Summary Proceedings

Third Annual World Congress on Industrial Biotechnology and Bioprocessing

Linking Biotechnology, Chemistry and Agriculture to Create New Value Chains

Toronto, ON, July 11–14, 2006

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Industrial Biotechnology and Bioprocessing

Not for Sale

Foreword

The *Third Annual World Congress on Industrial Biotechnology and Bioprocessing* convened in Toronto, Ontario, July 11–14, 2006, organized by the Biotechnology Industry Organization (BIO), the American Chemical Society (ACS), the National Agricultural Biotechnology Council (NABC), AGRI-FOOD Canada, BIOTECanada, the Chemical Institute of Canada (CIC), and EuropaBio. Some 200 presentations were made in five plenary and six parallel "break-out" sessions, and eighty-two presentations were made as posters. There were 1,135 attendees, an increase of 60% over the Second World Congress in 2005. The *Fourth Annual World Congress* is scheduled for March 21–24, 2007, in Orlando, FL.

The chief organizers—Brent Erickson (BIO), Peter Kelly (ACS), Ralph Hardy (NABC), Gordon Surgeoner (Ontario Agri-Food Technologies), Betsy Bascom and Philip Schwab (BIOTECanada), Roland Andersson (CIC) and Dirk Carrez (EuropaBio)—thank the Program Committee for their hard work and dedication in screening submissions, locating speakers and in organizing the program. Members of the committee were John Argall (BioAtlantech), Aristos Aristidou and David Glassner (NatureWorks), David Bransby (Auburn University), Doug Cameron (Cargill), Bruce Dale (Michigan State University), Larry Drumm (MBI International), John Finley (A.M. Todd), Rory Francis (PEI BioAlliance), Jack Grushcow (Linnaeus Plant Sciences), Colja Laane (DSM), Michael Ladisch (Purdue University), James Lalonde (Codexis), Mahmoud Mahmoudian (Rohm and Haas), Blaine Metting and Todd Werpy (Pacific Northwest National Laboratory), Glenn Nedwin (Dyadic International), Myka Osinchuk (BioProducts Alberta), Adrien Pilon (National Research Council of Canada), Manfred Ringpfeil (BIOPRACT GmbH), Sally Rutherford (BioProducts Canada), William Seaman (Florida Sea Grant), Brian Seiler (Eastman), Larry Walker (Cornell University), and Paul Zorner (Diversa). Also, a special expression of thanks goes to USDA Under Secretary for Rural Development Thomas Dorr.

The unflagging efforts and careful organizational oversight of Peter Kelly (ACS), Amy Ehlers, Matt Carr, Kimberley Scherr, Susan Spears and Jocelyn Modine (BIO) were vital 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 Summary Proceedings provides broad coverage of the plenary sessions and highlights of the breakout sessions. It is largely the product of first-rate work by Colleen McGrath, Sarah Munro, Aaron Saathoff, Navaneetha Santhanam and Deborah Sills (Cornell University) as recorders. Also, thanks are due to Susanne Lipari (NABC) for her excellent page-layout work.

Allan Eaglesham
Executive Director, NABC
Summary Proceedings Editor
September, 2006

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Welcoming Remarks

Dalton McGuinty (Premier of Ontario)

Mr. McGuinty, who represents the people of Ontario not only as premier but also as minister of research and innovation, expressed pleasure in hosting the *Third World Congress on Industrial Biotechnology and Bioprocessing* and welcomed the participants to Ontario. A culture of innovation is being created in Ontario, which is home to some of the most highly skilled workers in North America. The life-sciences sector, employing 40,000 people in 800 companies, is a global leader. Ontario's competitive advantage has always been its people, and the World Congress presented an excellent opportunity to form new partnerships and extend frontiers. McGuinty hoped that the delegates would enjoy the few days in Toronto and looked forward to working with them in the months and years ahead.

James Greenwood (President, Biotechnology Industry Organization)

Mr. Greenwood welcomed the delegates to the conference, expressing confidence that the companies represented will be those that will produce new fuels with less pollution, new agricultural and manufacturing processes, and new materials to enable us to live harmoniously with our environment. At BIO, he and his colleagues will do their part in Washington to help make the public-policy agenda in Congress as well in the states conducive to pioneering science, and through partnering meetings and technical sessions at the *Third World Congress* he expressed the hope that the attendees would succeed in what is perhaps the most exciting aspect of biotechnology.

Brent Erickson (Executive Vice President for Industrial and Environmental Biotechnology, Biotechnology Industry Organization)

On behalf of the Biotechnology Industry Organization and its more than 1,100 members—in all fifty states and thirty-six nations—Mr. Erickson welcomed the audience to the Third Annual World Congress on Industrial Biotechnology and Bioprocessing, saying that this was the biggest and most exciting *World Congress* yet. Over the following 3 days, he said, many potentially world-changing industrial biotechnologies would be highlighted, from new sources of clean-burning renewable energy to biorenewable industrial chemicals to the creation of new materials, pharmaceuticals and other biobased products through nature-based practices. Scientific and technical aspects of industrial biotechnology and bioprocessing as well as environmental benefits and great economic potential for investors, consumers, business and society as a whole would be discussed.

Erickson expressed particular pleasure in holding the third congress in Toronto. A very successful Agri-Food Forum had been held that morning—a fitting beginning to the proceedings. He thanked the Canadian co-organizers and invited Gordon Surgeoner (Ontario Agri-Food Technologies) and Betsy Bascom and Philip Schwab (BIOTECanada) to accept plaques expressing appreciation for their efforts in making the conference a success. Erickson also thanked the program committee for putting together a robust set of breakout sessions—mentioning Peter Kelly (American Chemical Society) and Ralph Hardy (National Agricultural Biotechnology Council) with appreciation for their help in starting the *World Congress* 3 years prior and for their stalwart support since—and the BIO team for their many excellent efforts.

Erickson likened the previous 12 months to a perfect storm of high energy prices, low energy security, rapid technology advances, market-pull development, increased global competitiveness and technology-products becoming market-ready. The confluence of these factors had created a tipping point. The record attendance at this conference was another indicator that a tipping point has been reached.

The past has seen much discussion of the potential of biotechnology, but with few examples of real economic impact. The fundamental technology has been developing rapidly. There is growing concern over global climate change. There has been a great deal of activity, with critical initiatives, in the European Union. Evolving policies in the United States have become more supportive.

Over the past 12 months, urgent need has materialized as energy prices have gone through the roof, and it remains to be seen how high they will go. Political instability in many parts of the world impinge on energy security, and hurricanes on the Gulf Coast have disrupted fuel supplies.

These factors are driving interest in industrial biotechnology. A comprehensive energy policy, signed into law by President Bush, authorized expenditure of over $3 billion for R&D and commercialization of bio-energy products. European Union and Asia biofuels initiatives were launched, and interest in biofuels and renewable feedstocks increased dramatically across the globe.

Energy insecurity is affecting government policies worldwide. We have seen US Department of Energy legislation, EU biodiesel initiatives, European Commission policies that will support coordinated use of biogas and the promotion of biofuels, in China a sustainable-energy initiative and in Indonesia a biodiesel "push."

Of course, climate change has been another driver, with increased interest all over the globe. In the United States, companies are not waiting for the government to take action, but are reducing greenhouse-gas emissions. This is spawning increased interest in pollution prevention and industrial biotech processes for various manufacturing applications.

Other key drivers? The chemical industry needs more-secure feedstocks and stable energy pricing. There is need for more-efficient process changes for companies to stay competitive in the global marketplace. Environmental concerns are increasing interest in green chemistry, and many BIO-member companies have won awards as a result of process conversions to methods that are cleaner and greener.

One of the most salient moments came in President Bush's state-of-the-union address in January 2006, when he voiced the goal of making cellulosic ethanol competitive with the price of gasoline within 6 years, which spun off a wave of increased political and financial support for the production of ethanol from cellulosic feedstocks. In subsequent speeches he talked about renewable energy, cellulosic ethanol, switch grass and other agricultural feedstocks, with dramatic effect on interest in industrial biotechnology.

In the eye of this perfect storm, timing for this conference could not be better. The tipping point has been reached and many are seeing hard work rewarded and dreams coming to fruition. We must maintain this momentum. The remainder of 2006 and 2007 promise to be exciting and gratifying for individuals and companies in this field.

