Summary Proceedings

The Second World Congress on Industrial Biotechnology and Bioprocessing

Linking Biotechnology, Chemistry and Agriculture to Create New Value Chains

Orlando, FL, April 20–22, 2005

Bio BIOTECHNOLOGY INDUSTRY ORGANIZATION

ACS

NATIONAL AGRICULTURAL BIOTECHNOLOGY COUNCIL

SOCIETY for BIOLOGICAL ENGINEERING
An AIChE Technological Community

EuropaBio
The European Association for Bioindustries

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Foreword

The second World Congress on Industrial Biotechnology and Bioprocessing convened in Lake Buena Vista, near Orlando, Florida, April 20–22, 2005, sponsored by the Biotechnology Industry Organization (BIO), the American Chemical Society (ACS), the National Agricultural Biotechnology Council (NABC), EuropaBio and the Society for Biological Engineering (SBE). Some 150 presentations were made in five plenary and five parallel “break-out” sessions, and more than sixty presentations were made as posters. There were 700 attendees—an increase of 50% over the first World Congress in 2004—from whom very positive feedback indicated that the meeting was an outstanding success. The third World Congress is scheduled for July 12–14, 2006, in Toronto, Canada.

The chief organizers were Brent Erickson (Executive Vice President, Industrial and Environmental Biotechnology Section, BIO), Peter Kelly (Manager, Industry Member Programs, ACS), Ralph Hardy (President, NABC), June Wispelwey (Executive Director, SBE) and Dirk Carrez (Public Policy Director, EuropaBio) thank the World Congress Program Committee for their hard word and dedication in screening submissions, locating speakers and in organizing a very dynamic program. Members of the committee were Aristos Aristidou (NatureWorks), David Bransby (Auburn University), Bruce Dale (Michigan State University), Clifford Detz (Codexis), Larry Drumm (Bio-Technology LLC), Roopa Ghirnikar (Genencor International), David Glassner (Cargill-Dow), Susan Hennessey (DuPont), Kevin Kephart (South Dakota State University), Mahmoud Mahmoudian (Eastman Chemical Company), Blaine Metting (Pacific Northwest National Laboratory), Marc Sampson (Bunge), Garrett Screws (Novozymes), Tony Shelton (Cornell University), Brian Seiler (Eastman Chemical), Larry Walker (Cornell University), Todd Werpy (Pacific Northwest National Laboratory) and Paul Zorner (Diversa). Also, special thanks go to John Benemann (Benemann Associates), Chris Deane (Organization for Economic Cooperation and Development), Jim Hetenhaus (CEA), Jack Huttner (Genencor International), Jeff Lievense (Tate & Lyle), William Seaman (Sea Grant Florida) and Michael Wang (Argonne National Laboratory).

The superior organizational skills and tireless efforts of Lauren Lamoureux (BIO) were essential to the success of the meeting.

Special thanks are due the US Department of Energy’s Genomes to Life program and the DOE’s Office of Energy Efficiency and Renewable Energy for their generous sponsorship.

This publication results largely from the excellent work of Erin Krause, Colleen McGrath, Scott Pryor and Aaron Saathoff (all of Cornell University) as recorders of the breakout sessions, and of Susanne Lipari (NABC) as layout designer.

Allan Eaglesham
Executive Director, NABC
Summary Proceedings Editor

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Welcoming Remarks

Jim Greenwood
President, Biotechnology Industry Organization

When modern biotechnology was born in 1973, with DNA transfer between microorganisms, no one could have imagined how things would progress over the ensuing thirty-two years. The growth of the World Conference in just a year—a 50% increase in attendance—is nothing short of phenomenal. Clearly, industrial biotechnology is the third wave in biotech.

Industrial biotechnology promises to deliver not only a bio-based economy, but solutions to the key environmental and energy challenges that we face in the twenty-first century. This is partially why Greenwood left Congress to join BIO. Initially he declined the offer, but after discussions with Carl Feldbaum, the outgoing president, he came to the conclusion that biotechnology is the single most transformational human endeavor in history. In terms of health care, food and agriculture and the industrial sector, we are changing the world at an incredible pace.

In the eleven years of BIO's existence, it has grown along with the biotech industry. Greenwood sees his role as taking BIO to the next level, to become a world-class advocacy organization.

"Advocacy" in Washington, DC, conjures up a picture of swarms of lobbyists within the Capitol, stalking and buttonholing their Congressional representatives. However, advocacy goes well beyond that. All of the participants in the World Congress are advocates for industrial biotechnology in one way or another, whether inside companies, in local communities or NGOs, in international organizations or even as government policymakers. We will need to act as advocates more as time goes by, in telling the amazing story of how biotechnology is improving people's lives globally, and what steps are needed to foster even more progress.

BIO represents the whole spectrum of biotechnology as it changes lives for the better around the globe, including health applications, agriculture and food, genetically modified organisms, and the industrial and environmental sectors. As sectors merge, new sectors emerge. Technology is the force that binds BIO.

When Greenwood announced that he would leave Congress, colleagues asked why and when they heard the word "biotechnology" their response was usually, "Ah, stem cells!" Members of Congress do not know what biotechnology is. Therefore, one of Greenwood's initial objectives is to change that. A ten-minute video is in preparation to tell the biotech story in dramatic fashion. When BIO's lobbyists are on the Hill, they will use DVD players to get their points across at the press of a button.

Leaders in the biotech industry are interested in business development. People in biotech share a passion to improve things—in human health, crops and the food supply, energy production, or in the broad spectrum of manufacturing processes—and to invent new, renewable consumer goods.

BIO's objective is to educate the general public about the benefits from all types of biotechnology and to help build and maintain public acceptance where there is lack of familiarity or where acceptance is weak. Investors and Wall Street analysts also need to be educated. To bring about positive change, companies must have access to capital.

In telling the biotech story, we must help bring about change in a responsible manner; we must be intellectually honest. Every technology has advantages and disadvantages. It is not enough to be a booster for biotech, we must candidly discuss risks as well as benefits. Collectively, we should help identify and frame the future that we want rather than avoid the future we do not want.

Greenwood intends to avoid the position in which the pharmaceutical industry has found itself: not anticipating the crises that would confront it, then fighting rear-guard actions. BIO will anticipate the policy issues and problems that the biotech industry will create, as well as the challenges and opportunities, and then be at the forefront of each of the issues, employing experts to develop policies and solutions that will be brought—ahead of time—to policymakers in countries around the world. Thus, they intend to provide intellectual leadership in developing policies to go side-by-side with the technology.

Enormous growth in the industrial biotechnology sector has been predicted for the next ten years. But there are challenges that we must all help overcome, and relatively soon. If commercial activity and business development are to be increased over the coming decade then more companies in non-biotech sectors must waken up to the new biotech tools that are available to them. A technology gap exists: a host of new enzymes is being developed for the manufacturing sector. Some visionary companies are capitalizing on these developments, yet others—in the pulp and paper, textile, and pharmaceutical sectors, and even in the energy sector—are either skeptical or are unaware of powerful new biotech options that could transform their products and make them more competitive. BIO intends to help address this technology gap, to educate those sectors, to act as human catalysts in bringing people together to learn from each other and to build scientific and commercial partnerships.

Another obstacle is how companies deal with financial risk. It takes hundreds of millions of dollars to build an ethanol biorefinery and the federal government must help companies reduce that risk. Such support must be coupled with wholehearted embracement of innovation by large companies. At some point, calculated business risks will be needed in moving forcefully towards a bio-based economy.

Industrial biotechnology involves numerous federal agencies—the Departments of Agriculture, Commerce, Defence, Energy, and the Environmental Protection Agency—all of which are covered by BIO. Some federal agencies are more familiar than others with industrial biotech. Last year, BIO released the report, *New Biotech Tools for a Cleaner Environment*. Many at EPA had heard little of biotech as a tool for pollution prevention. After release of the report, EPA contacted BIO and a memorandum of understanding is now being drafted for future collaboration.

Global climate change is much in the news. The use of industrial biotechnology to produce renewable energy and chemicals from agricultural feedstocks can be and will be a huge contributor to efforts to reduce greenhouse-gas emissions. Part of BIO's ongoing advocacy is to convince national policymakers that industrial biotechn must be part of any climate-change strategy. Climate change will stimulate investment in industrial biotechnology; it is only a matter of time before EPA, Congress, or the states require reductions in CO_2 emissions. It is likely that greenhouse-gas-emissions trading and marketable emissions credits will evolve, as with acid-rain pollution under the Clean Air Act. Carbon dioxide controls will positively affect the whole equation for renewable agricultural feedstocks.

Overall, industrial biotechnology has a great story to tell, which Greenwood will make a centerpiece of BIO's message: the cleaner, greener manufacturing process at reduced cost is a clear winner.

The next day, April 25, 2005, marked the thirty-fifth anniversary of Earth Day, a time to reflect on contributions to preserve our world by developing biotechnologies that help save energy, reduce toxic chemical pollution and replace fossil fuels with renewable feedstocks. As the global population increases, challenges to the environment become ever more acute. How appropriate that the World Congress was in full swing on that important day, with topics including producing renewable energy, increasing sustainability, preventing pollution, and use of agricultural feedstocks.

In the early decades of the twentieth century, George Washington Carver developed hundreds of uses for peanut, soybean and other crops. The concepts of sustainability that Carver helped conceive are being advanced anew. Greenwood announced that BIO and the BIO Development Association are creating the *George Washington Carver Award and Prize* for innovation in industrial biotechnology. This annual award will recognize significant contributions toward the advancement of a biobased economy, including industrial biotechnology, biological engineering, environmental science, economics and education. The award will emphasize the important goal of using biotech innovation in the development of sustainable value chains. By honoring those who have worked successfully toward this goal, this award will serve as a lasting memorial to the original vision of George Washington Carver, who, over a century ago, pioneered the creation and commercialization of products, materials and energy derived from renewable agricultural feedstocks. The first recipient will be named at the Third World Congress on Industrial Biotechnology and Bioprocessing.

Plenary Sessions

Plenary Session I

Paul Roberts (Author of *The End of Oil*)—*The End of Oil: On the Edge of a Perilous New World*
Thomas J. Vilsack (Governor of Iowa)—*Iowa's Vision for Integrating Biotechnology and Agriculture for a Stronger Economic Future*

Paul Roberts opened with the observation that it's an interesting time to be talking about energy. So interesting in fact, that even the Pentagon is talking about it; recently, intelligence analysts there called a meeting with representatives with other departments of government to discuss energy-economy trends in relation to national security. Their best-case scenario was a breakthrough in a fuel technology that will permit movement, smoothly and cost-effectively to an energy source that is cleaner and more efficient, and with less reliance on the Middle East. The worst case: even before initiation of the search for this new technology, a Middle-Eastern government would be toppled by fundamentalists who would cut off oil exports. Concomitantly, oil prices would rise and depress the global economy. It was suggested that the per-barrel price that would push the economy "into the basement" would be above \$50—which has prevailed for more than a year. Therefore, the energy economy is now more resilient than it was in the 1970s.

We are witnessing the end of a century and a half of cheap energy. Economies based on cheap energy are under strain, and as a result we are beginning to see a transformation in how we produce and use energy. At events like the World Congress, people are asking:

- What will the energy economy look like in 20 years?
- What will the fuels and technologies be?
- How much will energy cost and what will the impact be?

They want to know whether the transformation from fossil fuel will be smooth or chaotic. Will the transformation be managed proactively or will the *status quo* be held onto until the last minute? Energy has been central to economic growth and daily life for the past century and will become increasingly critical in the future as demands increase and fossil-fuel supplies diminish. Yet, these issues have been largely absent from the debate on energy policy at the national level. The US energy policy has changed little over the past 30 years—it has been reactive, not proactive, with only lip-service paid to alternative sources and new thinking. Despite vague promises from Washington about a future hydrogen economy, the US continues to emphasize fossil-based sources even as the oil economy shows signs of wear and tear. Why have policymakers failed to grapple with these issues? Why is there no national conversation about what we will do after oil? One reason is, many US policymakers genuinely believe that the *status quo* is fine, and another is that many policymakers—and Americans in general—see alternative energy as a joke. This allows us to talk about alternatives, like hydrogen, without setting specific deadlines.

The question we have to ask ourselves is: "What can we do about this?" A few years ago, when the White House was launching a new energy strategy, Vice-President Cheney stated that alternative energy may become significant in time, but the US is not in a position to stake its economy—its way of life—on that possibility. He stated that fossil fuels will, for years, continue to supply essentially 100% of transportation needs. Mr. Cheney is not a simple man; he knows a lot about energy, and a lot about oil, and he is right to note the inertia—political, economic and cultural—of the system. However, inertia cannot be the basis of a sound national policy, especially on energy. Because change is difficult, we need to talk about it at every level, every day. And the argument we need to make is straightforward: the oil-based *status quo* is not sustainable.

Conventional wisdom is that today's high prices are temporary—resulting from problems in Venezuela, Nigeria, Iraq, Russia, *etc.*—and will soon return to approximately \$30/barrel. Although reassuring, this scenario is getting harder and harder to square with reality. The chief exporters of oil are all politically unstable. In fact, a case can be made that oil revenues foster political instability. Saudi Arabia is the prime example. It is simultaneously the world's largest exporter of oil and the biggest reservoir of barely suppressed population: a powder keg. This should serve as a reminder of how vulnerable is our oil system, and of the risk involved in maintaining the *status quo* rather than transforming it.

Geological facts are as important as political factors. Although oil reserves are vast, maintaining production is problematic. Eighty-one million barrels of oil are consumed per day; that's a billion barrels every 2 weeks and the equivalent of North Sea reserves every 18 months. It's beginning to strain the system. Production peaks are being reached in various places, such as the North Sea. (The US production peak was reached in the 1970s.) Many smaller-producer countries are finding it difficult to maintain production far less increase it to keep pace with current demands. Despite high prices, the United States is consuming more gasoline than ever. And demand for gasoline is increasing in developing countries. China is mobilizing to obtain a greater share of current production and will—eventually—catch up with the United States in consumption.

Again: the oil-based economy is not sustainable.

What are our options? Examination of official US policy forces the conclusion that there are none. Policymakers are so cynical and dismissive of alternative energy sources that the alternatives that *are* discussed are, in fact, non-options. They like to talk about the petroleum reserves that exist in tarsands, ignoring the energy needed for extraction and refinement and concomitant releases of CO_2 Hydrogen is another example. The potential is there, but many issues will need to be addressed: storage, transportation, pollution from compressors, *etc.* Tarsands and hydrogen serve to distract us from real solutions using existing technologies, such as fuel efficiency (including hybrids) and biofuels. In the not-too-distant future, 25% of transportation energy could come from biofuels. Furthermore, there are national-security reasons for weaning ourselves from foreign oil. That this will not happen soon is no reason for doing nothing. Biofuels also present the potential for keeping energy dollars here at home for job creation, rather than spending them overseas.

A biofuel industry will not develop by itself. Like all emerging technologies, funding will be needed for R&D and to build awareness among consumers. The energy industry is unsure what to make of biofuels; in fact there is fear of biofuels. The environmental community, which should be excited about biofuels, instead is more concerned about genetically engineered crops. Farmers, researchers, engineers and politicians—currently disparate—must work together.

There are promising signs. Some energy companies are treating biofuels not just as part of their PR but as for-profit ventures. Some environmental groups are beginning to look favorably on biofuels. Some state legislatures are making commitments in funding. There is no room for complacency, but this is a good beginning. Perhaps the most positive sign is the attendance at this meeting, double what it was for the first World Conference.

Thomas Vilsack recently read *Innovate America,* a report by the Council on Competitiveness on the US economy that suggests that America needs a wake-up call. *Innovate America* opens with the following statement:

The legacy America bequeaths to its children will depend on the creativity and commitment of our nation to lead a new era of prosperity at home and abroad.

America's challenge is to unleash its innovation capacity to drive productivity, standard of living and leadership in global markets. At a time when macro-economic forces and financial constraints make innovation-driven growth a more urgent imperative than ever before, American businesses, government, workers and universities face an unprecedented acceleration of global change, relentless pressure for short-term results, and fierce competition from countries that seek an innovation-driven future for themselves.

For the past 25 years, we have optimized our organizations for efficiency and quality. Over the next quarter century, we must optimize our entire society for innovation.

The bioeconomy, biotechnology and bioprocessing are key components of a strategy for the United States and for the state of Iowa to lead the world into a new era of prosperity. Innovation is the key to creating an economic system that replaces fossil fuels with renewable resources—from agriculture and forestry—for the production of energy, chemicals and materials. There are three fundamental reasons why this is important and why it must begin now:

- Security. The United States consumes 25% of the world's oil supply yet has only 3% of the reserves. This constitutes a serious threat to national security if the United States continues to rely on resources outside of its control.
- Environmental. The issues of waste, smog and acid rain can be addressed by the adoption of a bioeconomy.
- Economic. Adding value to surplus crops and converting commodities into ingredients for specific purposes will provide opportunities to bring prosperity back to rural communities. Development of processing applications in locations where raw materials are produced can bring prosperity back to areas of the country that have long been economically depressed.

A community of about a thousand people in Eddyville, south-central Iowa, that has seen depressed land values and wages and economic and social stresses, reached out to Cargill with a request to make an investment in the production of ethanol. Cargill responded to the challenge with $700 million. Other international companies have linked with Cargill in this endeavor—using each other's processes and waste products to market a range of items, including food preservatives and enzymes—with a further outlay of $300 million. This billion-dollar investment in new construction has provided hundreds of good-paying jobs. A local community college entered the relationship; a technology school and laboratory were constructed on its campus to educate future workers. A new four-lane highway around Eddyville was needed to handle the extra traffic.

This is the future of Iowa and the future of the United States, and arguably the future of the developing world if it embraces the bioeconomy.

Iowa is the #1 producer of ethanol in the United States. This occurred as a result of economic incentives for the purchase of ethanol. In Iowa 70% of gasoline is now ethanol-based and they have tripled the number of in-state ethanol-producers. Current annual production stands at 600 million gallons and will be close to 900 million gallons when current plans come to fruition.

They are replicating this in the biodiesel area. The state of Iowa is using biodiesel in some of its vehicles as a promotion, and they seek additional ways to encourage purchase of diesel made from soy and other sources.

This innovation has spurred growth not only in terms of ethanol production, it has added value to corn in the state, benefiting farmers. It is one of the reasons that Iowa ranked second in *per-capita* income growth for 2004.

Three factors will favor Iowa, and the United States, successfully embracing the bioeconomy:

- fertile soil
- efficient farmers
- excellent public universities.

Today in Iowa, crops are being used to produce almost everything that an economy can use—fuel, electricity, materials and chemicals—and in the not-too-distant future they will be used to produce medicines.

Universities must be engaged nationally to help develop the biobased economy. The Plant Science Center at Iowa State University is focused on plant genetics. Progress is being made on producing minor crops able to tolerate environmental stresses. The Biofuel Program is a multidisciplinary effort at Iowa State. The University of Northern Iowa has an ag-based industrial blueprint program, helping the creation of products like biodiesel, and the University of Iowa is accelerating research on bioinformatics.

A fourth necessary component is government partnership to fund development of new companies. Iowa provides venture capital and encourages local communities to establish venture-capital programs focused wholly or in part on embracing the bioeconomy. Entrepreneurial centers are connected to their community colleges and universities to help make ideas marketable, and the Iowa Values Fund provides direct state assistance in business establishment, again with emphasis on the bioeconomy.

