co-PI: Tracy E. Twine, U. of Minnesota

Graduate Student: Andy VanLoocke

Undergraduate Student: Christina Burke

Regional Socioeconomic and Environmental Impacts of Alternative Biofuel Pathways

Principal Investigator: John Braden, U. of Illinois

Co-PIs: Madhu Khanna, Tom Theis

Graduate Student: Xiaolin Ren

The Impact of Global Trade in Biofuels on Water Scarcity and Food Security in the World

Principal Investigator: Ximing Cai, U. of Illinois

Graduate Students: Dingbao Wang, Xiao Zhang

Interactions Between Bioenergy, Carbon Allowances, and Water Quality BMPs

Principal Investigator: Ximing Cai, U. of Illinois

Co-PIs: Wayland Eheart, John Braden, George Czapar

Graduate Students: Tze Ling Ng, Aras Zygas

Collaborator: Jodine Tate

Market Context for Biofuels Microeconomics

Principal Investigator: Hadi Esfahani, U. of Illinois

Co-PI: John Vazquez

Faculty Collaborator: Clifford Singer

Graduate Students: Esra Ergul, Thorin Wright

Biofuels Research Initiatives and Extension: Synergizing Engagement with Stakeholders

Principal Investigator: Anne Heinze Silvis, U. of Illinois

Co-PIs: German Bollero, Maria Villamil

Graduate Student: Myles Alexander

Research Technicians: Beatriz E. Mira Rada, Ben Mueller

From a Global Oil Economy to a Global Biofuel Economy

Principal Investigator: Steven Weber, UC Berkeley

Graduate Students: Regine Spector, Bob Tammadon, Alan Dafoe, Amy Gurowitz

Assistant: Katherine Saad

Indirect Land Use Implications of Biofuels Programs

Principal Investigator: Michael O'Hare, UC Berkeley

Co-PI: Thomas Hertel, Purdue University

Graduate Student: Alla Golub

Soy Biodiesel Indirect Land Use Estimates for the California Air Resources Board

Principal Investigator: David Roland-Holst, UC Berkeley

Graduate Student: Frederich Karhrl

Microbially Enhanced Hydrocarbon Recovery (MEHR)

Programs

Microbial Enhanced Hydrocarbon Recovery (MEHR) Systems Biology Program

Principal Investigators: Terry C. Hazen, Lawrence Berkeley National Laboratory, John Coates, UC Berkeley, Bruce Fouke, U. of Illinois

Co-PI: Mary Firestone

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Graduate Student: Ana Cervantes

Postdocs: Kathy Byrne-Bailey, Saumyadityar Bose

Web Administrators: Sam Wright, Sherry Seybold

Assistant: Theresa Pollard

Consultant: Joseph Suflita, U. of Oklahoma

Distribution & Diversity of Metabolic Processes in Subsurface Microbial Communities Integrated with Reservoir Environmental Conditions and Geological History: A Universal Template for MEHR

Principal Investigator: Bruce Fouke, U. of Illinois

Co-PIs: Wen-Tso Liu, Nathan Price, Robert DeVille

Postdoc: Yiran Dong

Graduate Students: Philip Miller, Carly Hill

Researchers: Julia Waldsmith, Samantha Dwyer, Mike Nolte

Project

Geophysical Characterization of Microbial Activity in Reservoir Rocks

Principal Investigator: Jonathan Ajo-Franklin, Lawrence Berkeley National Laboratory

Co-PI: Seiji Nakagawa

Postdoc: Tae-Hyuk Kwon

Machinist: Steven Ferreira

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A GLOSSARY OF BIOENERGY TERMS

Biocatalysis
The stimulation of a chemical reaction by a biochemical agent such as an enzyme.

Biodiesel
A fuel that is chemically compatible with diesel refined from petroleum but is derived from biological sources such as rapeseed, soybean, and even waste oils, grease, and tallow.

Biofuel
A fuel made from biological matter. The term usually refers to liquid fuels rather than materials such as wood.

Biomass
Living or recently living biological materials that can be used as fuel; usually refers to plant matter.

Bioprospecting
The process of searching for and extracting previously unknown compounds and plant-derived chemicals in organisms.

Carbon Neutral
A product or process that does not add more carbon dioxide (CO_2) to the atmosphere over its life cycle. For instance, a plant consumes CO_2 when it grows, then releases it back out when it is transformed into and used as fuel.

Carbon Sequestration
The capture and long-term storage of CO_2 before it is emitted into the atmosphere, such as a system that separates CO_2 out of coal-fuel emissions and pumps it deep underground.

Cellulases
A class of enzymes produced chiefly by fungi and bacteria that catalyze the hydrolysis, or breakdown, of cellulose.

Cellulose
All higher plant cells are enclosed in cell walls composed primarily of polysaccharides (polymers of sugar) and lignin (a polymer of phenolics). The principal cell wall polysaccharide is cellulose, a fibrous material composed of hydrogen-bonded chains of the sugar glucose.

Cellulosic Biomass
The fibrous, woody, and generally inedible portions of plants that make up 75 percent or more of the plant material.