Erickson concluded by observing that this is no bubble; it's a megatrend. The limitless potential for industrial biotechnology has always been there and the fundamental technologies have been in place. But they will continue to improve dramatically, enabling novel, elegant solutions to the world's problems. The economics, markets and policies have not been in alignment until now. In 2005 and 2006, demand for energy and government policies pushed industrial biotechnology over the tipping point and we now have strong examples of technologies and products entering the market. The future really is now, and more will come quickly.

With the leaders, thinkers and doers of industrial biotechnology and bioprocessing from around the globe assembled for this *Third World Congress*, Erickson invited the delegates to make the most of their time by visiting the three exhibit areas, attending as many breakout sessions as possible, taking advantage of business-partnering sessions and opportunities to network and make new connections. The measure of success of the congress will be the dissemination of information and new ideas, of technologies and break-throughs that make this field so exciting, and the forging of new friendships, partnerships and collaborations that will positively affect the business and scientific communities for years to come.■

Plenary Sessions

I. US Policy Focus on Bioenergy and Biobased Products

Thomas C. Dorr (Under Secretary for Rural Development, United States Department of Agriculture, Washington, DC)

Mr. Dorr suggested that a celebration was in order to mark the emergence of biofuels and biobased products in the marketplace and the roles they will play in the extraordinary transformation that is in progress. A fundamental shift is occurring in our resource base. He stated that the Bush administration recognizes the enormous potential of industrial biotechnology as the enabling force for conversion of crops to fuels and renewable chemicals and materials of all sorts. The USDA is committed to seeing industrial biotechnology used more and more widely throughout the manufacturing and energy sectors. This is a transition for the USDA away from calories toward BTUs. From biotechnology to information technology to nanotechnology to materials science, we are literally challenging limits. Since the biotech revolution rests squarely on agricultural feedstocks, extraordinary opportunities exist for US agriculture and, more broadly, for investment, growth and wealth creation in rural America and across the globe.

The federal role is broadly based and encompasses taxes, regulatory and trade policies, tax reform and support for innovation. With respect to energy, the federal role is especially significant. As stated by President Bush, the United States has developed an expensive addiction for imported oil. Clearly, it is a national imperative to catalyze and accelerate the development of new sources of energy and of biobased products that constitute a significant opportunity to displace petroleum as the key feedstock. Federal funding for R&D continues to be largely a product of the Department of Energy. However, the USDA is also a source of grants, but, more importantly, the USDA's role is particularly significant in terms of commercialization. After all, biobased goods are value-added agricultural products. Since its formation in 1862, the USDA has taken on a wide range of functions related to food, nutrition, public health, safety, farms and rural development. Although the focus of Dorr's presentation was rural development, he pointed out that the Forest Service, the National Resources Conservation Service, the Farm Service Agency and the Office of the Chief Economist are aggressively involved in issues related to the biobased economy.

USDA Rural Development is an investment bank for rural America, with a loan portfolio of over $91 billion. It is the only federal agency that can build a community from the ground up. They have a substantial history in the area of energy lending; the utility portfolio amounts to $43 billion. The mission is to increase economic opportunity, and to improve the quality of life in rural America. They are easy to work with and accessible with over 800 state and regional offices, providing technical support on a project-by-project basis. They are driven from the ground up, reflecting what is going on in rural America.

Today, energy is a driver of economic development in many parts of rural America and in many rural areas globally. Therefore, it is a major growing area of activity for all involved in rural development. The 2002 Farm Bill created an Energy Title that included the Federal Biobased Products Program. The success of this program suggests that it will also be part of the 2007 Farm Bill. USDA Rural Development administers more than forty programs on renewable energy. With regard to alternative energy, they are involved in more than biofuels; they cover the spectrum:

- bioethanol
- biodiesel
- digestors
- biomass
- wind
- energy efficiency

In this environment, technologies compete for funding just as they will have to compete for market share. Between 2001 and 2005, USDA Rural Development invested over $350 million in 650 renewable-energy and energy-efficiency products. These investments were used to leverage over $1.2 billion from private partners.

Andy Karsner and David Garman at DOE and Secretary Bodman and Secretary Johans all recognize the important of partnerships. The scale of resources required to develop biobased technologies—especially in energy—will dwarf the resources that government can bring to bear. Government has a role to play, but as these new technologies reach marketability, private investors simply must pick up the ball. Dorr stated that, at USDA, their ultimate test of success is to put themselves out of the business of commercialization. They are not interested in building Potemkin villages that wither and die when the subsidy plug is pulled. Their goal is sustainable development and wealth creation in rural America.

The pre-eminent issue is the soaring price of oil. It's a critical, economic and national-security issue. Estimates for 2006 indicate that farm-sector production will amount to $267 billion and net farm income will be $56 billion. America's bill for oil imports is expected to be $319 billion; for the first time, the cost of oil importation will exceed the total value of farm production. The cost of oil importation is expected to be approximately six times farm income. In contrast, in 2001 it was a "2x" relationship. The DOE / USDA billion-ton report, published in April 2005, estimated that with the commercialization of cellulosic ethanol, the United States could meet as much as 30% of the current transportation fuel requirement by 2030. Using 2006 figures, this translates to a direct shift of $96 billion from oil imports to farm-sector production. Farmers have always been in the energy business, traditionally producing energy in the form of food calories, and now they have the opportunity to produce BTUs as well.

The United States produced 1.6 billion gallons of ethanol in 2000 and 3.9 billion gallons in 2005. A hundred and one refineries are in production, seven are expanding and thirty-four are under construction. When all are on-line, the annual production will be about 7 billion gallons. Still, this represents a small fraction, since America consumes about 140 billion gallons of gasoline equivalent.

From 1996 to 2000, ethanol accounted for about 4% of the increase in the total demand for gasoline plus ethanol. In contrast, between 2004 and 2008 it is expected that ethanol will account for 26% of that increase, which is a huge turn of events. This is important because pricing power frequently is in the hands of marginal producers, and the sooner the marginal producers are farmers rather than OPEC, the sooner we will all be better off. Alternatives can make a difference.

In 2004 and 2005 in NW Iowa, a bushel of corn cost about $2. With a yield of 200 bushels/acre, this extrapolates to $400/acre. Ethanol production runs at about 2.8 gallons/bushel of corn or 560 gallons/acre. At a conservative price for ethanol of $2.50/gallon, this extrapolates to $1,400/acre; using an increased cost of production of $1/gallon, the net value of ethanol is $840/acre. Bringing in the cellulosic fraction, a 3:1 conversion is likely in the refining of corn kernels. On top of that, the stover yield will be approximately 5 t/acre from no-till corn in NW Iowa. At 75 gallons/ton, this extrapolates to a total of 975 gallons/acre; at $2.50/gallon and with an increased production cost of $1/gallon, the farmer ends up with $1,462/acre.

However, not a single cellulosic ethanol production facility is operational. The breakthrough is likely to come from someone in the *World Congress* audience, and the sooner the better.

Biobased materials and chemicals represent another significant opportunity for replacement of imported oil with domestic agricultural feedstocks—for production of plastics, lubricants, fibers, cleaning agents, *etc*. Obviously price is the issue, but the price differential is being whittled at both ends. Industrial biotechnology is reducing production costs and high oil prices are driving incentives for feedstock substitution. The federal biobased products procurement program is an effort to accelerate this process. Four thousand biobased products have been identified as possibly eligible for the program; clearly the potential is there. The federal program can and will assist in building consumer-acceptance and in helping producers achieve the economies of scale that they need.

It is important to understand that rural America is awash in cash and equity. We all grasp the national implications for renewable fuels, and can readily understand the value-added potential of biofuels and other biobased products for producers. But the questions that arise are:

- How will these new industries develop?
- How will they be financed?
- Who will participate?

There is opportunity for everyone. Rural America has the financial resources to be a major player in the new biobased economy, not simply as a vendor but also as an investor and active partner with many attending this conference. A few cents per bushel more for corn and beans are not a big deal within the framework of $400 to $1,400/acre. Farmers already own the feedstocks, and within the full spectrum of renewable energy, farmers and other rural landowners control sites for capturing wind and solar energy. These distributed resources offer opportunities for new business and new investment models with potential for significant wealth-creation throughout rural America and throughout rural areas of the world. From USDA's standpoint, this has a high priority.