Not only has government the ability to provide resources and change its tax policy to encourage growth, but it is also a purchaser of goods and services. Iowa supports the federal government's Biobased Products Preferred Procurement Program and Iowa State University provides information on-line on sources of biobased products. Government can also encourage the formation of alliances. The Biosciences Alliance of Iowa and the BIOWA™ Development Association are designed to promote and market the concept of the bioeconomy, with the goals of developing ten regional biorefineries in Iowa in the next ten years and building at least five new biobusinesses, or major expansions of existing biobusinesses, each year beginning in 2005.

Our country is engaged in a fierce struggle: the war against terrorism. For the most part, our response has been in military terms. But the strength of the United States is not limited to, nor defined by, its military might, but also by its humanitarian endeavors. The bioeconomy has extraordinary potential in the latter regard. Imagine a world in which we literally grow the means with which to address the world's environmental stresses. Imagine how that could help change the face of America to the rest of the world. Imagine taking our science, our research and our ability to grow crops to produce cures for epidemics that ravage countries and continents. Imagine a diversified American economy that reduces reliance on oil, which, in turn, forces oil-producing countries to diversify their economies and increase their employment opportunities.

This is about making the United States more secure, it's about improving the environment for all of us, it's about bringing prosperity to depressed rural communities, and it is about putting a different face on this country, focused on our greatest strength, our humanitarian might.

George Washington Carver said:

I believe the Great Creator has put ores and oil on this earth to give us a breathing spell....As we exhaust them, we must be prepared to fall back on our farms, which are God's true storehouse and can never be exhausted. For we can learn to synthesize materials for every human need from the things that grow.

Plenary Session II *Overcoming Barriers for Biorefinery Commercialization*

Per Falholt (Novozymes)
Larry Russo (Office of the Biomass Program, US Department of Energy)
Thomas Pekich (Genencor)

Per Falholt stated the belief that not only is biotechnology the technology of the future, it is a key to a more stable world. With recent improvements, enzymes can be produced more rapidly than ever. The field has changed significantly in the past two years; many new enzymes are now available as a potential means of providing new solutions to problems. Although DNA sequences can be elucidated quickly, improved understanding is needed in terms of progressing from gene sequence to:

- structure,
- performance via function, and
- pathways, and from pathways to intracellular networks and from intracellular networks to cell-to-cell communication and how cells function in populations.

With continued R&D, new products will be tailor-made, including exploitation of synthetic organisms. With higher yields, economics will be improved and products will be affordable to all, benefiting developing and developed countries alike.

Products will be developed at an increasingly faster pace with financial savings, since time is money, using safe and sustainable technologies with benefits for the environment.

Novozymes' BioEnergy Project, sponsored by DOE since 2001—*Cellulase Cost Reduction for Bioethanol*—provides a good example of what is achievable through enzyme technology. In 2000, the cost of enzymes was the chief problem in the commercial production of ethanol from cellulose at >$5/gallon, whereas ethanol from starch cost <$1/gallon. Since then, enzyme production costs have been decreased by reducing feedstock costs, increasing enzyme recovery, and increasing fermentation yields. Cellulase activity has been increased by isolating more thermostable and thermoactive enzymes with higher specific activities; optimization of cellulase mixes has resulted in synergistic benefits.

Work continues in the improvement of cellulase synergism by culling fungi from nature. Isolates are grown on a semi-solid medium under various conditions with cellulose or lignocellulose as the sole carbon source. Vigorous growers are cultured in liquid medium and the broth is assayed for lignocellulose-hydrolyzing activity alone and in conjunction with cellulase from *Trichoderma reesei* to check for synergism.

As a result, by January 2004, Novozymes had achieved a twelve-fold reduction in enzyme cost, and, with improvements in pretreatment achieved at the National Renewable Energy Laboratory (NREL), a twenty-fold cost reduction was achieved by April, 2004. In April, 2005, the enzyme cost was $0.10–0.18/gallon, a thirty-fold reduction. Therefore, enzyme cost is no longer a barrier, although opportunities for improvement remain. Successful commercialization of biomass-based ethanol also depend on:

- establishment of formal collection system for biomass,
- further progress in technical biomass treatment,
- optimization of current yeast organisms, and
- financial incentives in industry for investments in biomass-handling facilities.

Biotechnology provides ever-increasing speed. In the 1990s, to go from gene bank to product at Novozymes took 5 years. In 2000 it took 2 years and in 2005 it took less than 12 months. They believe that they will meet future challenges through biotechnology and, in so doing, will conserve non-renewable resources for future generations.

Larry Russo stated that, about a year ago in the Department of Energy's (DOE) Biomass Program, the question was asked as to why research dollars spent on bioethanol development are not translating to commercialized products. In trying to understand the needs of the financial community, they consulted with people in Wall Street, venture capitalists in various parts of the country, and representatives of engineering and technology-development firms. They discovered a market barrier and a technical barrier.

There is a barrier in convincing the financial community to come up with funding for a new technology that is not broadly understood and which involves regulation by the Environmental Protection Agency and the Bureau of Alcohol, Tobacco and Firearms. As a result of the feedback, the DOE sees opportunities to streamline how they do things. Without rubber-stamping permits, they could provide help in terms of avoiding duplication of effort.

Representatives of companies that had successfully commercialized products said that they can accept technology risk or they can accept a market risk, but they cannot accept both.

What can government do to assist? It can provide incentives to address market risk. If we express the current ethanol tax credit of $0.52/gallon on a per-lb basis and if we make a product that is currently being made from petroleum or another non-renewable resource and apply that as a tax credit, then some products would immediately become commercially viable with potential for developing market share. In the Office of Biomass they cannot *cause* this to happen, but they can help to define such an incentive for a Congressional or White House initiative.

As far as addressing the technology barrier is concerned, DOE can make a contribution via competitive solicitations—which they are good at—to reduce the cost of core R&D, for example. The next phase is to attract interest in industry to show that the concept has technical viability, even if marketability is not yet demonstrated. At that stage, they look at solicitations and at a 20% cost-share with industry in order to prove the concept. Pilot scale is the next stage for examining technical feasibility.

However, responses from the financial world suggest that pilot-scale data are often insufficient to address concerns over technical aspects. Preferred would be a thousand hours of continuous operation or five thousand hours of intermittent, cumulative operation at about 10% of fully operational scale. DOE is planning a large solicitation that will build on recent product and prior-planning solicitations, to assist demonstrations of technologies at a large enough scale.

Having reduced market risk and demonstrated the technology, finding sufficient funding for commercialization may still be difficult. Loan guarantees can kill such programs. There needs to be an exit strategy.

Much of what they do at DOE is policy-driven. There are signs of market-pull to substitute petroleum-based products with those manufactured from renewable resources. But, we must be ready, not only from the technological standpoint, to ensure that nay-sayers will not be proven right.

Thomas Pekich stated that the biorefinery is the basis of the vision of a biobased economy. Carbon from plants is the raw material, converted by enzymes or cells into a multiplicity of organic chemicals. Protein engineering is advancing at lightning speed. Possibilities for new production methods to displace incumbent synthesis of bulk and fine chemicals are constantly increasing. We can now think of biotechnology for delivery of peptides, proteins and other biological molecules for mass-market applications.

Over the past year, the production of corn ethanol has grown significantly. With the recent increased price of crude oil spurred by increased demands from rapidly industrializing societies, it is likely that this trend will continue. In 2004, eighty-one plants in twenty states produced 3.4 billion gallons, an increase of 21% over 2003 and of more than 100% since 2000. In 2004, the corn-ethanol industry processed a record 1.26 billion bushels of corn, equivalent to 11% of the US crop. At the end of 2004, sixteen plants and two major expansions were under construction, representing an extra 750 million gallons of production capacity.

There is room to improve the production of ethanol from corn. At Genencor, they are focused on the introduction of raw-starch processing enzymes to remove the liquifaction and saccharification steps, which require heating and cooling of the starch slurry for conversion to glucose. With new enzyme systems, they are able to use raw starch for simultaneous saccharification and fermentation with significant energy saving. This technology can be added to dry milling as a bridge to biomass ethanol. The reduction in capital will make dry-mill plants even more profitable. The challenge is that the conventional process economics becomes a moving target for biomass ethanol to shoot for.

Over the past four years, Genencor and Novozymes have made strides in converting cellulose to glucose. The hydrolysis step is no longer viewed as the barrier to making biomass ethanol a commercial success. Great strides have been made also in other areas in biomass ethanol development, but those improvements have been made in "silo" fashion. Although further technological advances are needed, a key challenge is to test how well the process steps can be integrated.

It is hard to imagine garnering significant investment in biomass ethanol until venture capitalists see a demonstration plan that integrates the operations to produce ethanol as well as the associated economic projections. This needs to be address sooner rather than later.

Plenary Session III *Growing a Biobased Economy: Vision Is Not Enough*

Iain Ferguson (Tate & Lyle)

The excitement that pervades biotechnology as an industry-coming-of-age is reminiscent of the plant breeding industry in the late 1980s and early '90s.

Tate & Lyle is a renewable-ingredients business, and, by definition, they are a bioproducts company already. They use partnerships and are customer-centric. They want to know what the customer cares about and what the customer's customer cares about. As a global company they sell a service as a product bundled together; they don't just sell ingredients.

Focus on safety underlies their business philosophy, as do cost-control and high efficiency. They strive for leadership in selected areas of technology, and partnerships are central to their delivery to the marketplace.

They have broad innovation capabilities, mainly in development and application, and they partner to provide R&D. The Tate & Lyle venture fund was launched recently as a way of playing a part in sponsoring and helping to build new businesses.

With global sales of US$6 billion, Tate & Lyle does business in three main areas:

- food and industrial ingredients based on starch from corn, wheat and tapioca.
- sugar, the traditional business of Tate & Lyle in seven countries, including being international sugar traders,
- value-added relationships, producing SPLENDA® sucralose sweetener, Sorona® fiber, and Aquasta™ astaxanthin in partnership with McNeil (a division of Johnson & Johnson), DuPont, and Igene, respectively.

An old company—established 150 years ago—with a history of continuous reinvention, it started in sugar then moved into starches and is now moving into fermentation and bioproducts. They operate across the spectrum from basic commodities to advance biomaterials. Bringing together their commodity strength, their innovation ability and their ability to partner is the key organizational challenge that is critical to success in bioprocessing.

Food is still their biggest profit earner, which has been bolstered recently by the success of SPLENDA® sucralose sweetener.

Tate & Lyle has always been big in paper starch, one of the oldest bioproducts, which gives paper its strength and finish, and allows it to be processed.

They have recently accelerated investment in the biopolymer area, not only with DuPont but also in polylactic acid.

Their interest in what is important to the consumer is particularly relevant on the food side of their business. They do basic consumer research on habits and attitudes, and also on behavior—which differentiates Tate & Lyle from other food-ingredients companies. There is no point in making what the customer does not want; they are not about technologies looking for solutions, they are about markets and opportunities looking for technologies to make products. Health and wellness, obesity, diet and nutrition are among today's major drivers for consumers.

Tate & Lyle processes large volumes of agricultural products and they trade in many raw materials, being good at raw-material handling and at global sourcing. With their fermentation expertise, they lead the world in citric acid production, which provides scale and expertise for other fermentation activities. They produce xanthan gum and lactic acid, and are looking at the increasing potential to replace synthetic products with naturally produced analogues. Clearly, expertise in fermentation is not enough; separation and processing technologies are also essential. Strong customer relationships are also important; they deal with ninety of the world's top hundred food companies.

It was DuPont's initiative to find a bioprocessing company to complement their expertise and help the production of 1,3-propanediol that led to the joint venture with Tate & Lyle in the synthesis of Sorona®. A $100 million Sorona® factory is being built jointly in Tennessee; it will begin production in 2006, with strong customer demand from the carpet industry.

The joint venture with Igene for the production of Aquasta™, a natural astaxanthin, is structured differently; Tate & Lyle's production, distribution and sales are involved as well as the production of the glucose feedstock. Igene's role is in microbial strain development.

Ferguson described key elements that are driving ag-based bioproduts:

- Improved agricultural productivity, which is driving down the real cost of ag ingredients.
- High crude oil prices, being driven partially by increased demand in China (which now consumes more grain and steel, produces more refrigerators and has more cellular phone subscribers than the United States).
- Costs of renewable raw materials have been falling for the past decade.
- Consumer acceptance, albeit that attitudes to agbiotech vary from country to country.
- Favorable government policies.
- Technological improvements.

Having a vision of the biobased economy does not ensure that it will come to fruition. Biobusiness systems involve complex interdependencies that can be cemented or broken by government policies. Also, partnerships are key, but all partners must understand fully the assets, skills and resources of all concerned.

Vision into action for building biobusinesses requires:

- understanding distinct drivers (consumer, government, technology, relative input prices, and demographics)
- applying normal business rules
- aligning the entire system, and
- utilizing partnerships.

Plenary Session IV *Challenges and Outlook for the Future*

Bryan Foody (Iogen Corporation)
James Barber (Metabolix, Inc.)
Jay Short (Diversa Corporation)

Bryan Foody described Iogen as the leading developer of cellulose ethanol, a fully renewable, zero-CO_2-emission fuel that can be used in today's cars. Iogen also produces industrial enzymes for companies that process natural fibers, including those in the pulp/paper textiles and animal-feed industries. They have over twenty years experience in the development of cellulose ethanol.

The enzymes they produce for the pulp/paper industry help in the bleaching process. Through genetic and protein engineering, they have significantly reduced the temperature and pH necessary for bleaching in comparison with the chemical process.

Iogen is a full-service supplier, helping customers use enzymes effectively with computerized hardware. It runs a 420 m^3 enzyme-manufacturing (fermentation) facility that operates 24 h/day, 6 days/week, with only one shift manned. The rest of the time, operation is computer-controlled.

Iogen also operates a $40+ million prototype demonstration-scale cellulose ethanol plant that converts wheat straw to sugar and ethanol. It's the final step before scaling up for full commercial production. The first shipment of cellulose ethanol was made in April, 2004. Vehicle trials were run in Europe in June, 2004, in conjunction with Daimler-Chrysler, Volkswagen and Shell. "Mission Green" was a cross-Canada trial—14,400 km—in collaboration with General Motors in August, 2004; a GMC E85 Yukon SUV (285 hp, 5.3 L) running on 85% cellulose ethanol/15% gasoline had a CO_2 -emission equivalent to that of a Toyota Prius hybrid. As of December, 2004, cellulose ethanol fuels the Government of Canada's E85 vehicle fleet. And cellulose ethanol is part of Royal Dutch Shell's ad campaign.

Iogen expects to be in full-scale production of ethanol from wheat straw in 2006.

James Barber reported that Metabolix is using biotechnology to help meet US needs for plastics, fuels and energy using renewable resources. They have two technology platforms:

- microbial fermentation, and
- coproduction of plastics and energy from biomass crops.

The adoption of this technology will bring a range of environmental benefits, from reductions in consumption of fossil carbon and in greenhouse-gas emissions, to reduction in the solid-waste burden.

In the last six months, alliances have been established with Archer Daniels Midland and British Petroleum. The subject of this conference is essentially the creation of a new industry, linking commercial production to our agricultural base. Therefore, there will be alliances and partnerships "at every turn." This is seen already in such partnerships such as DuPont with Tate & Lyle, and Metabolix with ADM and BP. Proficiency in establishing and managing alliances will be a factor critical to success. A key component in establishing partnerships is to fully understand the other's critical needs. The strength of these human relationships will determine partnership success.

To what extent will public policy assist the development of industrial biotech? The US government already supplies funding , to a degree, for new endeavors in biotechnology. However, we are entering a period in which substantial capital investments will be needed. In its initial phase, during the Second World War, the thermoplastics industry was funded almost exclusively by the US government, which served also as the primary customer. Therefore, the asset base and operational capability were there in the late 1940s on which the industry grew commercially.

Education is needed to ensure that the public at large is aware of the benefits—potential and already received—from biotechnology in the industrial sector.

At what stage are the traditional chemical plastics companies? Some are probing and some are dabbling. It remains to be seen to what extent they will embrace the new technologies; there will be winners and losers.

Clearly, adoption of industrial biotechnology is inevitable. The question is not "whether?" but "when?" and "how?" Hydrocarbon cost is a fundamental driver as is the reliance of the United States and other industrial countries on increasingly unstable parts of the world for petroleum.

"An untapped microbial universe is a great source of new enzymes and new pathways for synthesizing molecules," said Jay Short. Less than one tenth of one percent of the microbes in seawater have ever been examined. At Diversa they develop discovery capability to investigate aquatic and terrestrial environments—including high-temperature, low-temperature, acid and alkaline conditions—and they are using evolution technology to optimize processes of interest for product delivery to chemical/industrial, agricultural and pharmaceutical markets.

Diversa was founded in 1994. Partnerships are fundamental to building a biotechnology business, and Diversa has (had) links with Danisco, Novartis/Syngenta, Danisco, Aventis, Dow, DuPont, BASF, *etc*. Revenue grew from $1.3 million for 1998 to $58 million for 2004.

Product areas being targeted are:

- animal care,
- fiber modification, and
- nutritional oils.

These present large markets with opportunities for technology improvement using enzymes.

Luminase™, an enzyme for improved pulp bleaching took only 30 months from discovery to launching the product, including fermentation development and obtaining regulatory approval. Diversa's products include:

- Cottonase™ for processing of cotton fiber,
- Phyzyme™ for poultry and swine diets, to improve phosphorus assimilation,
- Pyrolase™ for non-specific polysaccharide cleavage, *etc.*

Revenues are expected to reach $100 million for 2008.

Plenary Session V *Preparing for the Biobased Economy*

Richard Worzel (Futurist)

A futurist helps people prepare and plan intelligently; a futurist is a planner, not a prophet. It is easy to get caught up in your own specialty and miss other things that are happening.

Worzel summarized progress in biotechnology that may occur over the next two decades, including:

- human proteins synthesized in plants, such as potatoes,
- hoof and mouth vaccines from, *e.g.* alfalfa,
- skin-growth hormones from tobacco,
- human albumin, hemoglobin, interferon, and vaccines for hepatitis-B, cholera and other gastro-intestinal infection from brewer's yeast,
- epidermal growth factor as a treatment for lung cancer,
- monoclonal antibodies to target, asthma, Crone's disease, rheumatoid arthritis and lupus,
- therapeutic vaccines to jump-start the immune system in combating the AIDS pandemic,
- anti-sense products as treatments for cancer and heart disease, and
- gene therapies for cancer and cystic fibrosis.

What may prevent the items above from coming to fruition?

The first problem is the potential for adverse public opinion caused by a new "frankenfood" public-relations disaster. Worse, a careless organization could actually create an ecological disaster, *e.g.* unleashing a bioengineered organism equivalent in destructive power to the introduction of rabbits to Australia or kudzu to the United States. The industry would be well advised to continue devising and policing voluntary controls and safeguards before they are imposed by lawmakers who are ignorant of science but well versed in appeasing public sentiment.