Cellulosomes
Complexes of enzymes created by bacteria, but functioning outside the cell. They assist in digestion or degradation of molecules such as cellulose.

Deoxygenation
The removal of dissolved oxygen from a substance.

Depolymerization
Breaking down molecules into constituent parts. In biofuel feedstock, using the tools and techniques of biology and chemistry to convert polysaccharides like cellulose and lignocellulose into monosaccharides, or sugars, which then can be fermented into a fuel.

Energy Bioscience
The field of scientific study that seeks to discover ways to adapt knowledge of biological processes and materials to the development of improved technologies for energy production.

Ethanol
One of the most common types of biofuels, a liquid commonly produced by fermentation of sugar; also known as grain alcohol.

Feedstock
A raw material from nature that is in an unprocessed or minimally processed state and can be acted upon or used by organisms to create a product. Biofuel feedstock include grasses, wood, corn, sugarcane, sorghum, and other plant sources.

Greenhouse Gases
The gaseous constituents (water vapor, carbon dioxide, methane, nitrous oxide, and ozone, among others) of the atmosphere, both natural and human-made, that absorb and emit radiation within the spectrum of thermal infrared radiation emitted by the Earth's surface, the atmosphere itself, and clouds. While greenhouse gases are essential to maintaining the temperature of the Earth, an excess of greenhouse gases can raise its temperature, thus causing climate change and the resultant impacts on land and environment.

Hemicellulose
Heteropolymers (having many different sugar monomers, that may include xylose, glucose, arabinose, and others) present in plant cell walls along with cellulose. While cellulose is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure and is easily hydrolyzed by enzymes.

High-Throughput Genome Sequencing
Various technologies that are employed to lower the cost of gene library sequencing, allowing the large-scale production of thousands or millions of sequences at one time. DNA sequencing encompasses biochemical methods for determining the genetic "blueprint" of organisms by defining the order of their nucleotide bases.

Intellectual Property
So-called "creations of the mind"— in the case of the EBI, inventions and new technologies—around which various rights are provided for the inventor. Legal protections give the patent holder the right to control reproduction or adaptation of such creations for a certain period of time.

Life Cycle Analysis
The investigation and valuation of the environmental impacts of a given product or service caused or necessitated by its existence. The term "life cycle" refers to the notion that a fair, holistic assessment of, for example, biofuel development, requires the assessment of raw material production, manufacture, distribution, use , and disposal including all intervening transportation steps necessary or caused by the biofuel's existence.

Lignin
A compound that accounts for roughly 25 percent of the plant material that provides rigidity, and together with cellulose and hemicellulose forms the cell walls and the glue that binds them; an excellent fuel for providing heat, steam, and electricity.

Microbially Enhanced Hydrocarbon Recovery (MEHR)
Also called MEOR (oil recovery), using microbes to enhance recovery of fossil fuels.

Miscanthus

A genus of about 15 species of perennial grasses whose productivity, biomass yield, and growth capability on marginal lands makes it a prime candidate for development as a biofuel feedstock. The most widely used "species" is *Miscanthus x giganteus*, often referred to as "Miscanthus," which is a sterile hybrid of *Miscanthus sinensis* and *M. saccharifolius*. These species can be found throughout East Asia, from Papua New Guinea to southern Siberia, and through Japan and Taiwan. Because of their stature and silver flowering heads, all three are popular garden plants found in gardens throughout North America and Europe.

Net Energy

A fuel's energy, minus what is required to produce or obtain it. For instance, the net energy of gasoline is reduced by the energy lost in extracting oil from the earth, refining it, and transporting it to consumers.

Polysaccharides

Complex carbohydrates, such as starch and cellulose, consisting of a number of monosaccharides (sugars) joined together in long chains of molecules.

Second Generation Biofuels

First generation biofuels, like bioethanol, are produced by fermenting plant-derived sugars in a process similar to that used in making beer and wine. This requires the use of food crops like sugar cane and corn. Second generation biofuels, like cellulosic ethanol, are those produced sustainably with biomass comprised of the residual non-food parts of current crops and others, like grasses, grown for non-food purposes.

Synthetic Biology

The design and construction of new biological entities such as enzymes, genetic circuits, and cells, or the redesign of existing biological systems; builds upon advances in molecular cell and system biology.

Switchgrass

A warm season grass (scientific name *Panicum virgatum*) that is one of the dominant species of the central North American tallgrass prairie. Properties that make it a strong candidate for biofuel production include survival in drought conditions, perennial habit, and low nitrogen requirement when harvested in the fall.

Sustainability

The capacity to maintain a certain process or state indefinitely. To be sustainable, nature's resources must only be used at a rate at which they can be replenished. A sustainable biofuel feedstock is one that requires little energy input and produces few byproducts that impact the environment.

Xylose

A sugar with five carbon atoms ("C5") in each molecule, found in the cell walls of plants. Other sugars commonly found there include arabinose, mannose, galactose, fucose, rhamnose, galacturonic acid, and glucose, among others.

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