Many agendas are in play. National security comes first and economic competitiveness in the global market is an absolute must, and these will ultimately drive the national commitment. But, from a rural-development perspective, sustainable development, investment, jobs and wealth-creation in rural communities are USDA's goals. Biotechnologies in the form of biofuels and other biobased products offer rural America its largest new opportunity in history.

Dorr concluded by expressing gratitude to those who have persisted with dogged, persistent pioneering work to get this conference to where it is today. We cannot afford to miss out on the biobased revolution. The USDA looks forward to collaborating in turning opportunities into realities.■

II. A Comparative Analysis of Industrial Biotechnology Policy

Christian Patermann (Director for Biotechnology, Agriculture and Food Research, European Commission, Brussels)
Todd Werpy (Program Manager for Bioproducts, Pacific Northwest National Laboratory, Richland, WA)

Christian Patermann defined the bioeconomy as inclusive of all of the industries and sectors producing, managing and in any way exploiting biological resources. In Europe, it employs twenty-four million people and has a turnover of 1.6 trillion euros (almost US$2 trillion). It is important to understand that agriculture, forestry, aquaculture, food and fisheries amount to almost double the value of the chemistry and automotive industries in Europe. Therefore, increased importance is placed in these areas, particularly since R&D investment in the bioeconomy is still rather low.

The exploitation of biological resources is possible only with knowledge, which is produced by science, in particular the convergence of life sciences, nano-sciences, information sciences and engineering. The term "bioeconomy" implies the qualifier "knowledge-based." The issues underpinning the development of the bioeconomy can be solved, at least to some extent, with a knowledge-based approach. These issues are:

- The need for European companies—small, medium and large—to stay competitive.
- Society demands safer, healthier food.
- Renewable resources will gradually replace petroleum as the feedstocks for chemicals and materials.
- Society demands sustainable and eco-efficient production methods, which entail much more than biofuels.
- Europe, with 450 million people, will depend on other parts of the world to supply some feedstocks.

The elements of the knowledge-based bioeconomy will encompass industrial, societal and political factors as well factors that are specific to Europe. Europe has the luxury of being the only continent that has developed a strategy on life sciences and biotechnology in the form of the 2002 publication, *Life Science and Biotechnology: A Strategy for Europe*. Also there is the Biomass Action Plan, the EU Strategy on Biofuels, and an Energy Policy is under development. The objective of the Environmental Technology Action Plan is to reconcile preservation of nature with improvement in quality of life and maintenance of industrial competitiveness. All of this is underpinned by adequate investment in research.

The *Strategy for Europe* forecasts an increasingly important role for enzymes and the potential for agriculture to become a producer of non-food / feed crops such as industrial feedstocks and new materials such as polymers. It is under review and publication of a new edition is expected in 2007. Every year an action plan is produced, covering thirty aspects of the state of life sciences in Europe; it is reported to the European Parliament and the member states. This forces an examination of deficiencies, which, within the context of twenty-five members states is not always easy.

The Biomass Action Plan is intended to mobilize EU-wide activities promoting use of renewable feedstocks. The Biofuels Directive is also under revision, setting national targets for member states who are also encouraged to formulate National Biomass Action Plans.

The need for a technology platform is now understood—where industry, academia, stakeholders and policymakers can come together to strategize on biofuels for Europe. A critical aspect is relaying information to farmers to appraise them of the relevance and of the enormous potential of the biobased economy for them.

Key measures in promoting biofuels include stimulating demand by introducing incentives. Also, it is essential to ensure life-cycle sustainability—economically, competitively and environmentally—of the production and utilization methods. As feedstocks for biofuels, emphasis is being placed not only on methods of sugar production, but also on forest products. A comprehensive EU Forestry Action Plan was adopted in June, 2006—dealing *inter alia* with energy and biofuels. Importation of feedstocks will remain a key energy component, but it is vital not to replace one dependency with another. Increased funding for R&D is another key aspect.

A green paper on EU energy policy is being updated. The current edition predicted that 5.75% of transportation fuels would be replaced by biofuels by 2005. This goal was not reached due to unforeseen practical problems; it stands at approximately 2.5%. Another objective is to increase renewable electricity generation to 21% in 2010 (from 14% in 1997). Member states have been encouraged to accompany this process with detaxation of biofuels.

Since 2004, the EU's Environmental Technologies Action Plan (ETAP) has promoted eco-innovation and adoption of environmentally beneficial technologies through:

- promoting R&D,
- mobilizing funds,
- helping to drive demand and improving market conditions.

In 2005, the French Environment Agency published the *Bioproducts Guidebook for Greener Procurement.*

With respect to the issue of official and consumer acceptance, it is encouraging that, within the past few months, the European Parliament passed a resolution promoting crops for non-food purposes, including feedstocks for biofuels production, stressing utilization of organic wastes from farming and forestry and the need for research and improved coordination with the member states. It is important that, in all of our endeavors, we keep the legislative bodies in our respective countries fully informed.

The EU research policy has three key components:

- The Framework Program, which starts at the beginning of 2007, will have 55 billion euros ($72 billion) of funding for 7 years, concentrating on food, agriculture and biotechnology.
- Technology platforms will provide incentives for better communication among representatives of academia, industry and stakeholders.
- A forum will be provided so that the various programs of the European nations can synergize, *viz.* European Research Area networking.

Within the Framework Program, many new research activities will be open to the world. One program will be Food, Agriculture and Biotechnology, but nanotechnology, new materials, new production technologies, energy and the environment will also be covered. Three supporting "pillars" are as follows:

- sustainable production and management of biological resources,
- a fork-to-farm food component, emphasizing consumer interests as a driving force for farming—food, health and well-being,
- life sciences and biotechnology for sustainable non-food products and processes, concentrating on crop-improvement for feedstocks and biomass, including marine resources, for energy, materials and chemicals.

Novel farming systems will be included as will new bioprocesses (the biorefinery concept is crucial), biocatalysis, forestry and environmental remediation.

The technology platform on sustainable chemistry, managed by EuropaBio, is already producing practical results.

Progress in this field will be impossible without the help of the EU Parliament and without consumer support. We must learn from experience gained from green biotechnology to ensure public acceptance. Gallup and others are employed to generate the Eurobarometer and gauge acceptance of life-sciences and biotechnology. Two weeks prior, data for public acceptance of the following fields of biotechnology were published:

- genetic testing
- cloning of human cells and tissues
- industrial enzymes
- xenotransplants
- GE crops
- biofuels

Biofuels garnered the strongest support throughout Europe. The data as a whole promise social embracement of a truly sustainable future—economically, ecologically, and socially. Mr. Patermann invited the rest of the world to coat the enormous problems that lie ahead in this spirit.

Todd Werpy reminded the gathering of President Bush's allusion to America's addiction to oil in his January, 2006, state-of-the-union speech. From it has been born a biofuel-from-biomass initiative to help reduce reliance on foreign oil. Why biomass and biofuels? Dr. Werpy argued that this approach is the only short- or intermediate-term opportunity open to the United States to replace petroleum-based liquid transportation fuels. Furthermore, in addition to liquid transportation fuels, the opportunity to produce chemicals and materials from renewable feedstocks will also be critical in the future economy.

The Department of Energy needs to develop new technologies to produce an abundance of renewable energy as well as other products. The biorefinery concept is critical in that it will produce not only liquid transportation fuels but also chemicals and materials to help drive the overall economics. This presents opportunities also for the revitalization of rural economies.

A diverse energy supply is critical to US national security. In importing huge quantities of oil, wealth is being transferred to other parts of the world, which is fine except when it goes to countries unfriendly to US policies.

The goal of the DOE's biomass program within the president's initiative is to fund cutting-edge research activities for the production of ethanol. Over 90% of ethanol produced in the United States comes from starch-based material. One objective is to expand production beyond starch as the feedstock to include lignocellulosic materials. Clearly, biotechnology will play an important role. The overall goal is to replace 30% of US liquid transportation fuels with renewable fuels by 2030; this translates to 60 billion gallons of ethanol annually—a significant challenge.

Ethanol is the current model, but it likely that other fuels will become significant parts of the picture, including biodiesel and products of pyrolysis.