The next danger is political lobbying by well established opponents. The biotech industry is in its infancy with few heavy-hitting allies. A far-sighted, proactive association is needed for education, public relations lobbying in all countries. More than lobbying is needed, however, as demonstrated by what lobbyists did—or didn't do—for the tobacco industry and asbestos manufacturers in the United States. Fortunes were spent in lobbying Congress;

dueling lobbyists are not the answer to all problems. For example, much is made by companies such as Iogen of Canada about its introduction of ecoethanol to replace gasoline in automobiles. Ecoethanol—made from waste cellulose such as straw—has the exciting potential to reduce tail-pipe emissions. An extension to this is the work at the University of Minnesota, taking ethanol and converting it into hydrogen. That hydrogen may be converted to electricity tells a potentially win-win-win story about renewable recources. As promising as this is, imagine it in the hands of spin-doctors anxious to prevent ethanol impinging on gasoline sales, for companies that see themselves as selling oil rather than selling energy, or power utilities that see themselves as natural monopolies. By 2025, this transition will be well over; all the major oil companies will have production facilities devoted to renewable resources. But the transition will be difficult. At least some oil companies will fight a rear-guard action to maximize return on their existing petroleum assets, raising doubts about the environmental safety of ethanol and insisting that it be taxed to the same extent as gasoline in order to be "fair" ignoring the fact that oil production receives major tax write-offs as depletion allowances. And then, think of legislators reacting to job losses in their districts resulting from the switch to ethanol from gasoline, and local power generation away from utilities. Imagine Texas going up against Kansas or Indiana: who is likely to win that fight? Opposition to lower cost production and environmentally sustainable solutions from biotechnology may sound far fetched, but is already occurring. In an October 2004 issue, the *Economist* stated that the foes of genetic modification (GM) are many and they can be unscrupulous in their manipulation of information. GM needs skills and courage in its public relations. It will be best to be proactive. For example, in the issue of job losses, this industry is changing the rules so fast that traditional endeavors will be caught off-guard. Push-back from those traditional industries will result, therefore it is essential to trumpet the benefits of growing new industries. Biowa™ estimates that the ten biorefineries built in Iowa have created more than 22,000 jobs, with an \$11.6 billion/yr economic impact on the state increasing the tax base by \$367 million/yr.

The next threat is lack of access to capital. The financial markets rarely have the expertise to properly assess new technologies. This applies particularly to biotechnology projects, which typically require a PhD to understand. Venture capitalists may follow the enthusiasm of other investors. It's important to speak the financiers' language and understand the expectations of venture capitalists to play their game. Preparation, presentation, and particularly a strong management team are key. And it's important to understand the possible pitfalls of venture investing. Outside of the United States, the chances of finding venture capital drop dramatically. The United States has the broadest and the most sophisticated VC industry in the world (despite disparaging remarks above). In most other countries, government funding or a strategic partner are necessary.

China and India are industrializing quickly. Both have large populations and well educated researchers, and both are determined to reap as many manufacturing jobs and as many high-value R&D jobs as possible. Both are taking aim at genetics, and industrial biotech applications will not be far behind. Governments in both countries are less likely to introduce legislation blocking research in genetic or biotech areas than governments of North America or Europe. Accordingly, they are going to be world-class competitors. Worzel offered the cautionary note of being fully briefed in intellectual property laws in developing countries, especially China. The Chinese government has shown a hit-or-miss respect for intellectual property.

The greatest danger—and biggest opportunity—to this industry is information overload. Whoever figures out how to process information most effectively will win. The quantity of relevant data is growing in interrelated fields, such that data are accumulating dramatically faster than today's methods can manage. Not only is the rate at which we are drowning in information accelerating, the rate of acceleration is increasing. Not only is industrial biotechnology based on effective use of complex biological processes, it takes advantage of natural efficiencies that have been debugged by millions of years of evolution. It's flexible and has built-in feedback control. But, it is complex and requires insight, creativity and persistence to harness. Computing resources are cheap and broad-bandwidth communications cover the world. As a result, communications are now beginning to move at computer speeds rather than at *in-vitro* speeds. Whoever makes most effective use of these resources will have huge economic and business advantages. They will get to market faster. They will have fewer failures. And they will respond faster to changes in technology and the market place. Living entirely within one's own silo, unaware both of potential threats and of potential opportunities outside is a significant danger needing to be addressed in systematic fashion.

Here are two examples among many of groups devising new ways of dealing with information.

The Calgary automatic virtual environment (CAVE) at the Sun Center for Visual Genomics at the University of Calgary is a virtual-reality room where, when wearing special goggles, it is possible to "walk" through a projection of a molecular model of a new drug, a chemical reaction or the interior of the human heart. Its value is underpinned by the fact that humans evolved to integrate enormous quantities of information visually, which remains the most efficient way of absorbing massive quantities of data inherent in complex biological systems. In the words of Marshall McLuhan: "For the artist, information overload becomes pattern recognition." We are all artists now, and cannot afford to be just scientists.

At Genetics Squared in Ann Arbor, MI, clinical trial data are analyzed to produce diagnostics predicting who will respond to a given therapy or pharmaceutical. Genetic algorithms are used to search a give data space, mimicking evolution to find solutions when other approaches fail, making the solutions accessible to further analysis and development. Genetics Squared has shown that a drug discarded as ineffective by one of the largest multi-national pharmaceutical companies might be rescued if potential patients are screened to determine who will respond positively. And Genetics Squared has worked with data from the University of Southern California to identify specific stages in the progression of bladder cancer by better use of existing data rather than trying to invent better biotests.

A related genetic algorithm has been used outside of the medical field. It was employed first to produce a crop-optimization model for Pioneer Hi-Bred. Then it was used to develop a one-line function that characterized the kinetics of jet-fuel combustion for NASA that was more than 2,700 times faster than the differential equations that had been used previously. And then it was used to develop a dynamic controller for weaving paper products for a major consumer-products company. In each case, the cheaper, faster solutions that genetic algorithms produced were rejected as too easy, although it is the obvious consequence of a superior means of searching the data space. There are two morals to this story:

- Tools invented in one area of industrial biotechnology will probably have applications in many other areas, and tools invented outside of one's industry may be of value.
- Not only does the world beat a path to the door of the inventor of a better mouse trap, they would just as soon beat him to death.

Here is a selection of problems that needs to be tackled and may be best addressed with biotechnology:

- We are rapidly running out of fresh water in the right places. There is need for an inexpensive means of converting contaminated to potable water.
- Land-fills are becoming critically scarce in many countries. There is need to convert solid waste into a valuable resource.
- Greenhouse-gas elimination, especially for countries that signatories of the Kyoto Accord, which are committed to targets that are probably unrealistic.
- A breakthrough in energy storage is needed.
- Diabetes will become a healthcare crisis as a result of aging and fattening of rich-country populations.
- The need to produce food and feed with crops using less fertilizer, pesticide and water with less pollution of groundwater.

Farmers in developing countries will blossom as fierce competitors, producing surpluses in traditional crops. Agriculture in rich countries will need to constantly advance into new areas and adopt new technologies, including biotechnology.

The Dutch futurist Arie de Geus stated: "Learning faster than your competitors is the only sustainable competitive advantage in an environment of rapid change and innovation." If you are learning faster than your competitors, then the future promises to be exciting, interesting and prosperous. If you are not learning faster than your competitors, then you may not be in business in five years time.

At the outset, Worzel stated that no one can predict the future reliably and consistently. However, Alan Kay, a technological visionary disagrees in that he said that the best way to predict the future is to invent it. Industrial biotechnology is going to change the economy, dramatically affect world trade, and will have major consequences for the lives of individuals both in terms of quality and longevity.

Breakout Sessions

Manufacturing and Synthesis

The Cellulose Economy: Adding Value by Applying Industrial Biotechnology

Brian Seiler (Eastman)
Theodore Wegner (US Department of Agriculture)
William Winter (State University of New York, Syracuse)

The cellulose economy is real and growing. Contributions from the "forest biorefinery" are increasingly significant. Opportunities exist to derive substantial value from conventional by-products and/or waste streams as co-products. Biopulping employs enzymes that assist in de-lignification and isolation of hemicelluloses. Such lower-cost pulping contributes to the achievement of dissolving-grade quality, to produce nearly colorless cellulose esters at acceptable cost. Cellulose esters are high-value components of cosmetics, food thickeners, drilling muds, stabilizers and coatings for pharmaceuticals, and thickeners and performance enhancers for paints and coatings. The application of biotechnology to the pulp and paper industry will have environmental benefits. Opportunities exist to improve recycling of cardboard and office paper. And de-inking is improved with cellulase at neutral pH. Pulping may be improved by the presence of enzymes from fungi that degrade wood, as an alternative to delignification with toxic chemicals. The 30-million base-pair genome of the lignocellulose-degrading fungus *Phanerochaet*e *chrysosporium* has been sequenced and annotated. It is envisaged that currently operating mills will have products in addition to pulp—ethanol for example. New wood-processing factories will not produce fiber; the cellulose will be converted to sugars for production of higher value products.

Forests of the United States are overstocked. Fires in such forests are more destructive because higher temperatures prevail; 397 million acres are at risk. Forest management—urgently needed—would produce significant yields of wood essentially free.

Biotechnology has improved fermentation by yeasts, *e.g.* blockage of the main respiration pathway increases ethanol production. Genes for xylose metabolism have been cloned in yeast, thus C-5 and C-6 sugars may be fermented simultaneously. In addition to ethanol, wood sugars have significant potential utility for the production of polyhydroxyalkanoates, polylactic acid, acetic acid, and sulfur-free lignin.

New research is focusing on lignocellulosic fibers with unique multifunctional properties for nanotechnology applications.The United States possesses 2% of global oil reserves and consumes 26% of what is produced—more than the next five main-consumer countries combined. Biobased sources of energy would reduce US reliance on foreign oil. Wood is an attractive candidate as a source of ethanol, from several standpoints, including: abundant supplies, ease of storage, it is renewable, it has a higher yield/acre/yr than crop biomass with lower fertilizer inputs and a relatively high net energy ratio. Genetic engineering offers the potential to make trees more resistant to diseases and insects, with improved yields and chemical composition.

Biocatalysis for the Pharmaceutical Industry

Michael Homann (Schering-Plough Research Institute)
Birgit Kosjek (Merck)
Gjalt Huisman (Codexis)

Timely generation of metabolites of drug candidates is essential for understanding biological activity including toxicity. Traditionally, metabolites have been synthesized chemically, which can be time-consuming. Biological approaches directly produce metabolites, using microbial mimics of human metabolism. Preselected organisms are arrayed in multi-well plates, with significant savings in time and materials in comparison with conventional flask fermentation. Metabolite transformations involving regioselective glucuronidation have been successfully demonstrated with liver microsomes; this approach is particularly useful for synthesis of labeled metabolite standards. Generation of metabolites by biotransformation can be used in conjunction with discovery to expedite drug synthesis and evaluation.

Biocatalytic approaches offer promise for early-stage process development. Greater availability of enzymes that have been studied in detail and which may be purchased in large scale "off the shelf" is making possible the production of compounds of chiral complexity against aggressive timelines. To achieve biocatalytic reduction of dimethoxy-pyranone, nine ketoreductases and five alcohol dehydrogenases were screened; a ketoreductase (KRED-101 from BioCatalytics, Inc.) had high activity and displayed >99% ee *(R).* Cofactor NADPH was recycled by the addition of glucose dehydrogenase (GDH-101, also from BioCatalytics, Inc.).Although enzyme availability is steadily increasing, too few are available for catalyzing reactions of interest and many that are on the market have sub-optimal properties for industrial processing conditions. To improve biocatalytic reduction in the synthesis of hydroxynitrile, an intermediate of atorvastatin (Lipitor™), gene shuffling was used with an enantioselective ketoreductase and an efficient glucose dehydrogenase for co-factor regeneration; many-fold increases in enzyme activity and in stability at 40° and 50°C were achieved. Similarly gene shuffling was used also to improve cyanation steps with a halohydrin dehalogenase resulting in increased rate and improved stability and product tolerance. The reactions are now clean and conducted at neutral pH and mild temperatures. The reduction step gives excellent enantioselectivity, >99.9 % ee. There is no loss of stereochemistry in the cyanation step, which occurs under mild conditions, obviating any need for fractional distillation.

Integrating Microbial Genomics and Chemical Products

Blaine Metting (Pacific Northwest National Laboratory)
Mark Burke (Diversa)
Olga Selifonova (Cargill)
Todd Werpy (Pacific Northwest National Laboratory)

Two hundred microbial genomes have been sequenced. Within species, genome size may vary by as much as 1 Mb, approximately 800 genes. Bacterial-community sequencing is a nascent technology for simultaneous investigation of hundreds to thousands of unculturable species that act in harmony to undertake complex environmental chemistry. This underscores the diversity of biochemistry and opportunity afforded through understanding and manipulating microbial genomes.

Filamentous fungi—nature's recyclers—have hundreds of enzymes for plant-polymer hydrolysis and ability to utilize C-5 and C-6 sugars; they have particular utility for biomass conversion and fermentation. Studies on *Aspergillus niger* and *Phanerochaete chrysosporium* have revealed proteins that are specific to fungal morphologies. A particular morphology may correlate with pathogenicity or overproduction of metabolites *in vitro*, hence understanding the function of unique proteins may have utility in industrial processing. The genomes of filamentous fungi may be used to develop enabling technologies for chemical production in biorefineries.Genomic DNA may be used as a direct source of diverse genes by extraction from soil. Extensive libraries each containing millions of genes—potential sources of functionally diverse enzymes—have been constructed. High-throughput screening techniques (including robotics, flow cytometry and Gigamatrix™ technology) have been developed to screen billions of samples per day to identify promising candidates for specific processes. Directed evolution technologies (*e.g.* Gene Site Saturation Mutagenesis™, GeneReassembly™) are then employed to manipulate the best candidate(s) for optimal performance. This method was used to identify and improve a nitrilase—for a key step in the synthesis of Lipitor®—obtained from the Salak Mountains of Indonesia, in terms of pH tolerance, thermal stability, activity, and enantioselectivity at high substrate concentration.

3-Hydroxypropionic acid, not available in large quantities on the market, is a potentially useful platform chemical from which key compounds—1,3-propanediol, malonic acid, acrylic acid, *etc.*—may be synthesized. 3-HP has low toxicity, low corrosivity and high solubility. Its biosynthesis from glucose would be simplest via pyruvate, *a*-alanine, ß-alanine and malonate semialdehyde, which would need aminomutase transformation of alanine to ß-alanine. No such enzyme has been identified, therefore microbial lysine 2,3-aminomutase (transforms a-lysine to ß-lysine) was subjected to molecular breeding techniques to achieve acceptable levels of alanine 2,3-aminomutase activity for production of 3-HP from glucose.

Industrial Biotechnology for Pharmaceutical and Chemical Applications

Mahmoud Mahmoudian (Eastman)
Susan Truesdell (Pfizer)
Bruce Gaede (Abbott)

Biotechnology has evolved into three major areas:

- "red"—pharmaceutical and therapeutic/medical applications,
- "green"—agricultural applications, and
- "white"—industrial applications.

Increased petrochemical-raw material cost (*e.g.* natural gas prices have doubled since 2000), and competition from China and India have eroded profit margins in the chemical industry. Although white biotechnoloty is in the early stages in the chemical industry, many see it as a key driver. It will grow strongly over the next ten years and can no longer be ignored in certain product areas as it provides opportunities for new technologies and product development.

Success will depend on forging strategic alliances. Because it is often hard to predict the ideal product up front, raising resources can be difficult. It is necessary to focus on a few targets and a few tools, and to make a start.

Biocatalysis is often more environmentally friendly than is chemical synthesis, and may have yield and selectivity advantages. Molecular biology tools—including gene shuffling—now permit designing enzymes for improved performance in terms of activity, specificity, pH optimum, solvent and heat tolerances, *etc.*

At this time, the following reaction types are amenable through biotransformation: oxidations / reductions (hydroxylation, stereoselective ketone reduction, oxidation of alcohols to carboxylic acids, and oxidation of hetero functions), hydrolyses / condensations (lipases, esterases, nitrilases), and O- and N-demethylations. However, other types of enzymes may be developed by protein engineering.

Biocatalytic reactions can be run under milder conditions (*e.g.* at ambient temperature and pressure, in aqueous solvents) than can the chemical counterpart with benign reagents (*e.g.* no heavy metals, strong acids or bases). There is a long history of safe use of microorganisms and enzymes (*e.g.* antibiotic production, food uses) to draw upon. Furthermore, the stereoselectivity and regioselectivity of enzymes results in fewer byproducts, which may simplify recovery. Often it is possible to reduce the number of steps involved. The chemical route of synthesis of 2-quinoxaline carboxylic acid from 2-methyl quinoxaline was complex and involved mutagenic high-energy intermediates. A microbial route has been devised that replaces three steps with one, saving unit operations, processing time and cost, in the absence of hazardous intermediates.

Emtricitabine is the active ingredient of the antiviral drugs Coviracil® and Emtriva® for treatment of human immunodeficiency virus and hepatitis B virus. The biosynthesis involves an esterase isolated from pig's liver, which raises issues of possible contamination of the final product with animal viruses and transmissible spongiform encephalopathies.

Non-specific testing failed to detect virus in any lot of enzyme. In the absence of adequate tests for prions, the source enzyme comes with a certificate of origin from a country where risk of transmissible spongiform encephalopathies is minimal (Denmark, United States); also, liver is considered to be a "low infectivity" tissue. Animal-derived enzymes should be used only if no other choice arises from an extensive screening program; expert advice should be sought and extra time and resources invested to ensure product safety.

Biotechnology for Fine Chemical Production

Hans-Peter Meyer (Lonza)
Reinhold Öhrlein (Ciba)
Stefan Buchholz (Degussa)

Markets for biotechnologically derived intermediates and end products for the life-science and chemical industries are expected to rapidly increase. However, availability of microbial strains and enzymes is already limiting; thousands of new strains and enzymes of various classes are needed for the provision of substrates and products, especially new active pharmaceutical ingredients (APIs).

The Swiss industrial biocatalysis consortium—Novartis, Lonza, Givaudan, Ciba, Syngenta, Roche and Fluka—was formed to share microbial culture collections and related information. The objective is to overcome strain penury in the industry to help meet ambitious market targets and, above all, create new business opportunities. The consortium wants to see academic research focussed on finding new strains, enzymes and reaction types. The most pressing need is for dehydrogenases for the asymmetric reduction of ketones, ketoacids and olefins, and, less importantly, for the oxidation of alcohols. Oxygenases are also needed for mono-hydroxylations, especially of non-activated centers and of non-natural substrates. Peroxidases are needed as are transformation of ribonucleotides, stereospecific epoxidations and the oxidation of ketones to esters and lactones. And there is demand for lyases for synthetically useful enzymes for C–C bond formation (preferably asymmetric) using aldolases and hydroxynitrile lyases, for C–N (aminolyases) and C–O (hydratases) bond formation, and for lyases with broad substrate acceptance.Traditional chemical means of synthesis are often energy-intensive, have low selectivity and involve heavy metals and extreme conditions of pH and temperature, with impurities as by-products. In contrast, biocatalytic means of synthesis usually require little energy input under mild pH and temperature conditions, and are environmentally benign and highly selective. On the other hand, chemical synthesis is usually rapid and robust, involving low molecular weight constituents, and enzymic synthesis is relatively slow involving high molecular weight, relatively labile constituents. Alternative biocatalytic means of synthesis of proprietary compounds are being appraised—for production of photo initiators, phenol antioxidants, optical britheners, specialty monomer, olefine stabilizers and pharmaceutical intermediates—with some success; enzyme activity has remained constant for >9 months.

Needs include a variety of bulk, commercial hydrolases, more thermostable enzymes, improved carriers for biocatalysts, access to oxido/reductive enzymes on an industrial scale, and access to enzymes catalyzing the formation of C–C bonds besides aldolases Degussa has been using novel microorganisms and fermentation for some time, and currently markets enzymes, amino acids, polysaccharides, sphingolipids, *etc.*, for the manufacture of thickeners and ingredients for food, feed, cosmetics, *etc.* Degussa is structured in Project Houses of limited lifespan to provide access to new fields of technology to the company as a whole. Classical means of microbial strain development are still used as well as state-of-the-art techniques (*e.g.* directed evolution) to improve fermentation productivity.

Integration of Biotechnology in the Industrial, Household and Personal Care Markets

Vince Gruber (Arch Chemicals)
Nava Dayan (Lipo Chemicals)
Carl Podella (Advanced BioCatalytics)
Joseph Boothe (SemBioSys Genetics)
James Thompson (Procter and Gamble)

Tropospheric (air-pollutant) ozone has similar effects on cultured yeast as on human skin cells: heat-shock proteins are synthesized, lanosterol level increases (lipid repair), expression of 8-oxoguanine DNA glycosylase (OGG1) increases (DNA protection) and ATP production increases. These changes correlate with alterations in 2-D electrophoresis of proteins, genomic microarrays and protein/protein microarrays of yeast-cell lysates.

Biodynes® O3 is a lotion prepared from ozone-exposed yeast. Its application decreased degradation of skin DNA and cholesterol caused by exposure to ozone.

Pseudopterosins, isolated from the Caribbean sea whip *Pseudopterogorgia elisabethae* (a soft coral), are a mixture of diterpene glycosides that have anti-inflammatory properties. The sea whip has a symbiosis with the dinoflagellate alga *Symbiodinium*; pseudopterosin profile varies with location.

Skin irritation, characterized by epidermal edema, erythema and local elevation of temperature can be caused by primary chemical irritants or by allergens.

Gorgonian Extract™ is an anti-inflammatory agent that is added to skin-treatment products that have potential to irritate the skin (*e.g.* cleansers, retinol) or to products for sensitive skin and for sensitive areas (*e.g.* under the eyes), and after application of exfolients and keratolytic agents, both physical and chemical. Advanced BioCatalytics's products accelerate naturally occurring biochemical reactions at a fraction of the cost of conventional capital-intensive equipment and systems. The liquid formulations contain an optimized array of fermentation-based proteins, micronutrients and highly specialized surfactants. The synergistic effects of the ingredients has an uncoupling-like effect on indigenous microorganisms, resulting in acceleration of catabolism of organic contaminants into neutral end materials, such as carbon dioxide and water. Addition of ACCELL® to sewage lift stations and pipe lines controls grease buildup. Sodium metasilicate and sodium carbonate, the functional ingredients in commercially available bilge cleaners—at ph 11.5—are skin and eye irritants and toxic. Advanced BioCatalytics's prototype bilge cleaner contains a blend of anionic/nonionic surfactant and proteins; it is of low oral toxicity, is minimally irritating for skin and eye, and functions at ph 5.5. This technology has applications wherever surfactants are used: cosmetics, medical products, household products, institutional and industrial cleaners, industrial process aids, wetting agents and dispersants, water treatment, and agricultural products and processes.Oleosomes are oil-storage organelles in seeds, especially abundant in oilseed species. Comprised of on an oil core surrounded by a phospholipid membrane and protein shell, they emulsify freely in water. They are purified from milled safflower seeds by a process of flotation and separation, and marketed as 1–3 µm Dermaspheres™ by SemBioSys.Oleosomes have been formulated into a full range of skin-care products, the performance of which for various parameters—as exfoliating creams/lotions, moisturizing creams/lotions, lightening creams, sunscreens, ointments, color foundations, color blushes and eye shadows—is equivalent to or superior than leading commercial products. And they can be loaded with a wide range of actives, *e.g.* anti-oxidant, anti-inflamatory, anti-psoriatic, anti-acne, and topical anaesthetic compounds. Understanding a tissue/biological system at the cellular/molecular level and knowledge of how agents interact are key drivers of beauty-product innovation. P&G has attained the ability to rapidly obtain a "global" view of a biological system of interest and combine the knowledge with high through-put screening for more effective product discovery. Included in their toolbox are genome sequencing, gene-expression microarrays, bioinformatics, metabonomics (metabolite profile), matrix assisted laser desorption and ionization mass spectrometry (MALD-MS, peptide mass mapping), proteomics, and chemical libraries.

The genomics of a topical retinoic acid treatment (often used to improve the appearance and texture of the skin) were examined: >1,000 genes were up- or down-regulated. Up-regulated genes included some encoding structural proteins and defense proteins, and down-regulated also included some encoding structural and defense proteins.

P&G has developed automated high-throughput approaches in the discovery process, for rapid "benefit agent" identification; assays include enzyme activity and inhibition, ELISA, reporter-gene activity, cell adhesion (biofilms) and cell viability.

Industrial Biotechnology for Food and Feed

Hanne Vang Hendriksen (Novozymes)
David Maenz (MCN BioProducts)
Robert Hanchar (MBI International)

In 2002, acrylamide—carcinogenic in rats and mice—was found in a number of foods that are high in starch and cooked at elevated temperatures: potato and tortilla chips, French fries, cookies, toast, breakfast cereals, *etc.* Studies indicated that the most significant source of acrylamide in the human diet is coffee. It forms as a result of a Maillard reaction between asparagine and reducing sugars. By removing asparagine prior to cooking, the acrylamide level is concomitantly decreased; asparaginase, amino acid oxidase and glucose oxidase were examined and asparaginase found to be most effective.

Commercially available bacterial asparaginase was available. A search of Novozymes in house sequence databases revealed a gene from *Aspergillus oryzae*; it was cloned and transformed into a strain of *A. oryzae* developed for high-level expression. When tested in various bakery products, potato chips and French fries, acrylamide reduction was achieved with no adverse effects on sensory qualities. The technology can be applied to a wide range of products, offering the possibility of overall decrease in average daily intake. Novozymes is working towards broad market introduction of an asparaginase-based technology, making it available to all food manufacturers.

Removal of oil from canola seed leaves a 60% residual of low-value meal that has less protein, more fiber and more phytate than soybean meal. Therefore, although canola protein has a better amino acid balance than does soy protein, it trades at approximately 60% of the value of soybean meal and is sold as a low-value ingredient for pig, poultry and cattle feeds.

MCN Bioproducts has developed a process that generates an insoluble protein concentrate (65% protein), a soluble protein concentrate (60% protein) and a sugar-enriched syrup. The protein fractions are free of detectable phytate and much reduced in other anti-nutritional factors. The insoluble protein is a possible high-value alternative to fish meal for aqua feed and swine and poultry feed, and to animal-based protein and soy protein concentrates. Similarly the soluble protein is a potential alternative to dairy proteins, hydrolysed plant proteins and calf milk replacers. Performance trials with Atlantic salmon showed that replacement of 50% of the fish-meal in the typical commercial feed with canola soluble/insoluble protein concentrate did not affect growth. Replacement of 50% of fish-meal shrimp feed with insoluble concentrate resulted in a 30% increase in growth rate. Low-phytate feeds have environmental benefits for intensive aquaculture and land-based animal production.Ethanol production has increased 127% over the past five years and is expected to continue to increase. Most of that growth has occurred through increased numbers of dry mill plants in which, for every gallon of ethanol, 6 lbs of distillers dry grains and solubles (DDGS) are also produced. The value of DDGS relative to corn has decreased as a result of increased productions, although it contains 10% extractable oils and could theoretically generate \$50/ton DDGS as corn oil. De-oiled DDGS could increase revenues of dry mill ethanol by-products by 59%. De-oiled DDGS has a higher protein content and higher neutral detergent fiber level that unprocessed DDGS; energy, calcium, and phosphate availability are unaffected.

A stand-alone extraction facility, next to a 50 million gal/yr ethanol plant (located to eliminate DDGS transportation costs), designed to process 300 million lb DDGS/yr using hexane in a counter-current extractor to recover 99% of the extractable oil, would have a capital cost of approximately \$9.5 million and a unit production cost of approximately \$0.10/lb oil. It may be refined to edible quality, but with low yield; its greatest potential appears to be as biodiesel.

Cell Factories and the Pathway to Biodiverse Chemicals

Gopal Chotani (Genencor)
Colja Laane (DSM)

Brian McCormick (Procter and Gamble)Genencor's objectives include development of cell factories to convert plant material into value-added products. They are delivering cost-effective enzymes for low-temperature conversion of starch, sucrose and biomass to glucose that serves as a feedstock for cell factories.

Their cell-factory strategy includes transformation of a strain of *Escherichia coli* with genes from yeast and *Klebsiella* to induce synthesis and excretion of 1,3-propanediol, the monomer used in the production of Sorona® fiber, from glucose. Such rational biocatalyst design is more than a tool or a technique; it's a new way of thinking. Biorefineries integrate biocatalysts, feedstocks and product/process innovations, to pave the way for tomorrow.

DSM has developed microbial and mammalian cell-culture systems and processes for commercial production of citric acid, arachidonic acid, *ß*-carotene, vitamins B2 and B12, antibiotics, intra- and extra-cellular enzymes, fine chemicals, pharmaceutical proteins, live cultures, *etc.* Exposure of starchy food to asparaginase, converting asparagine to aspartic acid, decreases the formation of acrylamide during cooking. Brewers Clarex™ is a novel enzyme for cost-effective prevention of haze formation in beer. They have found that *Aspergillus niger* contains about 200 genes encoding proteases of various function, of which sixty are excreted. Peptides stimulate an insulin response causing faster glycogen refueling of muscles; this process is the basis of PeptoPro Sports energy-recovery drink, which was developed from concept to product in less than two years. DSM will continue to invest in industrial biotechnology as a means of combining social (people), environmental (planet) and economic (profit) benefits.

Petrochemical trends provide a backdrop for new biobased-product opportunities. Crude-oil import prices have been increasing steadily since 2001, providing a long-term-trend indication of what price to expect in the foreseeable future, since most oil trade occurs under long-term agreements. In 1994, 67% of the production cost of polyethylene was the olefin cost: ethylene. Today, with oil and olefin prices having doubled or more since 1998, ethylene comprises 80% or more of polyethylene production cost. And as the price trend continues upward, growth in high-value biobased products from renewable feedstocks will open up.

Some biobased products will require research to optimize the value equation

Value = Benefit / Costor cost barriers will limit market growth. At P&G they believe what has served biotechnology well in the past will be its mainstay in the future. The key is to forge strategic partnerships—*Connect and Develop*—and share knowledge, including knowledge of business risks. They recognize that they simply cannot afford to build capability in all aspects of biotech development, but instead must "connect the dots," linking appropriate expertise to solve technical challenges, in a highly cooperative manner. By combining the right capabilities, more products from renewable feedstocks will be introduced to consumers.

Production of Biopolymers and Intermediates

Adrie Straathof (Delft University)
Takaaki Maekawa (Tsukuba University)
Jian Yu (University of Hawaii)

Acrylic acid—annual production 4.2 million tonnes, valued at \$8 billion—is a commodity chemical synthesized for manufacture of polymers for flocculants, coatings, paints, adhesives, and binders for leather and textiles. Because it is prepared mostly from propene, production costs are rising; a sustainable alternative is needed. Although acrylate synthesis is known to occur in some microorganisms, metabolic engineering will be necessary to optimize the process, particularly in view of its toxicity. Hypothetical metabolic pathways have been identified for theoretical maximum yields from feedstock glucose without aeration. The toxic C=C–COOH sub-structure is present also in fumarate and itaconate and some cell types survive 35 g/L acrylate. Using selective pressure, genome shuffling, *etc.*, 50 g/L acrylate is seen as a realistic maximum concentration. Active excretion of acrylate is required, provided that the exporting process does not consume all of the ATP.

Continuous fermentation is envisioned using a genetically engineered strain of yeast at a pH of 7.0 (controlled by Na_2CO_3) yielding 0.72 g acrylate/g glucose to a concentration of 50 g acrylate/L and lactate at 1 g/L. Key information gaps include thermodynamic data of pathway intermediates, accessibility of suitable pathway and exporter enzymes, metabolic consequences of blocking competing pathways, potential tolerance to acrylate and equilibrium data for extraction.

Polylactic acid (PLA) is finding broad acceptance for many purposes although it is priced at approximately \$6/kg (\$2/kg is the economic price). PLA is relative fragile, needing addition of compounds such as gum-arabic for strength. Soy protein offers an feedstock alternative to PLA for manufacture of packaging film, with glycerol as plasticizer, sodium silicate as protein denaturant and emulsification agent, and agar for tensile strength. However, water solubility is a significant problem. Addition of calcium carbonate reduces the water solubility to some extent but presents problems in terms of mixing with the other components. Tapioca starch also helps, but further experimentation is required. Prehydrolysis and enzymic saccharification are necessary for conversion of lignocellulosic biomass to ethanol. Pretreatment of 2,000 tons of biomass yields 100 to 200 tons of toxic byproducts—furfural, 5-hydroxymethylfurfural (HMF), acetic, formic a levulinic acids, *etc.*—discharged to wastewater treatment. Production of thermoplastics from these byproducts would provide extra value. Bacteria such as *Ralstonia eutropha* can convert organic acids to short-chain-length polyhydroxyalkanoates (scl-PHAs, a carbon reserve) that can be molded like petrochemical counterparts; however, they are brittle and show poor ductility.

PHA plastics have been developed with superior embrittlement characteristics and good ductility by inclusion of 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV) and 4-hydroxyvalerate (4HV). Levulinic acid, the major byproduct, provides the precursors of 3HV and 4 HV. Production of thermoplastics would provide added value to ethanol production from lignocellulosic biomass.

Fermentation Routes of Renewable Chemicals

Hans Blaschek (University of Illinois)
Susanne Kleff (MBI International)
Joachim Venus (ATB Potsdam)

Butanol is an important industrial chemical and feedstock, used as a fuel extender and foodgrade extractant. Until the 1950s, it was produced commercially by fermentation from corn with acetone and ethanol (ABE). It has a higher boiling point and greater energy content than does ethanol. Strain BA101 of *Clostridium beijerinckii* was developed in the early 1990s by chemical mutagenesis together with selective enrichment on the non-metabolizable glucose analog, 2-deoxy-glucose. Pilot scale (20 L) fermentations using 6% glucose or maltodextrin demonstrated that BA101 produces twice as much butanol as does the parent strain. In addition, BA101 exhibits reduced acid production and increased carbohydrate utilization. Economic modeling studies indicate that the use of the hyper-butanol producing strain BA101in combination with improved recovery technologies can be competitive with the petrochemical route for butanol production.

Batch cultures are self-limiting due to product toxicity, resulting in low concentrations of butanol in the fermentation broth; high-energy inputs are required to recover butanol from dilute streams. When fermentation and gas stripping were integrated in (a) batch, (b) fed-batch and (c) continuous systems, 75.9 g/L ABE were produced in (a), 232 g/L in (b) and 460 g/L in (c).

Integrated gas stripping combined with fed-batch fermentation employing *C. beijerinckii* BA101 has demonstrated the potential for scale-up to industrial production. Genome-sequencing will assist post-transcriptional analyses of strain BA101 to promote understanding of hyper-butanol synthesis.

Succinic acid—at a possible production cost of \$0.42/lb—has the potential to act as an important chemical intermediate. With further reduction costs it will compete with commodity chemical feedstocks; biomass may act as an inexpensive carbon source. There is potential for integration of succinate production with existing wet-mill operations and the next generation dry-mill ethanol plants.In today's market, succinate may be a feedstock for pyrrolidinones. In the near-term, it could serves as a feedstock for butanediol-based polymers, such as polybutylene terephthalate and thermoplastic polyurethane. And in the long-term the market could expand even further to include replacing maleic anhydride in the synthesis of tetrahydrofuran, butanediol and *y*-butyrolactone.

Lowering the cost of glucose would lower the cost of succinate. Particularly attractive is corn-fiber biomass as a feedstock, which would have the added advantage of being already collected and a by-product of wet milling of corn. Treatment of the fiber by ammonia fiber explosion, enzyme hydrolysis and with dilute acid would yield C-5 and C-6 sugars for fermentation by *Actinobacillus succinogenes.*

The bacterium *Lactobacillus paracasei* strain 168 has a high production capacity for lactic acid, the salts and esters of which have many uses in the food, cosmetic, pharmaceutical and agricultural industries. Utilization of barley hydrolysate as a carbon source and substitution of expensive protein extracts with broth extracted from alfalfa or lupin provided good lactate production by batch fermentation in comparison with a well characterized synthetic medium. A continuous lactic acid fermentation process with cell retention is under development with substitution of expensive nutrients by green biomass that may be produced on under-utilized agricultural land.

Directed Evolution of Industrial Enzymes

Mary Yang (KAIROS Scientific)
Mark Burk (Diversa)
Gjalt Huisman (Codexis)

With directed evolution—*e.g.* recursive ensemble mutagenesis—novel biocatalysts can be created from diverse libraries of microbiological variants. Versatile high-throughput technologies have been developed to screen for diverse enzymes. Digital imaging spectroscopy combines image processing with spectroscopy so that spectral information is gathered for every feature or pixel in a two-dimensional scene, equivalent to hundreds of thousands of spectrophotometers operating in parallel. Simultaneous screening of multiple properties or multiple substrates is possible using substrate volumes of only 10–50 nL. Cells may be intact, lysed, or secreting. Throughput of a million variants per instrument per day is possible.Despite their potential contributions to industry, large-scale use of enzymes remains limited because of lack of availability for specific purposes. Diversa's discovery efforts are based on extraction of DNA directly from uncultured microbes obtained from terrestrial and aquatic sources. Extensive libraries have been constructed, containing millions of genes, and functionally diverse enzymes are now available. Techniques have been developed for high-throughput screening for identification of candidate enzymes for specific processes. Complementary directed evolutionary technologies have also been developed for optimization of enzyme phenotype: specific activity has been increased up to 39,000-fold, thermal tolerance has been increased, reversible thermal denaturation has been induced, up to a six-fold improvement in enantiomeric selectivity has been induced, up to a 30,000-fold increase in substrate specificity has been induced, *etc.* For example, using GeneReassembly™, *a*-amylase was altered to retain activity at pH 4.2 approximately equal to that at pH 5.5, and to retain activity at 90°C for an hour.

Several techniques are available for directed evolution of enzymes, involving generation of random mutations and screening mutants for improved function in terms of activity, stability, specificity, enantioselectivity, *etc.* Using DNA shuffling, successes include increased volumetric productivity of >1,000-fold, improved volumetric productivity of two-enzyme systems with co-factor regeneration for ketone reductions of fifty- to 100-fold, improved enantioselectivity of thirty-fold to >98%, stability improvement (*e.g.* no activity loss at 40°C for days), decreased product inhibition (*e.g.* enabling processes at >100 g/L), reduced impurity levels, and decreased enzyme cost contributions. The next step may be in finding better ways to accelerate the recombination of beneficial diversity. Quantitative structure-activity relationships—used in drug discovery—may foster identification of elements of diversity that contribute to improved function.

Bioprocessing of Agricultural Feedstocks

Crop Residue Assembly and Utilization

Mariam Sticklen (Michigan State University)
Richard Hess (Idaho National Laboratory)
William Lee (Chippewa Valley Ethanol Company)

A major issue in the emerging biobased economy involves the ability to easily and cost-effectively digest plant celluloses found into their constituent sugars. If plants were capable of producing and storing the enzymes needed to later digest their celluloses, lignocellulosic processing costs may be lowered. Transgenic lines of rice, maize and tobacco were created expressing a microbial E1 endoglucanase, which could be released from the plant for later downstream processing. Additionally, the flowering locus C (FLC) gene was engineered into E1-tobacco, resulting in higher biomass accumulation with delayed flowering, useful in helping to prevent gene transfer to unintended targets. E1-FLC plants also displayed enhanced uptake of lead uptake, a useful feature for phytoremediation.