A number of elements will support the development of necessary biotechnology and other technologies associated with fuel and chemical production under the president's initiative. Two major goals are involved:

- To reduce US dependence on foreign sources of petroleum equal to about 75% of what is currently imported from the Middle East by 2025.
- To make cellulosic ethanol cost-competitive by 2012.

The initiative is supported by the Energy Policy Act of 2005, which encompasses several aspects that will be critical to success: an alternative fuels infrastructure tax credit (a tax credit already exists for blenders and retailers of ethanol and biodiesel), mandatory federal-fleet requirements to use alternative fuels, and an ethanol / biodiesel producer tax credit. Title 17 of the Energy Policy Act of 2005 covers loan-guarantee programs to encourage the construction of facilities processing and converting cellulosic biomass to fuels and other products. In his budget, President Bush requested a 65% increase in funding over 2006, *i.e.* $150 million for 2007. In June 2006, the Senate Energy and Water Appropriations Subcommittee approved a $63 million increase above the administration's request. Clearly, there is strong political support for the development and implementation of biobased fuels and chemicals.

Several obstacles remain to be overcome. For example, the capital cost of pre-treatment of cellulosic material must be decreased, the fermentation-efficiency must be improved with more-robust organisms including utilization of five-carbon as well as six-carbon sugars, and the cost of enzymes for cellulose hydrolysis must be decreased.

The process has to be viable from a technical standpoint as well as from an economic standpoint.

In addition to technology needs, there is substantial need for infrastructural development and deployment. As of July 2006, there were 676 E-85 fueling stations in the United States, most in the mid-west, and 430 biodiesel fueling stations. Although these numbers are increasing, they represent only small fractions. One challenge is moving ethanol to parts of the country where it is most needed. At some point, a standard mixture has to be decided upon—E-10 for example—which will be available everywhere to allow optimization of engine performance.

Issues exist also in terms of biomass production, harvesting, collection, storage and processing. The DOE/USDA billion-ton study, which examined the potential for producing biomass on a sustainable basis, indicated that about 1.3 billion tons of biomass are available yearly, sufficient to meet the president's goals. On the other hand, the necessary infrastructure is lacking.

DOE has issued a major solicitation to fund two or three industrial partners to develop commercially ready technology for lignocellulosic-derived ethanol. We need to validate biomass-conversion technologies and integrate them into a biorefinery on a scale that brings credibility, *e.g.* approximately a 10%-scale facility.

If we are harvesting, collecting, storing and transporting biomass to a central processing facility, we must develop a portfolio of technologies that will allow utilization of all of that biomass. Biomass contains more than cellulose, therefore conversion of the non-cellulose fractions, *e.g.* lignin, to liquid transportation fuels and chemicals is also important. Accomplishment of that goal may be achieved by two technology pathways:

- biochemical—fermentation of lignocellulosic-derived sugars to ethanol,
- thermochemical—gasification to syngas or pyrolysis to a pyrolysis oil that may be integrated into the current petroleum-refining infrastructure.

Areas currently emphasized at DOE include feedstocks—primarily harvesting and distribution—development of organisms for improved efficiency of fermentation of sugars from lignocellulosic materials, syngas and pyrolysis technologies, a products R&D portfolio examining core-technology development including new chemical transformation, and economic analyses. Significant effort is being expended in maximizing the value of wet- and dry-milling methods, of pulp- and paper-manufacturing methods, and of agricultural waste as well as forest-residue processing. Ultimately the emphasis will shift to embracement of perennials as feedstocks for energy production.

A number of recent announcements have been encouraging. We continue to see increases in production of bioethanol and biodiesel by major companies including ADM and Cargill as well by co-ops, mostly farmer-owned. About 5 billion gallons of bioethanol are now produced annually and over 1 billion gallons of biodiesel. Similarly, biobased production of chemicals is rapidly increasing. Dr. Werpy stated that these are indicators that the United States is moving towards a biobased economy.■

III. Industrial Biotechnology: Turning Potential into Profits

Jens Riese (Partner, McKinsey & Company, Munich, Germany)

Dr. Riese observed that the industrial biotechnology scene has changed significantly since he spoke at the first *World Congress* just 2 years prior. He confessed to some concern that numbers he had used in his projections were overly optimistic, *e.g.* that the industry would double over 5 years. He thanked the delegates for helping to prove that his numbers were realistic; industrial biotechnology is growing at the pace he predicted.

Public awareness is increasing. Some 2,000 articles per month in the popular press make reference to industrial biotech. Increasing oil and gas prices mean that many biotechnology platforms have cost advantages. Polylactic acid (PLA) production has been costed at 20% less than that of polyethylene terephthalate, as has bioethanol in comparison with gasoline. However, internal forces are also driving the technology. Patents granted in this field have increased from 6,000 in 2000 to 22,000 last year; many are pending, therefore the final number for 2005 may be around 30,000.

On the other hand, certain realities must be faced. Customers are unwilling to pay high premium prices. "Green" products have to compete favorably with "traditional" counterparts in terms of cost and performance. Furthermore, in most instances plant-synthesized chemicals cannot yet be produced economically in large scale. Functionality breakthroughs will come as production costs are decreased and efficiencies are improved. And commercialization takes time. It has been suggested that commercialization of PLA has been unexpectedly slow when, in fact, its pattern of volume sold in the first 5 years has, if anything, been better than those of PVC, polypropylene and poystyrene.

What has been true for PLA is true for the industry as a whole: it is growing. The sales of products generated via fermentation or enzymatic conversion of biological feedstocks have increased from 50 billion euros in 2000 to 77 billion in 2005, and 120 billion euros in 2010 is a realistic projection. Although bioethanol constitutes a significant part of this, in fact a broad range of goods is being produced, including pharmaceutical ingredients, oleochemicals, biochemicals, polymers and enzymes—a broad spectrum of applications are being affected by industrial biotechnology. Riese is confident that penetration of the chemical industry will reach 10% of sales by 2010.

There is little dispute that bioethanol production from corn kernels results in a positive energy balance of about 20%, and biodiesel and cellulosic ethanol will be significantly better again. Also the biofuels' CO_2 balances are positive, which will contribute to meeting Kyoto targets.

The biofuels gold-rush is on, with many staking claims. Oil and technology companies are getting involved, whereas some in the agriculture industry have been there for a long time. The reason is not hard to find, considering oil prices and corn prices. Many governments are pushing conversion to biofuels, not only the United States and in Europe but also in China, India, Japan, Korea, Malaysia and Thailand—all have aggressive biofuel targets and mandates. Production capacity will double over the next 5 to 6 years, to about 24 billion gallons by 2012 as a result of these mandates, on top of economically motivated growth, in particular in Brazil. Market penetration is likely to grow from 2% today to 4% by 2012.

However, there are limits to this growth and to potential profitability. Biofuels are highly dependent of subsidies and/or high oil prices. This is not the case in Brazil, where the crude-oil threshold for profitability is $20/barrel. In the United States, without subsidies the corn-ethanol threshold price is $50/barrel and with subsidies it is $30. In Germany the without-subsidy threshold price is $80.

Negative aspects of ethanol as a transportation fuel include relatively low energy content, corrosivity, low vapor-pressure, cannot be pipeline-transported, and relative small net energy gain and CO_2-emission reduction with corn-kernels as the feedstock. The most serious limitation is feedstock availability necessitating the conversion of cellulosic biomass to bioethanol. Calculations indicate the availability of 2 billion metric tons of biomass annually, equivalent to about 160 billion gallons or 25% of total consumption of transportation fuels. This does not take into account possible genetic improvements to increase biomass production to optimize the conversion process. Today's technology indicates that conversion of cellulosic biomass to ethanol will result in a product of $2.00–$2.50/gallon. With optimization of the entire process there is potential to reduce this to $0.60–$0.80/gallon. Butanol, which has fewer inherent problems than ethanol but is not currently cost-competitive, could cost $1.30/gallon after process-optimization.

There is a long way to go, but provision of up to 50% of total transportation from renewable sources appears feasible. Business opportunities exist. A biofuel producer with a 5% share in a 20 billion gallon market 10 years hence, with a crude oil scenario of $40/barrel, would have a $2 billion/year business. Enzymes represent another business opportunity, as do fermentation organisms. Competitive advantage will come from:

- access to the best technology,
- access to low-cost feedstocks, and
- being part of an integrated value chain.