Biorefineries of the future will require relatively uniformly processed feedstock with suitable characteristics; however, future assembly systems are not yet well characterized and current ones will not meet biorefinery requirements. Three feedstock-assembly scenarios (all consisting of straw collection, roadside storage, grinding and transportation to refinery) were evaluated for performance since multiple straw collection systems will need to be accommodated. One way in which biomass feedstocks may be processed in the future is through a distributed or mobile grinding system. The distributed grinding concept was found to be logistically feasible in meeting both throughput (30–40 t/h) and bulk density (8 lbs/ft3) targets, although much opportunity remains for research and development of both the grinder and overall assembly system in order to optimize output. Importantly, grinder screen size was found to have a large influence on system capacity (and thus cost), but had relatively little impact on bulk density. Non-bale and distributed grinding systems appeared very promising for future biorefinery use.

One way advanced biorefineries may come into existence is through integration with existing bioprocessing plants such as those producing ethanol from corn starch. The Chippewa Valley Ethanol Company (CVEC) is attempting to add advanced biorefinery capability to their ethanol plant in order to have access to broader bioproducts markets in the future. Many barriers need to be overcome, including energy markets, fractured development, the feedstock conundrum, difficult technologies, policy vacuum, and the return on investment "leap of faith" with new technologies. CVEC is overcoming these through the high price of natural gas (energy markets), starting small in focus by using only gasification technology (fractured development), taking advantage of abundant feedstock options in its local area, using corn fiber from traditional corn processing and taking advantage of their farmer-owner structure (feedstock conundrum), showing return on investment through displacement of natural gas, and supporting biofuel-friendly policies (policy vacuum).

Report on Pretreatment for Biomass Refining

Bruce Dale (Michigan State University)
Michael Ladisch (Purdue University)
Richard Elander (National Renewable Energy Laboratory)

Although several pretreatment options exist for biomass prior to entering a biorefinery, only a few have shown significant promise. Any pretreatment option must fulfill several crucial needs: high accessibility of cellulose to the enzymes, high sugar yields from cellulose and hemicellulose, low capital and energy costs, and low cost or recoverable chemicals. The Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) was formed to methodically study leading pretreatment technologies using one source of biomass and a standard analytical technique to evaluate performance.

All of the pretreatment technologies (dilute sulfuric acid, lime, flow through, ammonia fiber explosion, ammonia recycle pretreatment, and controlled pH) were found to yield highly digestible cellulose at the enzyme-loading rates tested, 60 and 15 FPU/g glucan. Xylose results showed somewhat more variability; the higher pH pretreatments resulted in more xylose release in the hydrolysis stage than with the other pretreatment options, which tended to release more xylose prior to enzyme loading. The enzymes used, however, were effective in hydrolyzing this residual xylose from the solids, regardless of the pretreatment scheme. Lignin removal may not be important for successful hydrolysis. Future work will include process modeling, maximizing fermentation yields from yeast, as well as studies of capital and operating costs.

Many pretreatment options exist for cellulosic biomass conversion to sugars. A major part of CAFI's goals was to look at the leading options and perform rigorous mass balances and system analysis in order to understand the economics of each process. The pretreatments studied covered a wide range of chemical conditions, ranging from dilute acid to lime. One main difference among the technologies was in how they interacted with the xylan component of cell walls; dilute acid released most of the monomeric xylose prior to enzyme addition, while controlled pH and flow-through technologies tended to release oligomers. The higher-pH pretreatments, however, did not release xylose as effectively, making downstream enzymic steps crucial. Processes can still be optimized and enzyme cocktails improved.

After pretreatment and enzymic hydrolysis steps are completed, downstream fermentation of the resulting sugars presents some challenging problems as shown in small scale fermentations of corn stover hydrolysate obtained from a dilute acid pretreatment at NREL. For one, fermentation of dual substrates (xylose and glucose) is desired. Also, unlike pure sugar fermentations, inhibitory agents may be present. For instance, upstream processes can yield problematic levels of furfural, hydroxymethylfurfural (HMF), and acetate that serve to reduce fermentation yields. Attempted removal of these compounds using XAD-4 resin and overliming was apparently ineffective in removing all the active inhibitors as fermentation yields were still lower than desired. Much optimization work remains, but further research should dramatically improve results.

Good economic models of the various lignocellulosic-to-ethanol systems are needed in order to provide a cost comparison of the pretreatment options studied by CAFI. The pretreatment economic models were inserted into a base-case bioethanol plant model that used the 2001 NREL design case. Sugar yields are important to the models with each pretreatment process resulting in similar glucose levels but differing amounts of xylose oligomers and monomers; if the oligomers were assumed to be converted then the economic model showed little differentiation among the technologies. Capital costs were found to be similar for all pretreatment technologies when catalyst recovery and recycling were factored into the model; total fixed capital costs were also found to be similar. The models resulted in ethanol costing between \$3 and \$4/gal, which needs to be lowered to less than \$2/gal, the current cost of corn ethanol.

Industrial Biotechnology, A Chinese Perspective

Shi-Zhong Li (China Agricultural University)
Zhenjun Sun (China Agricultural University)
Runqing Hu (Chinese National Development and Reform Commission)

China faces many of the same energy problems as the United States: they import about 40% of their oil and their current usage is 5–6 million barrels and growing. Biomass-based industries are expected to provide much-needed income for poor, rural China. However, important differences exist for biomass utilization in China: corn for food is much more important than corn for ethanol, which means that species such as sweet sorghum and physic nut (*Jatropha curcas*) may be used as energy crops. The upcoming 5-year plan from the central government is expected to contain a major biomass program, and goals have been set that target production of 12 million t/yr each of bioethanol (~250 plants), biodiesel, bioplastics, and 50 million t/yr of pellet biofuel by 2020.

In order to achieve these goals, China plans on spending over \$1 billion on development. Agricultural residues and/or wastes are the main biomass product in China, where most of the crops grown are used for food. Currently, total wastes and residues amount to over 4 billion t/yr, much of which is underutilized; this amount is expected to increase significantly over the next 10 years. Thus, biofuel, bioenergy and other bioproduct development will help alleviate pollution problems as well as help achieve sustainability. Currently important or upcoming projects involve bioenergy production from the agriculture and forestry sectors, treatment and utilization of animal wastes, and further development of biogas, an important resource in China.

Government policy will be crucial for successful development of biopower, biofuels, and other bioproducts in China. Currently, there is no clear regulation of biomass energy on-grid price; it is normally done on a case-by-case basis with many difficulties. Tax policies are in place or being developed to favor biopower and biofuel production. Subsidies are currently used for research and development, and serve as an important incentive policy. The Renewable Energy Law, scheduled to take effect in 2006, represents a major policy step. This law specifically targets environmental protection, energy security and diversity, and promotes commercialization of renewable energy technologies through market enlargement as major goals. As a result, biomass development is expected to rapidly increase in China, and opportunities exist for private enterprise.

US Biomass Feedstocks: Current and Future Opportunities

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Edwin White (State University of New York at Syracuse)
Gene Quick (Southern Alliance for the Utilization of Biomass Resources)

When considering biomass feedstock options, a variety of plants could be used. However, as the nascent biobased economy develops, a useful way of categorizing feedstocks is whether their potential availability and development is near, mid- or long term. Additionally, optimal outcomes will be achieved only when conversion technology is matched with biomass type. Attributes of a good feedstock include low inputs, high yield and bulk density, easy harvesting, handling, and transport, good longevity in storage, low moisture, ash and silica contents, and low amounts of soil contamination. Harvesting crop residues may not be environmentally sustainable in the long term and may cost more than expected. Grasses avoid some of these problems; switchgrass has enormous potential but in the mid- to long term, while bermudagrass, bahiagrass, and tall fescue have immediate availability.

Willow is a short-rotation woody biomass crop that has been extensively researched and has commercial possibilities as a biomass feedstock. It has applicability in biopower and bioproducts and a net positive energy ratio. Some of the environmental benefits of this crop include low soil erosion due to a fine root system, non-point source pollution, and enhanced soil carbon storage due to its perennial growth. Breeding has increased the yield of willow by 18%, which translates into a 13% reduction in delivery cost, but overall cost of the system still needs to be reduced to make it economical; policy initiatives, improved conversion technology, further yield increases and harvesting-system development may help.

The forestlands of the southern United States represent a potentially large source of biomass for industrial use, and given that 60% of the south is forested, wood biomass will likely play an important raw-materials role. Additionally, biofuel and bioproducts markets represent excellent possibilities for revitalization of the US forestry industry. International competition in pulp and paper has resulted in large structural changes within the industry through merger and acquisitions and closure of many operations. This market is not expected to return to the United States, and, as a result, southern forests have expanded to cover 215 million acres. Public policy changes are needed to exploit this vast resource for biofuel and bioproduct development.

Meeting Large Biorefinery Feedstock Needs: A Regional Approach

Jim Hettenhaus (CEA)
John McGrath (Imperial Young Farmers and Ranchers)
Russ Pankonin (Imperial Republican)

For biorefineries to be successful, a substantial and well planned feedstock supply infrastructure must be developed. One area that is attempting to study and develop such an infrastructure for corn stover and wheat straw is Imperial, Nebraska. This rural area in southwestern part of the state offers abundant, high-quality cropland for biomass feedstocks and could provide up to 6 million dry tons of stover and straw annually. Additionally, the flat terrain and existing roads and rail lines could provide the needed transportation infrastructure to support a large-scale biorefinery.

The city of Imperial and surrounding area have a progressive farming community willing to embrace changes, if the economics and processes can be demonstrated. For instance, one grower is already producing switchgrass under irrigation. The current work force is about 11,000; of these, 2,000 or more have been found willing to consider a career change if opportunities, such as those presented by a biorefinery, were to become available. To be ready for such an event, Imperial has five or six industrial sites for potential development and financial incentives such as tax increment financing are available. The $3 million study is designed to develop the necessary models and technology needed for a regional large-scale biorefinery.

The needs of such a refinery would be many; the Imperial study has five main components: sustainable removal of stover and straw, establish farm value (what would it take to get farmers to remove stover?), develop innovative methods, produce a business model, and conduct an overall life-cycle analysis. The sustainability aspect will study carbon balance and maintenance of soil productivity. Development of an acceptable prototype one-pass harvester and validation of large-pile wet storage and transport will be key. The business model plans to study costs associated with switching the region to a no till, one-pass harvest farming system as well as handling, transport, and storage costs. Total system development costs may be around $30 million.

Plant Traits for Ethanol and Chemical Production

Susan MacIsaac (Monsanto)
Elspeth MacRae (HortResearch)
Bruce Ferguson (Edenspace)

As ethanol production from corn increases, finding varieties that are most suitable for conversion technologies will be important in order to maximize productivity. Monsanto is looking at corn-kernel attributes important in maximizing ethanol yield from starch. Differences among corn hybrids are only on the order of 1–2%. Simple attributes such as amount of starch in the kernel are not predictive of how well the hybrid will ferment; instead, factors such as starch-granule packing and protein coating may be of more importance. Physical barriers to degradation—cell-wall linkage to granules and enzyme-resistant chemical bonds, *etc*.—are among the factors that result in the 5–15% of residual starch not converted in dry-mill operations. Taken together, physical and compositional factors interact to influence ethanol yield.

In addition to ethanol, production plants can be used to manufacture many volatile compounds. These compounds can have a variety of functions including pollinator attraction, defense against herbivores, and specific signaling to other plants. Some of these compounds may have industrial, pharmacological or other important applications, and thus understanding—and manipulating—their production pathways is important. Using wild *Actinidia* and *Malus* species as genetic resources, biochemical pathways have been predicted and novel genes have been discovered using current genomics technologies. Once proof-of-function is established, pathway kinetics are studied and processes optimized to maximize compound yield. This approach has led to isolation of several novel, and potentially useful, genes.

Fuel and chemical production may complement another important use for plants: environmental cleanup. With such multiple uses, plants can be viewed as providing both services and products. In remediation applications, plants used for heavy-metal sequestration are currently disposed of in landfills, but genetic engineering approaches have yielded plants with increasingly desirable traits such as visual indication of exposure to heavy metal exposure, production of cellulases, and delayed flowering which helps contain horizontal gene flow. Cellulase-producing plants offer benefits in phytoremediation by aiding metal recovery and recycling as well as in biorefinery scenarios where they may help raise ethanol yields or lower production costs.

Commodity Agriculture Crops for Biobased Platforms

Jim McLaren (StrathKirn)
Sherman Reese (National Association of Wheat Growers)
Fred Yoder (National Corngrowers Association)
Harold Kraus (National Biodiesel Board)

A future biobased economy will likely rely on multiple plant species as feedstock sources. Dedicated energy crops may one day be widely available, but currently available commodity crops will also have important roles. One such crop is grain sorghum, which already has known genomics and well established management practices for optimizing yields. Sorghum offers better tolerance to drought and temperature extremes than does corn, making it an attractive alternative in drier climates. In order to carve a niche for sorghum in a biobased economy, a project focusing on its bioconversion to ethanol and other useful products is currently underway with goals of process optimization and germplasm improvement.

Iogen Corporation has sited the first US cellulosic ethanol plant in Idaho. The feedstock will be wheat straw, which has a carbohydrate content similar to that of corn, barley and sorghum, and has been contracted at about \$8 to \$20 an acre. Along with decreasing enzyme costs, the technology has improved to make cellulosic ethanol an advanced fuel with a 90% reduction in greenhouse-gas emissions over its life-cycle when compared to conventional gasoline. Although the potential of cellulosic ethanol is huge, certain challenges remain, including assembly, storage, and transport of biomass, and lack of government policies providing loan guarantees and supporting feedstock development.

Corn is also likely to be an important feedstock for biofuel and other bioproducts. Currently, corn kernels are the most widely used plant-derived feedstock for ethanol production: 13% of the total crop is used for starch-based ethanol. Energy efficiencies are improving: each BTU of energy is now said to yield 1.67 BTU of energy out and future processes are expected to be even more efficient. In addition to ethanol, many other products are currently made from corn such as wood-type flooring and plastics. Corn growers in the United States are currently urging the government to establish research priorities, audit current federal research to evaluate effectiveness of public and private investments in renewable energy and products, and provide funding to promote research for technology transfer to rural areas. Additionally, corn growers recognize that corn cannot do it alone and the future biobased economy will require an umbrella of diverse feedstocks.

Biodiesel represents another biofuel with potential to reduce US dependence on imported petroleum. Biodiesel is easy to produce and has a 3.2 to 1 energy balance, the highest for any of the biofuels. Additionally, biodiesel is a high quality fuel with high lubricity and ultra low sulfur content, resulting in about an 80% reduction in greenhouse-gas emissions when compared to standard diesel. Capital costs for a biodiesel plant are about half that for an ethanol plant and the current US capacity is approximately 60 million gallons/yr. Biodiesel tax incentives are expected to increase demand to about 124 million gallons/yr in the short term and by even higher amounts in the longer term, with B2 and B20 blends (2% and 20%) representing most of the usage. To meet this demand, many plants are being constructed with total industry capacity expected to be about 240 million gallons/yr by 2006.

Harnessing the *Populus* Genome

Stephen DiFazio (Oak Ridge National Laboratory)
Gerald Tuskan (Oak Ridge National Laboratory)
Jim Davis (University of Florida)
Timothy Tschaplinski (Oak Ridge National Laboratory)

Sequencing of the *Populus* genome will affect woody-plant biotechnology and biomass production. As the fastest growing woody plant in North America, this tree species represents an excellent potential biomass feedstock for biobased processes. *Populus trichocarpa,* the sequenced species, has a modest (and manageable) genome size and other characteristics that make it a good candidate for sequencing. The sequencing project—a collaborative effort involving many groups—will accelerate domestication of this species. With genomics, it is possible to bypass many breeding requirements and target specific traits, such as growth characteristics or disease resistance. One goal is to understand the many complex linkages between phenotype and genotype.

Populus has many traits that make it a potentially excellent species for domestication and use as a biomass feedstock. Currently capable of a growth rate of 5 dry tons/acre/yr, it is expected to undergo substantial yield improvements—projected at 13.5 dt/acre/yr—as domestication progresses. With genomics, the conventional cost vs. yield curve can be altered to show a sharper, more rapid decline in costs as yield increases. The ideal domesticated tree would be short (compared to current *Populus* stands) with a thick trunk as carbon is allocated to growth in diameter rather than height. Additional traits would include reduced branching, improved wood chemistry, pest resistance and improved photoperiod response. Current costs are about \$51/dt; with domestication this is expected to drop to \$21/dt.

In order to understand how yields may be improved, understanding is needed of carbon-allocation pathways and how they may be manipulated. Now that the genome is available, one approach is to elicit a phenotype response and observe changes in gene expression with microarrays. In an experimental system using *Populus* clones, a nitrogen response was stimulated by growing plants under varying levels of NH_4NO_3. This experiment revealed a diverse and coordinated response to nitrogen availability with nitrogen first accumulating in roots, then stems, and finally leaves; biomass accumulation was greater in shoots than in roots, resulting in a carbon increase in leaves and stems, but not roots. Cytokinins are crucial to regulation of this diverse response to nitrogen while manipulation of invertase may be promising for altering shoot/root carbon allocation.

Metabolomics is a developing discipline that can provide vital linkages between phenotype and genotype in *Populus*. Defined as the determination of metabolic consequences of a targeted change in gene activity, metabolomics represents the study of the exact end-products of biochemical processes. As such, when combined with genomics, transcriptomics, and proteomics, systems biology can be understood from genotype to phenotype. In *Populus*, random insertion of the *Bt* gene into three clones resulted in three very different metabolic profiles, which suggested a high sensitivity to the location of gene insertion. Similarly, some of the lines lacking a hexose transporter showed a two- to four-fold increase in phenolic acid conjugates. Thus, genetic insertion can have single or pleiotropic effects and stringent controls must be used for assessment of mutant phenotypes.

Energy from Biomass: Energy for the Future

John Sheehan (National Renewable Energy Laboratory)
Lee Lynd (Dartmouth College)
Yerina Mugica (Natural Resources Defense Council)

If biomass is to have a significant role in displacing petroleum and providing for US energy needs, it must receive a more serious commitment than it has in the past 30 years. In analyzing biomass possibilities and feasibilities, three major areas and their possibilities for change were considered:

- demand, where the assumption was rejected that it cannot be changed or controlled as demand (and supply) have technical and non-technical elements;
- supply, which involves rethinking the agriculture side of the equation; and
- technology state: avoiding comparison of "apples and oranges" where immature biomass technology is compared to mature petroleum processes—such a comparison doesn't paint a realistic picture, and thus a "high beam" perspective was used.

With the above concepts in mind, several biomass-technology options were analyzed using parameters resembling what they should be in their mature state. This analysis included both bio- and thermo-chemical processes with twenty-six combinations studied. Feedstock supply in a mature biomass environment was assumed to be switchgrass with a yield of 10 t/acre and a delivered price of \$40/ton. Processing plant size was between 5,000 and 20,000 t/day. Results from this ongoing analysis indicate that plants combining biological and thermo-chemical processes offer the best possibilities when considering energy-flow efficiencies and production-plant economics, which are very sensitive to plant size.

One question frequently encountered when considering biomass alternatives is whether or not they can actually meet our fuel or energy needs: how much land would be required and can enough biomass even be produced? Answers to these complex questions range from supporting biomass as a sound alternative to completely discouraging its use. Importantly, feedstock choice has a dramatic impact; future switchgrass energy yields of 155 GJ/acre compare favorably with corn (102 GJ/acre), and switchgrass has lower production costs and significant environmental benefits. However, some elasticity exists in how much land can be used and significant elasticity exists in how we use it, and other factors, mainly vehicle efficiency, can be markedly improved as well. By optimizing the system and looking at feasible mature technologies, biomass can likely meet most, possibly all, of our fuel needs if an aggressive policy is pursued.