This begs the question of why companies are not investing more enthusiastically in the necessary infrastructure. Many macro- and micro-factors are involved, including the question of the best technology. A recent DOE report presents what may be viewed by CEOs as 200 pages of problems. Key questions include:

- What is the competition doing?
- Who would make good partners?
- What will be the demand by region, feedstock and type of fuel?
- Will there be subsidies? Will the product be subsidized in other countries?
- Where are oil and gas prices going?
- Where are carbohydrate prices going?

McKinsey & Co. are assisting clients by building models to produce scenarios of how the industry may look in 2010 and 202, depending on oil price, cellulosic biomass productivity, *etc.* Dr. Reise expressed the hope of participating in fruitful discussions of these models with delegates.■

IV. Biofuels: Perspectives from the Oil and Chemical Industries

Justin Adams (Director of Long Term Technology Strategy, BP, United Kingdom)
William Provine (Research Manager, DuPont, Wilmington, DE)
Richard Zalesky (Vice President of Biofuels & Hydrogen Business Unit, Chevron Technology Ventures LLC, Houston, TX)

Justin Adams's presentation emphasized three aspects:

- BP's view of the drivers that will shape energy globally,
- the potential of energy biotechnology, and
- biofuels beyond the hype that is shaping the current debate.

There are four key drivers, the first of which is rapid growth in demand for energy resulting predominantly from strong GDP growth in many developing nations. The second is supply challenges. Thirdly, an increasingly important issue is security of supply, and the fourth driver is environmental constraints, chiefly underpinned by climate change.

At BP they believe that innovation is the key to addressing the challenges presented by these drivers. Innovation encompasses policy framework and business models as well as technology, requiring huge efforts from the public and private sectors—large and small companies.

The energy sector today involves billions of barrels of oil equivalent, trillions of cubic feet of gas and billions of tons of coal. Furthermore, projections indicate that energy demand—for transportation, electricity, heat, *etc*.—will grow by 60% between 2002 and 2030. The contributions of biotechnology must be viewed in that context.

A lot of fossil fuel remains in the world. Current reserves of conventional oil amount to 41 years and it is believed that unproven reserves will at least double this. Substantial resources of unconventional oil also exist, exploitable if economic and environmental constraints permit. The same is true for gas and coal; many hundreds of years of reserves of the latter are known to exist. The problem is not the quantity of hydrocarbons but their location, with the exception of coal. Looking to the future, it is likely that coal will play an increasingly important role in providing energy in, for example, the United States, if security of supply is the key challenge.

The evidence is increasingly strong that elevated CO_2 levels in the atmosphere are linked to global warming. The challenge lies in reversing our enormous dependence on hydrocarbons, and thus reduce atmospheric CO_2; it is not a trivial matter.

At BP these issues have been under consideration for many years. Novel technologies emerge and radical breakthroughs occur when science is developing most rapidly. Currently, biology is developing most rapidly and without doubt will bring “disruptive” technologies. They see enormous potential for the biosciences and biotechnology. Today, 85% of the world’s energy is carbon-based and all of life is carbon-based. The world has had 4.5 billion years to develop means of using that carbon, therefore there are likely to be synergies as we progress.

There has been enormous funding in the biomedical sector and to a lesser extent in the agricultural sector, but support for industrial biotechnology has lagged behind. Stronger support for energy bioscience will bring exciting developments.

Nature has evolved many mechanisms to harness energy. By exploiting some of nature’s designs there is enormous potential to meet the energy challenges of the future. BP is interested in the whole domain of energy bioscience—not merely biofuels—ranging from hydrogen production to increased recovery of oil and bioremediation.

Today, biofuels contribute 2% of the global transportation fuel pool. That 98% comes from oil demonstrates that hydrocarbons will remain the main source of transportation energy for many decades. Hydrogen may come, but it will be at least mid-century before it makes a significant impact. Possible options include turning coal and gas into liquids, but—unlike biofuels—these approaches make no contribution to CO_2 reduction.

Major questions remain. It costs a lot to produce biofuels, for example. Will they be able to compete economically without subsidies? Will biofuel use reduce CO_2 emissions, and is their production sustainable in terms of water use, other inputs and maintenance of soil quality?

Adams stated that making ethanol from corn, let alone from lignocellulosic biomass, gives a twenty-two times multiplier on each barrel of oil used. If the priority is displacing imported oil, the reality is that biofuels can play a huge role.

To address these challenges we must move to low-cost abundant feedstocks, and find cost-effective technologies to convert lignocellulosic materials to biofuels. Currently it is uneconomic to produce bioethanol from biomass. On the other hand, the opportunity is enormous and biotechnology holds the key to reducing production costs—from increased crop yields, lower costs for enzymes and new fermentation organisms. Many factors must come together to drive down costs. BP is not committing dollars to biofuel-plant construction because significant work is needed to develop these technologies for economic deployment on a large scale. If industry moves too early or too fast, it could set the effort back by years. It is important to guard against over-hyping; hydrogen is a good case in point—the “bubble” is slowly deflating with realization of the challenges involved. We must avoid creating unreasonable expectations for lignocellulosic ethanol and invest in the technologies that will drive costs down quickly. BP is committing to this with its Biosciences Institute.

Although bioethanol and biodiesel have important roles in the short-to-medium term, these molecules are not ideal for automobile transportation. Butanol has many advantages over ethanol, and it may represent just the beginning in the search for superior liquid fuels.

What chemistry did in the twentieth century, biology will achieve in the twenty-first century. The petroleum value chain is complex, yet it runs efficiently and well. As we look to biofuels, we have to understand the whole of the new value chain. It must be an integrated system to compete with the mature petroleum sector. By addressing the issues highlighted here, industrial biotechnology and, in particular, energy biotechnology will become the chief drivers of innovation and growth.

William Provine reminded the audience that DuPont’s subsidiary company Pioneer Hi-Bred International, Inc., is one of the largest producers of corn and soybean seed in the world. Pioneer also markets protection chemicals to assist corn and sugar-cane growth. In that regard, they are creating new products specifically for the biofuels and biochemicals industries. DuPont is researching the conversions of starch and sugar, and most recently cellulose (collaboratively with the DOE). They are interested in creating novel fermentation processes for biofuel and biochemical synthesis, specifically for production of ethanol, cellulosic ethanol, biobutanol and bio-PDO (propanediol). DuPont is using modern technology—metabolic engineering tools—to build better biofuels and biochemicals; choices are available that were unavailable to industry a decade ago.

DuPont’s $100 million bio-PDO plant, located at Loudon, TN—a joint venture with Tate & Lyle—is mechanically complete and under test. Production is expected late in 2006, and maximum annual output is expected to be 100 million gallons. Market opportunities for bio-PDO extend beyond Sorona polymer; with a new product, sometimes the world comes to your door to suggest new uses.

With respect to DuPont’s efforts in biofuels, they are moving from “differentiated” to “transformative” products. DuPonts sells more than $300 million worth of goods to the biofuels industry, and it is hoped that this will increase, partly through Pioneer’s hybrids of corn that will maximize ethanol production per acre. In conjunction with BP, DuPont has realized initial targets for the production of biobutanol. Testing it as a transportation fuel is in progress in the United States and Europe; the current focus is on bringing it to market in the United Kingdom.

Butanol has the advantage of pipeline transportation, thus it is easier to move to the consumer via current infrastructure. It can be used in automobiles without the retrofit issues that apply to ethanol use, and it has a higher energy-value than ethanol.

DuPont / Pioneer has a collaborative effort funded by DOE for development of cellulosic ethanol in conjunction with the National Renewable Energy Laboratory (NREL), John Deere and Diversa, focussing on integration of key elements: corn-stover milling, pretreatment, saccarification, fermentation of five-carbon and six-carbon sugars, and separation of lignin as a source of energy for the biorefinery.

About 390 gallons of ethanol can be generated per acre from corn starch. If kernel fiber were to be used as a cellulosic feedstock rather than as animal fodder, another 20 gallons/acre may be generated, as demonstrated by DuPont. The stover in total represents 310 gallons, thus there is the potential to approximately double the ethanol yield by including cellulosic sources of carbon. The critical process is the efficacy of conversion of cellulosic feedstocks to soluble sugars, not just for ethanol synthesis but also for other products. Research collaboratively with NREL on this aspect, in progress at DuPont's experimental station in Wilmington, DE, indicates that 100% conversion is feasible.

"If you believe that there is a liquid-fuels gap relative to supply options, how might we fill that in?" asked Richard Zalesky. Chevron is looking at biofuels as a realistic part of the solution. Sixteen million barrels per day may be met with what some call "unconventional" sources: extra-heavy oil, coal-to-liquids, gas-to-liquids, and biofuels. Biofuels should not be seen as a silver bullet, but as part of the solution. Chevron already blends 300 million gallons of ethanol into gasoline per year, for sale in the United States—similarly in Australia and other countries. Of the 9,300 Chevron / Texaco service stations in the United States, some 20% are involved in blending. Entrepreneurial dealers in their network are free to sell E85—albeit not as a Chevron product—and some are doing so.

Much about bio-derived fuels has yet to be learned. After 125 years, much is known at Chevron of traditional fuels; however, there is no better way to learn than by doing. Therefore, they are involved in demonstration projects in collaboration with the State of California, General Motors and Pacific Ethanol, supplying E85 to vehicles in the state fleet. Chevron wishes to understand mileage, emissions and maintenance aspects and, most importantly, consumer acceptance. There have to be value advantages to consumers who make informed choices in how they use their time and money. The consumer should be able to drive from Spokane, WA, to Key West, FL, and have appropriate fuel available throughout. In the future, fuel availability may vary on the way, with E85 in Minnesota and a biobutanol blend in Delaware—which will work as long as the consumer's assessment is favorable and vehicle technology and fuel-delivery infrastructure work.

Biodiesel is also of interest to Chevron. Experience has shown that it is critical that the market, raw material, manufacturing capability and distribution all are mutually compatible, possibly more critical in this case than in some more conventional businesses. Furthermore, biodiesel's attributes must be understood so that equipment manufacturers can certify it for use in their vehicles at increasing concentrations and so that energy companies—such as Chevron—can market uniform products.

A general rule of thumb in commercial production is: the bigger the better. The larger the unit, the greater the throughput, the lower is the unit cost and the more likely the enterprise will be successful. The reverse applies to hydrogen as a transportation fuel and to biofuels. With advance manufacturing techniques they were able to make hydrogen from natural gas at a price competitive with gasoline. No basic science breakthrough was involved, rather innovative engineering was brought to bear, auguring well for the future of biofuels in general. Chevron seeks partnerships and collaborations to complement their own capabilities.

Biobutanol goes a long way to addressing issues arising from integrating a new fuel into the current infrastructure. Although building a new pipeline might be part of the process of adoption of bioethanol, in reality, replication of distribution systems is unlikely to occur in most developed countries. Although inability to integrate is likely to be a show-stopper, there is no reason to believe that fuels derived from biomass feedstocks cannot be formulated to meet the challenge, and biobutanol serves as a good example.

Chevron is investing in innovative technologies in this area, spending $400 million dollars/year in total. Supportive, flexible economic policies that protect the environment will be essential. Great strides have been made recently—shown at this conference—in energy-efficiency improvements in biofuel plants, which must be maintained. At the same time, efforts to reduce greenhouse-gas emissions are essential; Chevron shares BP's commitment to that endeavor.■

V. An International Perspective on Biofuels and Cellulosic Ethanol

Suzanne Hunt (Biofuels Project Manager, Worldwatch Institute, Washington, DC)
Fernando Reinach (CEO, Allelyx, and Director, Votorantim, Sao Paulo, Brazil)
Lee Lynd (Professor, Dartmouth College, Hanover, NH)

Suzanne Hunt stated that ethanol production globally had doubled within the past five years, and although biodiesel production is much smaller, its rate of increase has been ever greater. Bioethanol production per hectare from sugar cane in Brazil is approximately twice that from corn kernels in the United States, and productivity of biodiesel from palm oil is four to five times that from soybean per unit area of land.

In Brazil, half of the sugar crop produces 40% of their transportation fuel needs. In the United States, 15–20% of the corn crop produces 2–3% of non-diesel fuel, and in the European Union approximately a quarter of the rapeseed crop provides about 1% of transportation fuel. Globally, despite rapid increases in production, biofuel meets only about 1% of transportation needs.

Recent encouraging developments include significant venture-capital investments and new federal-funding opportunities.

The fundamental question relates to the maximum potential displacement of oil by biofuels. Estimates vary widely, depending on assumptions of effects of climate change on agricultural productivity, of population growth and human-dietary changes, *etc*. But some detailed studies are encouraging. A conservative estimate in Germany, assuming rigorous sustainability criteria, showed that 25% of their national petroleum needs may be met with biofuels. Similarly, the USDA/DOE billion-ton study indicated that, by 2025, a third of US transportation needs may be met with a combination of current and next-generation biofuels.

Important factors that will affect biofuel production include ability to utilize new agricultural feedstocks, including waste streams. A Worldwatch study indicates that solid waste from an average US city of a million people could be used to produce fuel to meet the needs of 58,000 people. Importantly, in France the same amount of biofuel would meet the needs of 360,000 people, and in China of 2.6 million people. Energy-crop breeding for increased biofuels productivity will also be important.

Publication of an analysis of trade issues is expected in September, 2006. At this time only about 10% of biofuels are traded internationally, chiefly from Brazil and within the European Union. Technology transfer will be important for adoption of biofuels globally. At this time, international quality standards for biofuels have yet to be determined. The United States and the European Union have established their own standards, and other countries are following suit, but internationally accepted standards will be needed to foster trade.

Discussion of sustainability issues is on-going, accelerated by concern, especially in Europe, over rainforest destruction in Southeast Asia resulting from palm-oil production. The EU parliament has passed legislation requiring that all imports of bioenergy be certified as produced under sustainable conditions; what those standards should be and their implementation are now under consideration.

Regarding WTO issues, it is uncertain whether biofuels will be treated as agricultural or industrial products. There is discussion also of liberalization of trade in environmental goods and services, the definitions of which remain to be clarified. The politics of basing trade preferences on production method rather than on the nature of the end-product has been contentious, although the case law is clear: at least in theory, biofuels can be considered as environmental goods. Agricultural subsidy reforms will certainly impact the market.

Bilateral agreements will be important, as will the issue of whether governments will protect their domestic industries or foster development globally. Austrialia, the United States, the European Union and Brazil have levied hefty import tariffs.

Opportunities concomitant with significant replacement of petroleum with biofuels include:

- energy security,
- rural development in the United States,
- job creation, and
- economic development in the developing world.

A study by the World Bank suggested that for every unit of energy from petroleum replaced by biofuels, 100-fold more jobs are created.

Risks associated with production of large quantities of biofuels include land conflicts, as exemplified by disputes in Brazil. Water has not received due attention; reduction of usage in production of bioenergy will be increasingly important. Industry consolidation has occurred in Brazil and efforts to resist that will be needed in the United Stated if maximum rural development is to be realized.

Ms. Hunt is commonly questioned on the “food versus fuel” issue: what will be the implications of increased commodity prices on food costs for the poor? It is likely that the effects will be mixed. However, considering the negative impacts that commodity dumping has had on developing countries, increased pricing of commodities may be expected to have positive effects.

Other important environmental risks associated with increased biofuel production are agricultural expansion into sensitive habitats and soil degradation. Degree of environmental impact is determined mainly by the feedstock crop and associated management practices. Land-use change is also an issue; for example, conversion of virgin rainforest or prairie to agricultural production of feedstock releases large amounts of carbon dioxide, thus diminishing or eliminating positive carbon balances gained from biofuel consumption in place of petroleum. Energy use in infrastructural changes associated with biobased fuels and in the production process must also be taken into consideration.

Assessment of energy balances have shown that virtually all of the current commercial biofuels present favorable energy balances in terms of the energy contained in the product per unit of fossil fuel input (*i.e.* ratios of 1.5:1.0 to 36:1.0).

The transportation sector produces about 25% of greenhouse gases globally. While greenhouse-gas emissions are beginning to level off in total, they continue to increase from the transportation sector. While biofuels represent attractive strategies for mitigating greenhouse-gas emissions for heat and power, they offer the only feasible approach for transportation.

Further reading:

Biofuels for Transportation: Global Potential and Implications for Sustainable Agriculture and Energy in the 21st Century. Washington, DC,Worldwatch Institute (2006).