Development of a large-scale biofuels industry has many potentially positive impacts, from reducing or ending US dependence on foreign oil to benefiting rural America's struggling economy. Cellulosic ethanol offers significant long-term benefits, but the debate should not be about corn ethanol versus cellulosic ethanol: corn ethanol is the current technology and is part of a biofuel scenario. WTO trade policies as well as future climate policies make biofuels look increasingly attractive both for the farmer and for the United States. In order to reach a goal of 1 billion gallons of ethanol production capacity by 2015, \$2 billion in investment would be needed between 2006 and 2015. A renewable fuel standard mandating specific amounts of cellulosic and total ethanol and greenhouse-gas policies internalizing greenhouse gases in the fuels market would provide steady drivers of demand.

Advancing the Forest Biorefinery

Bill Hancock (Diversa)
Arthur Ragauskas (Georgia Institute of Technology)
Lori Perine (American Forest and Paper Association)

Forests represent an enormous source of lignocellulosic material that could be used for production of fuel and chemicals. Although pulp mills represent an excellent existing platform for retrofitting, forest biorefineries have not yet been developed. Biotechnology includes tools to add value to the cellulose, hemicellulose, and lignin streams in a pulp mill, which will help biorefinery development. Finding and/or developing optimized enzymes is important. By using nature's inherent biodiversity and coupling it with ultrahigh-throughput screening, directed evolution and host engineering, new enzymes or enzyme mixtures can be customized for a bioprocess. These technologies are being brought to bear on the problem of rapid and efficient cellulose hydrolysis for forest biorefinery development.

If forest biorefineries are developed from existing pulp-mill operations, certain aspects must be taken into account. Perhaps most importantly, any added processes must first not interfere with the core business of pulp and paper production. In a sense, biorefinery processes must be carefully integrated to add new value streams while not disrupting old ones. Notably, cellulose would not be a candidate material for use since it is the key component of paper; some hemicellulose would also have to be reserved for paper production. Two fundamental approaches exist that would transform pulp mills into forest biorefineries: prior to pulping using a hot-water extraction process and post-pulping (after Kraft digestion) where the black liquor serves as a source of chemicals, followed by gasification of liquor residuals.

In recent years, the domestic forest products industry has suffered from stiff competition overseas, which has resulted in mergers, acquisitions, and significant downsizing. The American Forest and Paper Association formed the Agenda 2020 Alliance to examine the competitive issues facing the industry. The alliance has six primary platforms, one of which is focused on developing the forest biorefinery and has a goal of having one or more major demonstration facilities in place by 2010. However, biorefinery concepts represent a disruptive technology to a conservative industry that is capital constrained. The technology strategy consists of sustainable forest productivity for sustainable forest management, value prior to pulping by extracting useful wood components, and new value streams from residuals and spent pulping liquors.

Overcoming Challenges in Ligno-Cellulosic Fermentation

Hans van Dijken (BIRD Engineering)
Lee Lynd (Dartmouth College)
Jan de Bont (TNO)

For efficient and economically viable production of ethanol from cellulose, both glucose and xylose must be fermented in a relatively short timeframe. However, development of an organism capable of fermenting both of these sugars has been challenging: good xylose fermenters have trouble growing in acidic lignocellulosic hydrolysates while good glucose fermenters can grow in such an environment but do not use xylose. This has pointed to a major research need that may have been met with development RWB 218, a strain of *Saccharomyces cerevisiae* engineered to effectively ferment both sugars. Cloning of a newly isolated fungal xylose isomerase, coupled with additional genetic and evolutionary engineering has led to RWB 218, capable of fermenting a mixture of xylose and glucose.

Consolidated bioprocessing (CBP) may represent another significant step forward in reducing costs of production of cellulosic ethanol. The idea behind CBP is to create an organism capable of hydrolyzing cellulose and hemicellulose while fermenting the sugars released from the hydrolysis. Benefits include elimination of a separate cellulase production step, higher hydrolysis rates and potentially reduced contamination and yield loss and high solvent tolerance. Total costs could be reduced from \$0.19 to \$0.04/gallon ethanol. The thermophiles *Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum* are promising candidates for metabolic engineering, and acetate and lactate production. *T. saccharolyticum* knockout mutants have been created that show promise in realizing the potential of CPB. Work is ongoing in creating double knockout strains of both organisms.

Chemical toxicity, either from chemicals present in the fermentation broth or from fermentation end-products are important to consider in industrial settings since production efficiency or time-to-market can be affected. The "Log P" rule (logarithm of solvent concentration in octanol/water) can be used to predict the concentration of a compound in an organism's cell membrane; high membrane concentrations indicate compound toxicity. Effective efflux systems are important in enhancing tolerance of a microorganism to a given compound. Additionally, selection processes can result in organisms with markedly higher tolerance, after several generations, when compared to the original wild-type strain, but this technique is species- and substrate-specific.

Sustainability Issues

Are Healthcare Deal Structures Relevant to Industrial Biotechnology?

Michael Sundman (Sundman Business Development)
Hans-Peter Meyer (Lonza)
Jean-Marie Sonet (Proteus)

Traditional pharmaceutical-biotech collaborations may be suitable for in dustrial biotechnology in structure but not in the actual profit made on each alliance. Industrial biotechnology offers products that are more tailored to intermediate consumers, rather than end-users, making it difficult to obtain a high rate of return on any single product. Deals must be centered around making smaller amounts of money on a more diverse array of products, as opposed to the conventional one-product-large-profit model employed by many pharma-biotech alliances.

The variety of products from industrial biotechnology is broad. Fine chemical companies are beginning to employ white biotech processes due to needs for reduction in waste and pollution, and due to high expectations for large growth in the industrial biotech sector. There is no typical white biotech deal structure due to the heterogeneity of products and companies. The best approach is to start with a small project and extend the scope as confidence and trust grows within the partnership. The ultimate goal for increasing white biotechnology is continued innovation, particularly in new enzymes for new molecules.

Early-stage collaborations between white biotech companies and their industrial partners can have significant benefits, including reduced costs, extended product lifecycles and expanded IP portfolios. With preexisting market and application knowledge along with production and marketing capabilities, the industrial partner can help to optimize program aspects such as research, development, industrialization, production and marketing. Sharing trust allows the biotech company to benefit and aid in further development of the process: alternative routes and products and faster development timelines. These partnerships must establish market needs, project planning, execution, reporting, milestones, project leaders, technical committees, and legal provisions.

Public/Private Sector Cooperation to Develop Biobased Products Markets: The Federal Biobased Products Preferred Procurement Program

Marvin Duncan (USDA)
Ramani Narayan (Michigan State University)
Carl Muska (DuPont)

The Federal Biobased Products Preferred Procurement Program, designated under the Farm Security Rural Investment Act of 2002, states that federal agencies must purchase biobased products identified in this program, such as biobased hydraulic fluids, janitorial cleaning agents, industrial cleaning agents and gas and diesel additives. This program was designed to stimulate demand for new biobased products, increase demand for agricultural commodities, encourage development of processing and manufacturing in rural communities, capture environmental benefits and enhance energy security. Vendors and manufacturers of biobased products can benefit from this program by the creation of a preferred market, large-scale demonstrations of biobased products performance and use, and the development of new biobased products. Excluded from this program are products that are not reasonably available, fail to meet performance standards, are unreasonably priced or are produced outside the United States.

The issue of increasing levels of atmospheric carbon due to the use of petroleum-based products has been one of the main reasons why proponents have encouraged the transition to biobased products. Although petroleum is considered "organic," the problem with using petroleum-based products concerns the timeframe in which this organic carbon was fixed from the atmosphere. Biobased products are made of organic carbon that was fixed much more recently, making their use much more beneficial in terms of life cycle analysis (LCA), which is used to determine the biodegradability and recyclablity of a product. Carbon-14 signatures form the basis of a test method to qualify as a biobased material.

Standards for biobased products are required due to the rapidly growing industry and a need for a common set of definitions, methods and procedures. These standards also help consumers make informed choices. Dupont's Sorona® polymer is made from 1,3-propanediol, which can be manufactured using a petroleum-based process or by fermentation using corn. The corn-based process requires 40% less energy inputs and compares better via lifecycle analysis to the petroleum-based product. Sorona® can be used to make apparel, fibers, carpet and automotive components.

IP Issues Related to Trends in Agri-Business

Peter Mascia (Ceres)
Butch Mercer (Dow AgroSciences)
John Howard (Applied Biotechnology Institute)

Biotechnology can be used to improve every aspect of the crop value chain: production, harvest, storage, transportation, processing and sale. Enhancing the plant itself has become a major focus. First-generation genetics traits were developed to increase herbicide resistance, insect resistance, and viral-disease tolerance. Second generation traits, involving the translation of traits across species, are just now coming online. Large numbers of plant genes have been sequenced and now require functional determination. Ceres has filed patents on over 50,000 plant genes and 10,000 promoters.

There are endless opportunities in the field of plant-made biologics. New technologies must provide societal benefits and be economically viable. Because plant-made biologics require outstanding skills—scientific, academic, and business—and the intellectual property effort surrounding these new technology platforms spans boundaries of plant science and immunology, Dow AgroSciences is focused on the scientific discipline interface. Dow AgroSciences is committed to innovation, pursuing new scientific fields and recognizing the new value of intellectual property.

Recent progress in biotechnology has caused the creation of a new patent division focused on bacterial and yeast systems to produce industrial enzymes, therapeutics and vaccines, animal cell cultures to produce therapeutics and antibodies, eggs to produce vaccines, and plants. Thus far, focus has been mainly on improvements in plants, not the use of plants to produce functional proteins. Four functionally equivalent plant-synthesized proteins have been commercialized to date. The main challenge in plant-producing these proteins is economic; however, through innovation, better technology will not only decrease the cost of manufacturing these proteins, it will also increase the value of products made through plant biologics.

Industrial Biotechnology to Improve Your Triple Bottom Line

Joseph Cunningham (Industry Canada)
Chris Hessler (AJW, Inc.)

Technology Roadmap (TRM) is a process tool that helps identify key technologies that an industry, sector, or company needs. The TRM-management structure is led by an industry champion, with a principal goal of providing a measure of sustainability using a systems concept. Canada has a natural advantage in realizing its priorities of reducing greenhouse-gas emissions and enhancing national energy security due to the enormous amounts of marine, forest, and agricultural residues that, through technological advancements, can help to replace many currently used compounds that are considered toxic. Through a hands-off strategy, many other programs and organizations are being put in place to help industry promote sustainability and reduce global climate change. Government must keep up with industrial changes in order to keep policy in line with technology.

The report released by the Biotechnology Industrial Organization in June 2004—*New Biotech Tools for a Cleaner Environment*—utilizes OECD case studies on industrial sector pollution data from the pulp and paper, textile finishing, pharmaceutical and vitamin production, plastics and chemical production, and fuel production industries, to analyze the potential savings in energy costs, water pollution, air emissions, and reduction in petroleum usage by replacing traditional technology with biotechnology. The report found that public policies are not yet responsive to opportunities brought on by industrial biotechnology. In order to increase the support for industrial biotechnology in the public-policy sector, flexibility of policies must be increased, along with a strengthening of outreach programs and funding towards research. Product success will be a key element in fostering public support for industrial biotechnology.

Towards a European Strategy for Industrial Biotechnology

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Wim Soetaert (Ghent University)
Colja Laane (DSM)
Stan Roberts (Centre of Excellence in Biocatalysis)

The European knowledge-based bioeconomy encompasses all aspects of biotechnology, from feedstock production to biomass processing to material utilization. Recent European policy promoting biotechnology includes the Lisbon Strategy, which encourages sustainable economic growth, social cohesion and respect for the environment, and the Common Agricultural Policy, which advocates the safe production of high-quality agricultural commodities, food safety and animal welfare standards. Through these types of policies, the European Union hopes to improve public health, decrease local impact on climate change, raise awareness of nature and biodiversity, and increase renewable energy sources, all through the use of industrial biotechnology. A life-science and biotechnology strategy, which includes a thirty-point action plan, has been accepted by all member states.

A SWOT (Strengths, Weaknesses, Opportunities and Threats) Analysis of Industrial Biotechnology Research in Europe revealed existing barriers and potential advances in Industrial Biotechnology across the European community. Europe has a strong research sector, with an active industrial biotechnology community and top-level biocatalysis expertise, along with being the world's largest chemical producer, manufacturing one third of the chemicals produced globally, with a high degree of specialization, an excellent work force, and high environmental standards. However, there seems to be poor integration and cooperation between the European research community and industry, resulting in fragmentation and little coordination of research efforts. The regulatory framework is often slow, complicated, and variable among member states, with a complex patenting system. Other barriers to increased white biotechnology across Europe include the poorly developed biofuels sector, farmer resistance to changing agricultural policies, and high toll barriers for sugar and other feedstocks. Recent phenomena presenting opportunities for growth in the biotechnology arena include the high price of oil, increasing public acceptance, and a recent objective set forth to produce 20% of transportation fuels through renewable means by the year 2020. Particular threats to this growth are lack of funding, poor integration and cooperation among stakeholders and decreased oil prices.

The white biotech value chain in Europe consists of renewable feedstocks that can be converted to simple sugars, and subsequently processed to biofuels such as hydrogen and ethanol, biomaterials like polylactic acid, 1,3-propanedial, and polyhydroxyalkanoates, and biospecialities such as food ingredients, pharmaceuticals, and fine chemicals. The European Union's vision includes increasing the number of chemicals and materials produced through white biotech processes, increasing the proportion of biomass-derived energy, and increasing economic efficiency and the use of renewable resources. Significant technological, economic, and public-acceptance hurdles must be overcome in order to realize this vision.

The UK Center of Excellence in Biocatalysis, Biotransformations and Biocatalytic Manufacture has set up a new research center in Manchester, along with satellite laboratories equipped with 500- to 1000-L fermentors with the ultimate aim of exploiting biocatalysis for cost-effective conversion of simple feedstocks into high-value specialty chemicals, including pharmaceutical intermediates. Technology platforms include enzyme discovery, biocatalysis development, and process optimization, with a focus on biocatalysts for redox reactions and pathway engineering. Outreach will present many opportunities for industries to participate in directing research and company-company discussions.

Sustainable Biomaterials: The Path Forward

John Ranieri (DuPont)
James Stoppert (Cargill, Inc.)
James Barber (Metabolix, Inc.)

DuPont's energy goals for the year 2010 consist of reducing the production of greenhouse gases by 65%, to flatten total energy usage, to achieve 25% of revenue from renewable sources, and to source 10% of energy from renewable resources. In order to make the new paradigm of the integrated biorefinery a reality, Dupont is working in partnership with Pioneer, Diversa, and John Deere towards the production of low-cost feedstocks. The materials and energy, applied biosurfaces, and biomedical markets are key targets for DuPont's presence in the industrial biotechnology sector.

With the increasing volatility and higher prices of today's hydrocarbon markets, process efficiencies for bio-based products have improved dramatically as more dependable, renewable, and lower-cost feedstocks become readily available, especially in the United States. These products will ultimately be more dependable and economical for consumers and industry alike.

Metabolix is commercializing a family of natural plastics based on renewable resources that is comparable in properties to existing plastics and is widely applicable. The process by which these plastics are made is strongly patent-protected and will ultimately reduce reliance on fossil carbon, lower greenhouse-gas emissions, and lessen solid-waste generation. The current plastics market is growing at 5%/yr, a rate that traditional petrochemical plastic production will ultimately not be able to meet. Metabolix has developed two separate technology platforms to produce renewable plastics: the first, in conjunction with ADM, uses microbial fermentation, and the second produces natural plastics directly in crop plants.

A Worldwide Strategy Towards Sustainability

Christian Bertsch (German Parliament)
Harry Aiking (Vrije University)

Germany has a successful track record in the renewables sector as seen by the introduction and compliance with the Renewable Energies Act in 2000, which has created 130,000 new, sustainable jobs, and has made Germany a technological and market leader in the wind-power and photovoltaic sectors. In order to further the shift away from dependence on fossil-fuel resources, Germany has introduced the Renewable Resources Act to promote production and use of bioproducts. Similar to the Renewable Energies Act, the Renewable Resources Act uses a fixed-charged system and does not depend on long-term subsidies.

The PROFETUS research program set out to study the hypothesis that a transformation from meat to plant protein for human nutrition is more environmentally sustainable, technologically feasible, and socially desirable. To date, the study has concluded that the switch from animal to plant protein could feasibly have a 3- to 4-fold reduction of land use, greenhouse gases, pesticides and antibiotics, and a 30- to 40-fold reduction of water use and eutrophication. The main transitional barrier found in this study was general conservatism, in addition to other social, economic, and political forces. Due to the increasing price of meat, consumption is bound to decline, a transition that can be mitigated by innovation.

Energy and Environmental Issues in Industrial Biotechnology

Bruce Dale (Michigan State University)
Daniel Musgrove, Universal Entech
Gregory Bohlman, SRI Consulting

In calculating net energy values associated with ethanol production from biomass, system boundaries must be defined and careful attention paid to cropping scenarios. One study at Michigan State University revealed that bioconversion of switchgrass to ethanol had a higher net energy value, crude oil displacement, and greenhouse-gas reduction than using either corn grain or corn stover as a feedstock. Using E85 instead of E10 shifts the balance more in favor of corn-based ethanol production.

The economic barriers to commercializing bioethanol include collection and storage of low-density biomass, lignocellulosic pretreatment costs, net enzyme costs, and complexities of co-products. Using municipal solid waste (MSW) to produce ethanol introduces a potential cost benefit in that there is a well established infrastructure of MSW facilities, the potential reduction or elimination of pretreatment needs, and the low volume of byproducts produced. Universal Entech has an R&D project to demonstrate the unit operations of an MSW-to-ethanol process, gaining more knowledge of the mass/energy balance associated with the process, and assessing inhibitory and problematic issues. This continuous-flow ethanol reactor technology, called the CMSRS, operates on high-concentration feeds and allows ethanol removal from the vapor. Average cellulosic conversion rates over the span of the trial were 71% and sludge separation was not problematic.

Examples of biorefinery projects funded by the USDA/DOE in 2003 are sugars from lignocellulosics, an integrated corn biorefinery, corn-fiber separation and conversion, and a starch and biomass conversion pilot plant. Biorefineries are very capital-intensive, approximately three times more so than dry mills, but co-product revenues do introduce economic synergies. Process economic issues include feedstock cost and consumption, producing or purchasing of enzymes, recovery costs and market value of co-products, and waste-treatment costs. Solving the technical challenges associated with wastes such as lignin, gypsum, and wastewater will make these types of projects much more feasible in the future.

Bioprocessing: Business, Law and Ethics

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Tom Jacob (DuPont)
Leif Christoffersen (Diversa)
Stephen King (Napo Pharmaceuticals)
Jeffrey Kushan (Sidley Austin Brown and Wood)

The subject of bioprospecting brings up many complex issues for the pharmaceutical, biotech, cosmetics, and waste sectors, involving access limitations, concerns about conservation of biodiversity, misappropriation of resources and knowledge, concerns about prior informed consent upon access, and inappropriately issued patents. Attempts are now being made at an international level to mandate a new disclosure requirement that involves disclosure of source/country of origin of traditional knowledge and/or genetic resource, proof of prior informed consent, and proof of benefit-sharing agreement. The United States, along with Japan, is not in favor of the new disclosure requirements, but US applicants will be affected by them even if it does not alter its laws in accordance with the international modifications.