Fernando Reinach urged the audience to remember this “take-home” message: it is impossible to buy gasoline in Brazil. Only blends are available. The choice is between E25 and E100, and this situation has prevailed for about ten years. However, automobiles have been running on ethanol from sugar cane since the 1920s.

Votorantim is the largest conglomerate in Brazil, a $9-billion company, which, since 2001, has been developing technologies to reduce the cost of production of ethanol from sugar cane.

Sugar cane has been grown in Brazil since the 1600s. Efforts to convert sugar for human consumption to the feedstock for energy production began in the early 1970s as a result of the OPEC crisis. Now ethanol is becoming a global commodity and, furthermore, sugar cane may become the feedstock for products other than ethanol.

Brazil will remain a leader in ethanol production since it is in the tropics with plentiful arable land for biomass production, sunshine, warm temperatures, adequate rainfall and relatively inexpensive labor. Also, it has the ability to increase the scale of production, more so than other sugar-producing countries, such as Cuba, India and Australia.

Sugar cane generates more ethanol per unit of land area than any other species. Brazil’s average production is in excess of 6,000 litres/ha, at a cost of $0.16/litre. Ethanol production from sugar cane has not been subsidized since 1999. About 75% of cars in Brazil are now of the flex-fuel type, running on any combination of ethanol or E25. Imported cars run on E25 without requiring retrofitting. Dr. Reinach is of the opinion that technology concerns in the United States are overblown.

Global demand for ethanol, partly as a result of the Kyoto Protocol, is projected to double over the next five years. Reinach expects that Brazil’s competitiveness globally will improve beyond the current $0.16/litre, resulting in part from utilization of bagasse as a feedstock for cellulosic ethanol. Unlike corn, all of sugar cane is routinely harvested and transported for processing, and after extraction of the juice, bagasse is a by-product, disposal of which is a serious problem. "Mountains" of bagasse have accumulated. Some is burned to supply the energy for mill operations; in 2005, 122 million tons were produced. Brazil could increase ethanol production two-fold via cellulosic conversion of this biomass.

Again in 2005, Brazil produced 436 million tons of cane on approximately 5 million ha, which represents a relatively small area; three times this hectarage is planted to soybean. Of this total production, 55% is used to produce sugar and 45% is converted to ethanol, *i.e.* 17 billion liters in 2005. The 122 million tons of bagasse at the mills in 2005 is theoretically equivalent to 25 billion liters of ethanol. Also, if 30% of straw is collected from the field, an additional 6.6 billion liters is possible, and the lignin present in the bagasse contains sufficient energy to supply the necessary steam, electricity, *etc*.

Planting of "fiber cane," products of Votorantim's breeding activities aimed at increasing biomass rather than sucrose content, may result in a further two-fold increase in ethanol yield. Production of 22,000 liters/ha appears to be feasible. To realize this goal, two companies have been established: Allelyx and CanaVialis. The former was founded by scientists who sequenced the genomes of *Xylella fastidiosa* (a bacterial pathogen), sugar cane and eucalyptus, and the latter by traditional sugar-cane breeders. CanaVialis produces elite plant germplasm by breeding, whereas Alellyx scientists are involved in gene discovery. Alellyx takes the scientific and regulatory risks, and CanaVialis takes the risks involved in commercializing new varieties of sugar cane. Allelyx's transgenic varieties are synthesizing 40–80% more sucrose than the current best variety in trials, and CanaVialis has five chief breeding objectives:

- increased sucrose content,
- early maturation,
- adaptability to new environments,
- optimization for mechanical harvesting (rather than tradi tion manual collection), and
- fiber cane.

Reinach concluded by reminding the audience that gasoline cannot be purchased in Brazil.

Lee Lynd suggested imagining a sustainable world within which human needs for food, energy and materials, organic and inorganic, are met. Relatively few potentially sustainable resources are available—closer to half a dozen than to dozens—and if we imagine connecting them to human needs, the processes involve either photosynthesis in the form of biomass or some form of renewably produced electricity. Plant biomass is the only foreseeable sustainable source of food and the only sustainable source of organic fuels and organic materials.

Energy conversion, utilization and access underlie many of the great challenges and opportunities of our time. Abundant evidence indicates that energy conversion and utilization are the largest contributors to human effects on the environment. And several economic dimensions are related to access to energy, including rural economies, world poverty, balance of payments and technology exports.

With reference to national security, Dr. Lynd stated that ex-CIA Director James Woolsey and Senator Richard Lugar have described oil as "a magnet for conflict," necessitating foreign-policy compromises.

It is important to take a long-view perspective to meet energy-supply challenges and grasp concomitant opportunities. A multi-institutional project involving Dartmouth, the Argonne National Laboratory, the National Renewable Energy Laboratory, the Union of Concerned Scientists, the University of Tennessee, the National Resources Defense Council, Michigan State University, Princeton University, the Agricultural Research Service and Oak Ridge National Laboratory, sponsored by the DOE, the Energy Foundation and the National Commission on Energy Policy has the following objectives:

- Identify and evaluate paths by which biomass can make a large contribution to future demand for energy services; and
- Determine what can be done to accelerate biomass energy use and in what timeframe associated benefits may be realized.

They have made thermodynamic appraisals of energy balance, for analysis of material flows for environmental analyses, and for economic analyses. This represents a ten-person-year effort, in which twenty-four scenarios—including many products and combinations—have been considered, including electrical power, Fischer-Tropsch fuels, ethanol, hydrogen, dimethyl ether, light gases and animal feed. The work is unprecedented in scope, particularly with respect to mature-biomass conversion technologies, focusing on long-term possibilities.

When energy contents of feedstocks are expressed as $/gigajoule, oil (at the relative modest price of $50/barrel) comes out at $9/GJ, which is less than that of soybean oil ($0.23/lb, $14/GJ), but somewhat more than that of corn kernels ($2.25/bu, $6.50/GJ) and decisively more than that of cellulosic crops ($50/tonne, $2.5/GJ). Their work is focused on cellulosic materials, which would be competitively priced with oil even at $15/barrel. Therefore, the primary economic barrier to be overcome is the cost of processing cellulosic biomass. The most costly step is in converting recalcitrant biomass to reactive intermediates, such as sugars and syn gas; the downstream steps of conversion of intermediates to products, such as ethanol, are relatively mature, relatively high yielding and relatively inexpensive. Research and development on new crops and cropping systems will be vital to realizing the potential for large-scale sustainable production of biomass, for reasons that go beyond production economics.

A key to development of effective simultaneous saccharification and fermentation (SSF)—biomass hydrolysed simultaneously with fermentation—will be the use of exogenous biocatalysts, specifically cellulases. Mesophilic strains of yeast or bacteria that are genetically engineered to utilize xylose as well as glucose, will have a requirement for extra cellulase. This approach may be replaced with thermophilic xylose-utilizing bacteria that, at higher termperatures, require substantially lower inputs of cellulase. Eventually, consolidated or "one-step" bioprocessing (CBP) may be possible, using cellulytic bacteria engineered to improve their production of ethanol or even butanol, or using organisms that already produce ethanol and engineered to hydrolyse cellulose, which would not require exogenous cellulase. Gene-transfer methods are now available for thermophilic anaerobic bacteria and there has been significant progress recently in fundamental understanding of the bioenergetics of cellulose catabolism and sugar metabolism and in development of novel ethanol-producing organisms. Models indicate that substituting CBP for SSF+advanced cellulase has the potential to reduce ethanol production cost by 50%.