The Convention on Biological Diversity aims to promote the equitable sharing of the benefits gained by the use of genetic resources. The Bonn Guidelines presented a major step in establishing an inventory of potential measures. Risks of current circumstances are the potential failure to implement Bonn Guidelines, premature shift to mandates, and danger of parallel processes. Challenges shaping the access (to genetic resources) and benefit sharing (ABS) revolution will be addressed in Vienna in 2006, including country ABS regimes, the FAO's standardized Material Transfer Agreement, certificates of origin/source/legal provenance proposals, codes of conduct among commercial, public and non-profit entities, traditional knowledge and indigenous and local-community obligations, and patent-disclosure proposals.

Bioprospecting—often misconstrued as biopiracy—is the exploration of biodiversity for valuable genetic and biochemical resources. Diversa is collaborating with institutions in many countries, including Kenya, Ghana, and Costa Rica, to collect nucleic acid sequences from the environment and create gene libraries based on these sequences. Through these collaborations, Diversa is providing environmental metagenomics technology and training, research support, monetary and non-monetary benefits to the institutions and communities in which the bioprospecting occurs.

Napo Pharmaceuticals is establishing collaborations with indigenous villages to develop medicines based on locally isolated compounds. An agreement has been reached with a village in Samoa based on prostratin, a compound used by traditional healers to treat hepatitis. Important considerations that must be addressed when entering such agreements are: prior informed consent and access to benefit-sharing agreements need to be established both at the community and at the national level, and that western legal concepts and sense of equitability should not override or ignore the customary laws of local communities.

Bioprospecting and the equities surrounding it are trade and political issues that involve a significant activist community who are against intellectual property authorization, and rules of trade that do not properly regulate bioprospecting activity. Several developing countries—Brazil, India, Venezuela, Argentina, Egypt, *etc*.—are pushing for reforms under the aegis of the Convention on Biological Diversity. Pressure is building in Geneva to accept the patent-disclosure obligation presented by these countries. Industry is being portrayed as the major obstacle to addressing the concerns over bioprospecting and biodiversity in developing countries.

Assessing the Transition to a Biobased Economy

Chris Deane (Organization for Economic Cooperation and Development)
Bruce Dale (Michigan State University)
Jan E.G. van Dam (Wageningen University and Research)

The OECD is an organization of thirty member countries, seventy nonmember countries, NGOs and industry representatives that represent a forum for the collection and analysis of data, for debate, and for making internationally agreed policy recommendations and best-practice guidelines. The OECD division on biotechnology, providing the tools to analyze the future of biotech for stakeholders, published *The Application of Biotechnology to Industrial Sustainability* in 2001, a report that cited twenty-one case studies and concluded that biotechnology can reduce costs and environmental impact of industrial activities. The OECD will publish a report in 2006 addressing policy measures to be taken at the local level to leverage the transition to a bioeconomy, and key issues affecting this transition.

A life-cycle analysis (LCA) model was used to assess the material/energy inputs and outputs of both products and processes of biobased product systems. Some early conclusions from the analysis are: corn-stover removal provides less soil organic carbon but reduces soil nitrous oxide emissions and inorganic nitrogen losses by leaching; a winter cover crop enhances soil organic carbon, reduces nitrous oxide emissions and also reduces inorganic nitrogen losses by leaching; using lignin to generate electricity and steam further reduces crude oil use and greenhouse gas emissions; and sources of biomass must be local.

Major drivers for the transition to a biobased economy are the Kyoto Agreement, the decline of petrochemical resources causing market growth for renewable energy, concern over feedstocks for chemical industries, and consumer demand for more sustainable products. Currently, 50% of arable land is being used to produce food across the globe, posing the question of whether enough arable land remains to produce crops for energy and chemicals, taking into account population growth and improved standards of living. In the biobased economy, unlike the petroleum-based economy, there need be no waste. The entire crop be exploited to produce food, feed, energy, materials and chemicals.

New Opportunities Need New Business Models

Rick Norland (Thorington Corporation)
Yali Friedman (New Economy Strategies)
Rick Smith (Dow AgroSciences)

Many opportunities exist in rural communities to build businesses based on conventional bioproducts such as building materials, paper and textiles, and energy and heat produced from combustion of wood or other biomass, and on unconventional bioproducts like fuels, plastics, chemicals, and composites produced from biologically derived materials. Bringing these products to market requires a comprehensive evaluation of the technologies, products, and companies involved in the production process. Considerations of existing infrastructure, customer base, and product value must be made to ensure a strong business platform that will entice early-stage business investors.

New Economy Strategies works with community, academic, and entrepreneurial leadership to develop and help implement strategies to support regional development of technology-based economies by identifying target opportunities, organizing resources, creating portfolios of near- and long-term opportunities, and facilitating implementation of action plans. Working with venture-capital firms can be good for projects with large R&D requirements, capital-intensive growth, and near-term exit (3–5 years), but bad for basic science, due to often poorly defined paths to market. Biodiesel and bioethanol projects represent good markets for venture-capital investors. Locating a business in close proximity to venture-capital firms or to other startups is one key measure to ensure the best chance of obtaining interested investors.

Dow AgroSciences produces WOODSTALK™, a composite material made from wheat straw that can serve a variety of product lines, including the do-it-yourself, industrial, flooring and construction markets. The straw used in the production of this bioproduct is produced by over 450 farmers on 200,000 acres of land in Canada, representing the largest baling operation in the world. Notable attributes of the product are: it was rated a Top Ten Green Product in 2002 by Building Green, Inc.; it contains a formaldehyde-free, high-performance polyurethane resin; it is a low-emissions, low-VOC product made from 93% renewable wheat-straw fiber; its use reduces greenhouse gas and particulate emissions by diverting waste wheat straw from annual burning; and it represents a new cash crop for local farmers.

Novel Applications

Biohydrogen Production

John Benemann (Benemann Associates)
Jim Swartz (Stanford University)HideakYukawa (Institute of Innovative Technology for the Earth

Three main problems are associated with direct biophotolysis of water ($H_2O \rightarrow O_2 + H_2$) that must be addressed in order to produce sufficient H_2 for use in transportation:

- the low efficiency of the photosynthetic process,
- the generation of explosive O_2 gas, and
- the O_2 inhibition of H_2 production from hydrogenase.

One approach to solving these problems is to bypass O_2 production, thereby effecting an increase in H_2 production. This can be accomplished via a two-step process in which algae/cyanobacteria are used to fix CO_2 into storage polysaccharides that are converted into H_2 during dark fermentation. Further applied research with microalgae is encouraged because they have the potential to be a greater source of H_2 energy than oil, gas and coal combined.

Genetically modified organisms can be used to increase H_2 production during direct biophotolysis. The issue of O_2 inactivation of hydrogenases can be addressed by designing enzymes with tighter active sites to inhibit O_2 entry, as well as by using directed evolution to develop more-O_2-tolerant hydrogenases. Increasing the amount of reduced ferrodoxin (electron carrier) may also drive significant H_2 accumulation.

Genetically modified *E. coli* can be used as a biocatalyst of H_2 production from formate. The bacteria are grown to high density and are artificially inhibited, potentially by quorum sensing, and are used to continuously generate H_2 gas at approximately 200 L H_2/L/h. The advantage of this process is the ability to produce large quantities of product with a relatively small reactor.

Marine Biomes as a Source of Industrial Innovation

Robert Bidigare (University of Hawaii)
Eric Mathur (Diversa)
Mark Hildebrand (Scripps Institute of Oceanography)

The Center for Marine Microbial Ecology and Diversity (CMMED) is searching the oceans for novel compounds for treatment of cancer and infectious diseases. Two examples of compounds under investigation are ciguatoxin (CTX), a neurotoxin responsible for food poisoning, and beta-N-methylamino-L-alanine (BMAA), which has been linked to Alzheimer's disease. These studies may elucidate the genes responsible for toxin production and aid the development of broad-based screening methods.

Only 0.01–0.1% of microbes in seawater can be cultured *in vitro*. Diversa is developing high-throughput methods for culturing and screening recalcitrant microbes. Operating on the theory that organisms grow better when separated from competitors, single cells are isolated and incorporated into microdroplets and allowed to grow under various conditions on columns. Colony growth can be measured using flow cytometry, with signal varying with colony size. Diversa is also working on non-culture based methods of cloning and characterizing genes for subsequent screening to identify novel compounds.

Diatoms are unicellular photosynthetic algae whose cell wall is composed primarily of silica. Their small size and complex 3-D structures make them ideal for generating low cost micro/nanoscale structures rapidly and with high fidelity. Studying the genes responsible for silica wall deposition may allow the generation of tailor-made nanoscale particles with practical applications in coatings, ceramics, chemical catalysis, information storage, optics, and microtags.

Nanobiotechnology: Thinking Globally and Acting on a Very Local Scale

Harold Craighead (Cornell University)
Shi-You Ding (National Renewable Energy Laboratory)
Michael Ladisch (Purdue University)

Nano/microscale fabrication offers the opportunity to examine single-molecule behavior in real time. Microfluidic channels and photon-counting optics coupled with single-molecule labeling can be used to track molecule-binding events or obtain relative molecular sizes. Other applications of nanotechnology include nanoscale-size exclusion separations and tracking particle diffusion in microwells.

The National Renewable Energy Lab (NREL) is using quantum dots to study nanoscale chemical reactions. Of particular interest are quantum-dot interactions and how quantum dots can be used to characterize enzyme/substrate kinetics. One application of quantum dots is the visualization of cellobiohydrolase I (CBHI) processivity along a cellulose molecule. Work with microcantilevers will quantify the force necessary for enzyme processivity.

Nanoscience involves the design and production of flexible/adaptable structures no larger than 100 to 200 nm in one dimension. Combining biomolecules with solid substrates can be used to produce portable biosensors. Microfluidics coupled with derivatized microchannels can be used to screen small quantities of dye-labeled proteins, with unknown function, for selected affinities. Microfluidic techniques will have many practical applications in drug discovery.

Novel Exploration and Discovery in Diverse Marine Biomes

David Kyle (Advanced BioNutrition)
Mike Lyon (Deep Ocean Expeditions)
Govind Nadathur (University of Puerto Rico)
David Drahos (Novozymes)

Aquaculture requires the use of fishmeal or fish oil, but natural catches do not meet the commercial demand. Substitution of fishmeal or fish oil with omega-3-fatty acids (DHA), derived from algal fermentation, is sufficient to sustain growth. Fish require probiotics or vaccines to help promote growth and protect against infection. A novel micromatrix system in which the probiotic is encapsulated in an alginate matrix is used to ensure post-gastric delivery of compounds without degradation in the stomach. The micromatrix constricts in low pH environments (stomach) and is relaxed to release probiotics in high pH environments (intestine). The future of this technology is to incorporate probiotics into infant formula.

Less than 2% of the world's oceans have been explored with most of those regions near wealthier countries. Even more surprising, better maps of Mars have been compiled than of Earth's ocean floors. Deep Ocean Expeditions is working in conjunction with the P.P. Shirshov Institute of Oceanography and the Russian Academy of Sciences to organize The New Challenger Global Ocean Expedition. The expedition will use the largest fleet of underwater submersibles for a mission of this kind. The group will study previously unexplored regions of the ocean with an emphasis on discovering new organisms and ecosystems. The Expedition's submersibles were used for the filming of the new IMAX film "Aliens of the Deep."

Several marine organisms display high tolerance of extreme saline conditions. Work with the yeast *Debaryomyces hansenii* has led to the discovery that glycerol phosphate dehydrogenase gene (GPD1) is induced under high salt conditions. Mutations of GPD1 have been identified that cause higher levels of induction. The ultimate goal of this work is to fuse genes of interest with salt-inducible promoters to produce high levels of protein expression for drug molecules, industrial enzymes, growth hormones, *etc*.

One of the biggest problems associated with large-scale shrimp farming is the toxic accumulation of ammonia and nitrite in ponds, causing decreased shrimp size and higher fatality rates as a result of disease. A consortium of bacteria, *Nitrosomonas eutropha*, (capable of oxidizing ammonia) and *N. winogradskyi* (oxidizes nitrites), when added to shrimp ponds dramatically decreases accumulation of toxic nitrogenous compounds.

Can You Build a Solid Product on a Wet Protein?

Karl Sanford (Genencor)
Jonathan Dordick (Rensselaer Polytechnic Institute)
Ken Sandhage (Georgia Institute of Technology)

Genencor is working to create protein polymers, specifically designer proteins that link discrete amino acid sequence modules with known properties. For example, silk proteins may be combined with elastin sequences to create polymers with unique properties. This particular polymer is capable of forming clear films or gels and is non-allergenic. The ultimate application of this technology would be to combine silk proteins with antimicrobial peptides and generate nanofibers to make antimicrobial textiles with practical applications in the medical field.

Functionalized nanostructures can be designed to incorporate biological molecules with specific activities. Binding proteins to nanostructures has the advantage of protecting enzymes from denaturation under conditions that normally cause proteins to unfold. Functionalized nanostructures can also force proteins into specific orientations. These functionalized nanostructures can be used to manufacture antifouling materials that can self-clean and detoxify.

BaSIC (bioclastic and shape-preserving inorganic conversion) is the process by which the chemical composition of diatom exteriors, silica, is converted to another compound without altering its 3-D shape. Diatoms are excellent tools for generating micro/nanoscale structures because they can create complex 3-D structures with high fidelity. Diatom silica has been replaced with magnesium, titanium, and mixtures of europium/barium and titanium. Some of these new structures are capable of photoluminescence and could be used in optical devices, micro-sensors, or hi-definition television production.

Nano-Biotechnology as an Emerging Platform Driving Innovation in Material and Electronic Applications

Eric Mathur (Diversa)
Dan Luo (Cornell University)
James Turner (State University of New York at Albany)

The gene CanA has been isolated from a hyperthermophilic archaeon. The protein product of CanA, in association with cations, self-assembles into hollow nanotubes (named Nanodex™). The nanotubes have potential use in separation of racemic mixtures because they are inherently chiral. Functionalization of the nanotubes may allow their use in antigen presentation, drug delivery, or medical device implantation.

DNA molecules have been synthesized as building blocks for nanobarcodes. DNA is an attractive material because it self-assembles, is stable, is non-toxic, and is water-soluble. Several DNA strands can be assembled into single Y- or X- shaped molecules. Dendrimer-like DNA (DL-DNA) can be constructed from these building blocks using ligases. With the addition of different fluorochromes and probes (DNA, RNA, protein) to DL-DNA, nanobarcodes can be created. The ratio of various fluorochromes to one another indicates the population size of a particular component in a complex mixture.

Nanofiltration membranes composed of alumina or cellulose acetate can be designed with controlled filtration characteristics, including flow and molecular-weight cutoff. These nanofilters can be used in the purification and enrichment of DNA from blood, where heme normally inhibits PCR amplification. Nanofilters have been used also in the study of Parkinson's disease. Employing the filters to separate neuronal cells from microglial cells led to the conclusion that small reactive molecules and not cytokines are responsible for neurone death.

Marine Biotechnology: Promising Drugs from the Sea

William Kem (University of Florida)
Frank Mari (Florida Atlantic University)
Peter McCarthy (Harbor Branch Oceanographic Institution)

Paranemertes peregrina is a nemertine worm that is a carnivorous predator that uses venom to paralyze its victims. The venom contains several alkaloid compounds; one particularly interesting alkaloid is anabaseine, which is valuable because it is an agonist of nicotinic receptors (nAChR). Anabaseine is non-specific in its reactivity towards the various types of nAChRs; therefore, the alkaloid is being used as a reference for designing other specific nAChR agonists. DMXBA is one such agonist showing promise in treating schizophrenia.

Conopeptides are small, stable, and highly modified peptides present in the venom of cone snails. Cone snail venom composition varies between species, resulting in more than 100,000 bioactive conopeptides available for study. They have applications in treating conditions ranging from chronic pain to epilepsy. The first FDA approved drug from a marine source is Prialt® (for the treatment of chronic pain), a w-conotoxin, which was isolated from cone-snail venom.

Sponges are a potential source of a variety of lead drug compounds. They are also host to large numbers of microorganisms. Research suggests that compounds attributed to marine invertebrates may actually be derived from their microbial symbionts. Many microbial isolates are difficult to culture; they are fermented on a small scale using multiple types of marine media containing chitin or kelp. Extracts are then taken from the fermentation process and tested for activity, including antimicrobial and anticancer properties.

Impacts of Morphology and Strain Development on Fungal Fermentation

Richard Burlingame (Dyadic)
Mark Marten (University of Maryland)
Linda Lasure (Battelle Pacific Northwest National Laboratory)
Peter Punt (TNO)

Dyadic works with a commercial strain of *Chrysosporium lucknowense* fungus that produce alkaline cellulases. The strain has been selected for increased protein expression and low viscosity. The low viscosity is due to the fact that the fungi do not form large filamentous clumps but rather discrete units termed transferable elements. Lower viscosity results in higher aeration, increased nutrient transfer, and greater levels of automation for microtiter assays/culturing.

The colony morphology of the fungus *Aspergillus oryzae* can be altered based on nutrient input. Pulsing glucose into the culture medium instead of continuous feeding leads to smaller, low-viscosity, fragmented cells. The altered cell morphology—generating higher production of industrially relevant enzymes—is thought to induce the fungus into sensing an onset of starvation (between glucose pulses) at which they begin to self-metabolize older filaments. In the fermentor, these partially hydrolyzed filaments break away resulting in smaller cell size.

Work on *A. niger* is focused on determining the genes responsible for generating pelleted cell morphology (no filaments). Low levels of Mn^{2+} are critical for maintaining the pelleted morphology. Using global proteomics studies incorporating mass spectrometry, ubiquitin homologs have been identified as being upregulated in the pelleted cell morphology.

Fungal proteases can decrease production of industrial enzymes by degrading proteins as they are produced. Mutagenesis studies have been conducted to select for low-protease strains of fungi. Large-scale screening of low-protease strains has been made possible with a suicide-selection process. Vacuolar proteases are believed to negatively affect protein yield.

Department of Defense Initiatives and Activities in Industrial Biotechnology

James Valdes (US Army)
Morley Stone (Defense Advanced Research Projects Agency)
John Frazier (Air Force Research Laboratory)
Harold Bright (Office of Naval Research)

The US Army is interested in applying biotechnology to non-traditional tasks including sensors, fuel, armor, and camouflage. Biopolymers are being examined as a means of stabilizing soil for landing planes, flexible body armor is being developed to protect extremities, and ways of utilizing waste generated by soldiers as biofuels are being investigated.

DARPA's mission is to prevent technological surprise from harming national security and towards that goal they are aiming to advance defense systems with biology. Project areas include designing robots to mimic animal behavior or attributes (octopus arm, bug climbing wall) and using bees to sense explosive devices.

The goal of the biotechnology program of the Air Force Research Laboratory is to understand and exploit life forms and biological systems at the molecular level to provide revolutionary products. Research is being done in nanofabrication for filtration systems to detect and prevent contamination of fuels. In addition, self-assembling bioconstructs are being investigated with tailor-made sensors, as are biopolymers containing thermal sensors and dynamic coloration (for use in camouflage).

The Office of Naval Research has projects in a variety of areas including biosensors, energy-harvesting, microbial "green" synthesis of materials, next-generation antibiotics, microbial fuel cells, biosynthesis of precursors to TNT, and optical biosensors to replace antibodies.

Advanced Genomics as a Powerful Tool for Innovations in Industrial Biotechnology

Jeroen Hugenholtz (Kluyver Centre for Genomics of Industrial Fermentation)
Uwe Sauer (ETH-Zurich)
Han de Winde (DSM)
Eric Johansen (Christian Hansen)

Kluyver Center uses several genomics approaches in its research to optimize industrial fermentations. Focus is on lactic acid bacteria and their role in starter cultures for making cheese. The enzymes involved vary among lactic acid bacteria and selection methods have been developed to identify mutants that may be used in new cheese starter cultures. Other areas of work include boosting the level of folic acid production from a strain of lactobacillus, studying vitamin B-12 production from lactic acid bacteria, and modifying a strain of yeast to ferment xylose.

The fluxome is the pattern of flow through metabolic pathways in a given organism. C13-labeled molecules can be used as substrates for tracking of compounds through metabolic pathways. Large-scale flux networks have been designed to show responses of the fluxome as a product of genetic perturbations. *Bacillus subtilis* is the current model system for studying the fluxome. Key issues relate to events occurring at metabolic branchpoints and changes in redox-cofactors in response to flux perturbations. Fluxome studies have identified a novel gene regulator (CcpN) that can be modified to improve recombinant riboflavin production in *B. subtilis*.

DSM is studying the genes induced in *A. niger* in response to protein-production stress in an effort to optimize strains for industrial enzyme production. Under stress, protein is synthesized faster than it can be folded and secreted; consequently, proteins are misfolded and degraded. Two-D gels coupled with mass spectrometry have identified a number of proteins involved in vesicular trafficking and folding, as well as endoplasmic reticulum chaperones that may be responsible for the cell's stress response. Similar techniques were used to evaluate genes involved in central carbon metabolism in *Saccharomyces cerevisiae* to determine what controls the fermentative capacity of yeast.

Lactobacillus lactis starter cultures, used in the dairy industry, have traditionally been produced by anaerobic fermentation. Recent work culturing the bacteria under aerobic conditions (with the addition of heme and O_2) has led to an increase in cell biomass with subsequent benefits in starter cultures. Microarray analysis has been used to compare gene expression in the anaerobic and respiring cultures to identify the metabolic processes responsible for increased biomass production.

Specialized and Radio Frequency Assisted Fermentation

John Carvell (Aber Instruments)
Steven Splinter (Radient Technologies Inc.)

Radio-frequency impedance (RFI) can be used to obtain real-time biomass measurements in fermentation systems. RFI probes subject cells to low-frequency radio waves and then measure the resulting capacitance of the fermentation system. The signal varies as a function of live biomass. This technology is valuable to brewing because the concentration of yeast at the beginning of the fermentation process is critical to the composition of the final product.

The microwave-assisted process (MAP) is a novel extraction technique used for increased recovery of natural products from biomass. Microwaves are used for selective heating of moisture contained within the natural product of interest. Localized heating is rapid and causes a build-up of pressure within the biomass. The pressure drives the natural product from the biomass into the surrounding solvent. In addition to the increase in natural product recovery, the technique allows faster extraction with reduced energy and solvent usage. It is being developed for antioxidant extraction from rosemary herbs, removal of essential fatty acids from oil seeds, and extraction of pigments from microalgae.

Advances in Bioprocessing

Progress in Bioethanol Commercialization Using Wheat Straw and Corn Stover

Kevin Wenger (Novozymes)
Joel Cherry (Novozymes)
Quang Nguyen (Abengoa Bioenergy)

A stepwise commercialization approach (from utilization of grain starch to fibers to lignocellulosic biomass) is required for the increasing bioethanol industry. Although cellulase production and cellulose hydrolysis have received much attention, collection, pretreatment, hydrolysis, fermentation, and recovery are all important aspects of economic cellulose conversion. Developments in each of these areas can have significant impacts on the other processes. Keys for successes in process improvements include partnering between companies with different expertise and working synergistically with existing technologies such as corn dry milling.

Enzymes accounted for approximately 80% of bioethanol production costs in 2000; therefore, Novozymes used a wide array of biotechnology tools to improve fungal strains and enzyme activity for cellulose conversion. They were successful in reducing enzyme loading by a factor of six for the same glucose-conversion levels and brought enzyme costs down to an estimated \$0.10/gallon ethanol. Further work includes production scale-up, process integration, and capital-cost reductions. It was noted that performance was significantly affected by minor pretreatment changes and source of corn stover.

Cellulosic biomass will be an important feedstock for bioethanol production but utilization is challenged by the feedstock complexity, severe pretreatment conditions, relatively slow and expensive hydrolysis, and co-product development. Such barriers need to be addressed to reduce operating and capital costs associated with cellulosic ethanol production. Improvements can be made by: improving feedstock processing to reduce contamination; developing on-site enzyme-production facilities; increasing yields by decreasing inhibitors and developing robust organisms; decreasing capital costs through continuous high-solids processing using proven equipment in an integrated process; and through the development of high-value co-products.

Pacific Rim Developments in Industrial Biotechnology

Bernd Rehm (Massey University)
Yusuf Christi (Massey University)
Trevor Suthridge (Forest Research)

Biotechnology has been identified as a key growth area in the New Zealand economy. There is a particularly strong agricultural industry with 40 million cows and 60 million sheep and a uniquely high standard of animal health. The biomedical sector is growing rapidly and many biotechnology companies starting as university- or government-research spin-offs.

Protein and metabolic engineering tools were used to control production of polymers and surfactants from pseudomonads. A Massey University spin off company used such techniques for the design of biopolymer-based nanoparticles for drug delivery, drug targeting, and protein production. Bottlenecks in alginate (valuable compounds for use in food additives and pharmaceuticals) expression were identified using gene-knockout technology with specific enzyme subunits.

Microalgae and cyanobacteria can be used for the production of high-value chemicals such as neutraceuticals, pharmaceuticals, insecticides, pigments, *etc*. A closed tubular photobioreactor cultivation system is an alternative to the shallow, open-system raceways for organisms grown in relatively non-selective environments. Knowledge of growth kinetics, photoinhibition, and the system fluid mechanics were used to model biomass production as a function of reactor geometry, location, season, and time of day. High capital costs for such reactors require production of high-value compounds.

Utilizing existing infrastructure in wastewater-treatment facilities, zero-value wastes can be used for the production of value-added chemicals and biopolymers. Eco-Smart Technologies considers the environmental footprint and long-term sustainability in designing novel microbial production systems. Their systems overcome existing technological barriers by designing for low energy requirements, low- or zero-value feedstocks, and robust mixed cultures without using genetically modified organisms.

Lignin for Commercial Products

Frank Guffey (Western Research Institute)
Bozena Kosikova (Slovak Academy of Sciences)
Mary Lopretti (University of Uruguay)

Lignin can be mixed with asphalt at concentrations as high as 7% to increase roadway life by 40 to 100% without affecting other important roadway properties. Although refinery analyses estimate lignin value at \$35 to \$50/ton based on its fuel value, its use as an additive to asphalt could be worth \$260 to \$800/ton. Benefits include decreases in highway maintenance costs, increased highway service life, and an added revenue stream for biorefineries using lignocellulosic feedstocks.

Research was conducted to explore the use of lignin from the wood-pulping industry as an alternative antioxidant to commercial phenolic compounds. Lignin supplementation was shown to decrease oxidation of polypropelene. Further, experiments on lignin-feed rates showed that lymphocytes and testicular cells exhibited similar resistance to oxidative damage.

Kraft lignin, a by-product of the paper industry, can be used for the manufacture of industrial polymers such as polyurethanes and resins. Various fungi (*Phanerochaete chrysosporium, P. ostreatus* and *Gloeophyllum trabeum*) were used to convert kraft lignin to monomers and co-monomers at 1-L and 50-L scales. Physicochemical characterization of the kraft lignin structure throughout fermentations was done using gel permeation chromatography, infrared spectroscopy, and UV spectroscopy

Transforming the Ethanol Industry—The Bridge to Biomass Ethanol

David Johnston (US Department of Agriculture)
Bill Dean (Genecor)
Larry Russo (US Department of Energy)

New corn-milling processes have been developed that incorporate enzymes into traditional wet-milling and dry-grind processes. The enzymic wet-milling process leads to higher starch yields and reductions in usage of SO_2 and water, process time, and overall costs. Enzymic dry-grind ethanol processing allows the removal and recovery of co-products such as germ, pericarp fiber and endosperm fiber prior to fermentations. Removal of these constituents yields increases in fermentation capacity and compositional improvements in distillers dried grains and solubles (DDGS).

Genencor has worked to reduce costs of saccharification for cellulosic ethanol production through improvements in enzyme-production economics (\$/g enzyme) and enzyme performance (g enzyme/gal ethanol). Process improvements have brought enzyme costs down to \$0.15–\$0.30/gal ethanol with additional cost savings still expected. No-cook ethanol processing will lead to energy savings, capacity increases, inhibitor reductions, cost reductions, and revenue increases from value-added co-products.

Goals of the Biomass Program within the US Department of Energy include reducing dependence on foreign oil and creating new industries within the United States in the form of integrated biorefineries. Current barriers include feedstock characterization, infrastructure, and cellulose-conversion technologies. The government cost-sharing program is set up so that government sponsors a relatively large portion of the high-risk, bench-scale research activities with decreasing contributions for developed lower-risk, pilot- and demonstration-scale projects. Unfortunately, discretionary budgets within DOE have been decreasing with increasing earmarks, causing a further barrier to research in this area.

Transitioning into a Biobased Economy: From 3G to the Future

Ka-Yiu San (Rice University)
John Pierce (Dupont)
John Frost (Michigan State University)
Oliver Peoples (Metabolix)

Metabolic engineering approaches for increasing succinate production from *E. coli* in both aerobic and anaerobic environments were presented. Succinate is typically produced only as an intermediate in the TCA cycle in aerobic growth, but this pathway was altered to yield average productivities of >1g/L/h with minimal byproduct formation. Manipulation of the anaerobic pathway for succinate production yielded increases in productivity to 1.6 g succinate/ g glucose consumed. Similar technology could be used to improve production capability of other valuable compounds such as fumarate and malate.

In the complex field of biobased products, picking the right target products can be difficult, but is critical for effectively managing production costs and revenues. The technical complexity of biomass conversions requires a multi-disciplinary approach and partnerships are essential to compete in the bioproduct and biomaterials fields. Process chemistry, engineering, and material science need to be integrated in order to deal with the unique challenges posed by biological production.

Phloroglucinol derived from the fermentation of glucose using *E. coli* can be a flexible substitute for benzene as a starting point for organic chemical production. Microbial synthesis of phloroglucinol is important to the manufacture of triamino-trinitrobenzen (TATB), a thermally stable, highly energetic material. Phloroglucinol is also used in production of resorcinol, widely used in resin and adhesive formulations.

Metabolix has used metabolic engineering techniques and large-scale bioprocess engineering to control production of polyhodroxybutarate (PHB) copolymers with a broad range of material properties (thermostability, stiffness, *etc*.). The robust technology allows the production of alternatives to synthetic plastics within bacterial cells from renewable feedstocks at costs of $2/kg. Production of polyhydroxyalkanoates (PHAs) in plants will bring costs down further and could serve as an ideal biorefinery co-product. It is estimated that 20 acres of switchgrass could yield up to 10 tons of polymer per harvest.

New Frontiers in Oil Seed Engineering

Duane Johnson (Montana State University)
Toni Slabas (University of Durham)
Jack Grushcow (Linnaeus)

Canola oil was used as a model to determine the chemical composition required for a biobased oil to function in engine applications. By increasing the oleic acid composition, oxidative stability increased. The addition of hydroxyl fatty acids and wax esters enhanced lubricity and minimized cold-engine friction, respectively. These advances show the potential for modified biobased oils to outperform conventional oils.

Vegetable seed oils show a great degree of diversity in chemical structure and function and are an increasingly attractive feedstock for the oleochemical industry. Information about the genomics, proteomics, and enzyme-functional selectivity in oil-seed production is being used to garner information about the biosynthesis of unusual fatty acids with potential high-value as industrial feedstock. This approach should lead to increased production of these fatty acids in seeds by the selective introduction of new biosynthetic pathways into plants.

Castor oil has excellent material properties and >1,000 patented applications but has problems; its use in the United States is limited in that it is difficult to grow in temperate climates, and extraction yields the highly toxic and potentially bioterrorist weapon ricin. By genetically engineering conventional oilseed crops to express castor oil, a safe dependable supply of this valuable oil would be available for industrial purposes. Biobased lubricants can provide significant reductions in emissions of CO, CO_2, hydrocarbons, and NO_x, and improvements in vehicle fuel economy. Regulatory issues remain as the largest bottleneck to the use of modified canola for production of castor oil components.

Advances in Fuel Cells

Ron Cascone (Nexant)
Rose Ritts (PowerZyme, Inc.)
Shelley Minteer (Saint Louis University)

Although there is skepticism about the viability of a hydrogen economy, fuel cells (FCs), and biobased FCs in particular, have the potential to make cleaner, cheaper, and more efficient electricity. The vehicle market is greater than three times the residential, commercial, and manufacturing needs for FCs. There are several potentially viable options for biobased FCs including the utilization of enzymes, whole microorganisms, biofuels and biobased hydrogen in various designs.

An enzyme embedded in a perforated membrane transfers protons yielding a power flux of >200 mW/cm^2. With this power density, a 70-cm^2 membrane could power a laptop computer and serve as a replacement for conventional lithium batteries. Possible future markets for this technology include portable power for everyday electronics, energy-scavenging systems, and transportation.

Microbial FCs provide complete fuel oxidation, yielding high conversion efficiency with low power density, whereas enzymic FCs, which do not completely oxidize fuel, have low efficiencies but high power density. A novel approach to immobilize enzymes at the electrode surface can extend enzyme lifetimes in biofuel cells. Recent research in enzymic biofuel cells shows possibilities for small scale, portable power applications.

Biobased Chemicals and Materials: From Bioethanol to Secondary Metabolites

Lonnie Ingram (University of Florida)
Matthes Koffas (State University of New York at Buffalo)
Hal Alper (Massachusetts Institute of Technology)

Using agricultural residues as feedstock, up to half of the imported petroleum could be replaced by renewable biobased products. Other possible feedstocks include biosiolids, municipal solid waste, and dedicated energy crops. Barriers to cellulosic energy conversion include: high risk and cost involved in a first-of-a-kind full-scale plant; uncertainty in petroleum prices; and co-product development. Other technologies, including increasing fuel efficiency, will be needed to reduce our growing need for imported petroleum products.

Plant metabolic pathways for the production of antioxidant flavonoids have been engineered into *Escherichia coli* and *Saccharomyces cerevisiae* to increase production capability and reduce downstream processing. Yeast was shown to be a significantly better production platform with yields up to ten-fold higher than in *E. coli*. Biosynthesis of several novel flavonoids, with possible improved function, was demonstrated with recombinant strains.

Systematic (model-based) and combinatorial (transposon-based) models were used for the identification of gene knockout targets to increase lycopene biosynthesis in *E. coli*. These search strategies yielded distinct gene sets afecting product synthesis through the increase in precursor molecules or other regulatory mechanisms. Exploration of the possible combinations of gene knockouts found in the two search methods yielded sixty-four knockout strains including two with an eight-fold production increase over the wild-type strain and a two-fold increase over the engineered parental strains.

The Role of Biotechnology in Improving the Health Benefits and Nutritional Value of Foods

Egon Bech Hansen (Danisco)
Mark Hanover (Tate & Lyle
Mark Zoller (Senomyx)

Recognizing obesity as a serious public health concern in many western countries, Danisco is looking for ways to help the food industry do their part to curb this problem through the development of food ingredients with well recognized health benefits. Technologies employed in the development of such products include intestinal microbiology, intestinal immunology, genomics, and nutrigenomics. Examples of products include prebiotics, probiotics, and enzymes allowing a wider use of whole grain flours.

Biotechnology tools can be used to improve the taste, appearance, shelf-life, and nutrition of foods, thus benefiting farmers, consumers, developing nations, and the environment. Much of the advancement in the market of functional foods is being driven by health and wellness concerns. Despite the promise and potential of these new products, questions remain concerning food and environmental safety, ethics, and government regulation.

Senomyx is utilizing biotechnology for the discovery and development of novel flavors, enhancers and taste modulators, allowing food processors to improve the nutritional quality of their products while retaining or enhancing taste. Flavor enhancers allow reduction in sugar, salt, or MSG without sacrificing the taste associated with each. By incorporating taste receptors into the screening process, increased throughput in the product screening process is possible by a factor of 200–300, thus decreasing overall product-development time. Olfactory receptors are much more varied and complicated, and have not been used in a similar manner thus far.

Each group stated that although consumer acceptance is important to the functional food market, this type of marketing was not the role of these companies. It was acknowledged that America and Americans tend to be more accepting of new food products for a variety of cultural, economic, and political reasons.

Student Papers

Scott Pryor (Cornell University) *Optimization and Modeling of Antifungal Lipopeptide Production during the Solid State Fermentation of* Bacillus subtilis

Response-surface modeling was used to optimize production of the antifungal lipopeptides iturin A and fengycin by *Bacillus subtilis* grown on wheat middlings in 0.5-L solid-state fermentation bioreactors. The addition of peptone and nitrate supplements and extra pH buffering had either negative or negligible effects on reactor performance. Maximum production occurred at the highest substrate moisture content and lowest reactor incubator temperatures with increases of 210% and 220% in production of iturin A and fengycin, respectively, under optimized conditions.

Reza Yegani (Kobe University) *Effects of Illumination Conditions on Growth of Photosynthetic Cells*

Stability of *Rhodobacter capsulatus* growth rates was studied in semi-continuous photobioreactors. The growth rate was modeled as a function of light intensity with a Monod-form equation and maximum growth rates were found to decrease with increasing cell concentrations. High growth rates could be sustained with supplemental illumination on the rear side of the flat-plate photobioreactor or with ferrous ion supplementation.

Lizbeth Laureano (Michigan State University) *Factors that Limit Enzymic Hydrolysis of Biomass*

Spectroscopic analysis was done to determine crystallinity, lignin, content, and acetyl content of poplar-wood samples and to model their effects on hydrolysis yield. These factors have been cited as important in the enzymic digestibility of lignocellulosic materials. Multilinear regression and principle component regression (PCR) techniques were used to predict glucan conversion based on spectral data. PCR yielded a better fit.

Wei Liao (Washington State University) *Fumaric Acid Production from Agricultural Residues Using Pellet-Form* Rhizopus oryzae *(ATCC 20344)*

Fumaric acid, a compound with a wide range of industrial applications, was produced in liquid fermentations using pelletized Rhizopus oryzae grown on pretreated animal manure. As *R. oryzae* growth can be controlled under nitrogen-limiting conditions, it is critical to separate the solids stream from the nitrogen-rich liquid stream. The pelletized form of the fungus yielded high fumaric acid production than did clump or filamentous morphologies.

Yan Liu (Washington State University) *Enhanced production of L(+)-LacticAacid by Repeated Batch Culture of Pellet-form* Rhizopus oryzae *(NRRL 395) with Cull Potatoes as Raw Material*

Pelletized R. oryzae was grown in 5-L liquid fermentors with a cull-potato substrate for the co-production of chitin and lactic acid. Pelletized fungal morphology led to a two-fold increase in lactate production over the clump or filamentous morphologies because of decreased mass-transfer limitations. In repeated batch cultures, the fungal-pellet size increased slowly allowing increases in lactic acid productivity. Pellet size and fermentation broth dissolved oxygen were found to be important operating factors for lactic acid production.