One of the scenarios that has been developed indicates that for 100 units of energy in cellulosic biomass, CBP produces 54 units of energy as ethanol and 39 units as residue and biogas. These 39 units may be processed thermochemically to produce power or Fischer-Tropsch gasoline and diesel. Thus, for the 100 units of energy in the feedstock, about 75 units of energy emerge. Considering that about 5 units of energy are used in farming and feedstock transportation, the output:input ratio is 15:1. These data are consistent with others that the energy balance for cellulosic biofuel production will be dramatically positive, indeed higher than the energy output:input ratio for current petroleum processing.■

Breakout Sessions

Biofuels and Bioenergy

Meeting the Energy Demands of Biorefineries

Michael Ott (BIOWA)
Robert Brown (Iowa State University)
Jerard Smeenk (Frontline Bioenergy)
Ken Ulrich (ICM)

Commercial ethanol production from corn is a fast growing industry, and ethanol production in 2005 consumed the equivalent of 20% of the natural gas use in Iowa. The projected growth of the ethanol industry could overwhelm natural gas supplies. Efforts are being made to reduce energy demands of the corn-ethanol process, but another approach to reduce natural gas consumption is to use alternative fuels (such as biomass gasification) to power the corn-to-ethanol process. Gasification is a high temperature process (750–800°C) that converts solid carbonaceous fuels into flammable gas mixtures called syn gas. Biomass gasification produces almost no greenhouse gases and has the advantage of "keeping renewables in renewable fuels." Gasification of biomass can potentially increase efficiency of ethanol plants by converting by-products into additional motor fuel. Gasification is an immature technology that is currently more expensive than coal combustion (but less expensive than using natural gas); it needs more research and development.

Potentially, biomass gasification could improve the profitability of corn-ethanol plants and keep energy dollars local. Frontline Bioenergy is planning a gasification project to fuel a dry-grind corn ethanol facility in Minnesota that will produce 45 million gallons/year. The project will use a staged approach for feedstock logistics and technology scale-up. The first phase will produce 25–100 tons per day (tpd) of syn gas and use primarily wood as a feedstock, but will also test corn stover and distiller's dry grains (DDGs). The second phase will integrate gas conditioning for multiple gas applications and work on improving conversion efficiency. The third phase will produce 300 tpd of syn gas, and will be provided to all major natural gas appliances present in the ethanol plant, including boilers and dryers. The third-phase gasifier will use corn stover, wood, and DDGs as feedstocks.

ICM is responding to requests for various forms of alternative energies to fuel corn-ethanol plants. They are interested in integrating the following alternative energies: coal, steam, gasification and cogeneration. Coal has the advantage of being a readily available and inexpensive feedstock. It is possible to gasify coal as well as utilize DDGs as a gasification feedstock. Using gas in existing ethanol plants makes sense, because gas-burning infrastructure already exists. Utilizing a heat-recovery steam generator will increase efficiency, and is capable of handling fly ash. ICM guarantees 37,00 BTU/gallon of coal and 34.000 BTU/gallon of biogas using existing technology.■

Biorefineries: Technology, Risks and Policy

Anna Halpern-Lande (Cyrnell)
Gregory Bohlmann (SRI Consulting)
Paul Zorner (Diversa)
Eric Bowen (Sigma Capital)

There is a lot of public interest in biotechnology, but further progress will be necessary to develop commercial biofuels industries. We need to move from first-generation bioethanol produced from corn to second-generation bioethanol made from cellulosic biomass. Moving from corn ethanol production to large-scale cellulosic ethanol will require investments in a nascent technology with unknown value chains, which makes investment risky. Sources of funds are institutional investors such as venture capitalists, private equity and project financiers, banks, and corporate investors. Different types of investors work under different terms and require different returns depending on the risk they are willing to take. Risk can depend on markets, policy support, start-up costs and forecasts for second-generation technology, competitive technologies and exit strategies. In order to obtain investment, it is important to assess risk as accurately as possible and to create the best value possible.

The Process Economic Program conducted an in-depth independent evaluation of the implementation of the biorefinery concept in Brazil and North America. The National Renewable Energy Lab (NREL) gives the following definition: "A biorefinery integrates biomass conversion processes to produce fuels, power, and chemicals from biomass." Ethanol production within a biorefinery scenario would require that biomass be utilized both for fuel production and to provide energy for the production process, and the biorefinery would produce additional co-products such as distiller's dry grains (DDGs) from corn and sugar from sugar cane. In order to develop biorefineries, the industry requires sufficient agricultural resources, government support, a stable ethanol market, investment capital, and technology development. Brazil produces ethanol from sugar cane, which has a higher yield than corn used for ethanol production in N. America. Both countries have sufficient land to develop large-scale commercial biorefineries, but this study claims that there is more available land for growth in the biofuels industry in Brazil. The national government of Brazil subsidized ethanol production with the National Fuel Allocation Program in the 1970s, and price supports ended with the liberalization of the alcohol industry in 1999. In Brazil today, fuel ethanol competes with oil successfully, and Brazilian ethanol producers are seeking export markets, while in North America the bioethanol market is small and still growing. It appears that Brazil is well positioned to develop biorefineries due to the efficiency of sugar cane, the established ethanol market, and high sugar prices that are assisting funding.

Worldwide demand for energy is driving growth in alternative energy sources, and the potential of biofuels to replace a substantial portion of transportation fuels is stimulating interest from "big players," including Steve E. Koonin (Chief Scientist, BP). With regard to biofuels, he stated recently that, in the jargon of the petroleum industry, the "size of the prize" is too large to ignore. Diversa is a biotechnology company that is aware that much R&D is needed to achieve the "prize" of a commercial cellulosic ethanol industry. The success of the biomass-to-ethanol process will require synergy among the following unit operations: biomass collection, pretreatment, enzymatic hydrolysis, and fermentation. Incremental improvements in the different biomass-ethanol processes should substantially contribute to overall success. For example, large-scale ethanol production in North America will require locally grown highly productive energy crops as feedstocks. Diverse lignocellulosic feedstocks will require different enzyme cocktails that can work under varied conditions, and Diversa is in the process of developing such novel cellulase enzyme mixtures. One source of cellulolytic enzymes that Diversa is mining is the termite gut and the microbial community that inhabits it. Termites may be a source of novel cellulases that can be optimized through Diversa's direct-evolution tools to perform on specific cellulosic feedstocks under a range of industrial conditions. Improved synergy among the processes will result in overall increases in ethanol-production efficiency.

Sigma Capital is a boutique investment bank that advises renewable-energy companies; one focus is project finance for biofuel production. In general, project finance depends on the cash flow of the project and the biofuels industry currently has very little cash flow. Government cash assistance will be necessary to develop cellulosic ethanol into a large-scale commercial process. There has been a lot of legislative action recently in the United States including the Energy Policy Act of 2005, which contains a number of incentives designed to spur next-generation biofuels. For example, a renewable-fuels standard was legislated that includes a credit-trading system designed to give cellulosic ethanol a larger renewable credit than corn ethanol, but it is still not clear how this system will work and whether it will accomplish its goals of increasing biomass-ethanol production. Another example is the Farm Bill, which mandates the procurement of biobased products by the federal government. These and other legislation have increased hope for needed government economic incentives in the biofuel industry, and though appropriations have been frustratingly slow, they are expected soon.■

Twenty-First Century Biology for Biofuels Production

John Houghton (United States Department of Energy)
Art Ragawkas (Georgia Technical Institute)
Sharon Shoemaker (University of California at Davis)
Jim Fredrickson (Pacific Northwest National Laboratory)

In 1898, Rudolf Diesel manufactured an automobile that ran on peanut oil, and in 1908, Henry Ford came out with his bioethanol-fueled vehicle. Today only 2% and 0.01% of transportation fuel used in the United States are bioethanol and biodiesel respectively. Biofuel production is the grand challenge for the new millennium, and the objective of biofuels research is more, bigger, and better. In order to substantially increase production of biofuels, we need to utilize biomass resources and not rely onlyon corn as a feedstock. An integrated biorefinery approach will ensure that all valuable components of biomass will be utilized. Faster and cheaper ways to characterize cellulose, hemicellulose, and lignin in biomass must be developed to enhance fermentable sugar production via pretreatment and enzymatic hydrolysis. And both five- and six-carbon sugars need to be fermented into ethanol. All of these improvements in bioethanol production must be realized together with parallel life-cycle assessments that will describe the cradle-to-grave impact of the technologies involved.

The model needed for implementing biofuels will include understanding requirements, meeting expectations, and working within existing infrastructure. In the ′70s, ′80s, and ′90s, scientists and engineers used a reductionist approach to understanding biofuel-production processes. In the new century new technologies such as genomics, imaging, and rapid throughput assays are helping to advance relevant technologies.

The United States Department of Energy has declared the need to identify scientific barriers to rapid expansion of cellulosic biofuel production. During a DOE biomass-to-biofuels workshop, general scientific needs associated with biofuel technology were defined. Biomass crops need to be characterized more fundamentally, from the synthesis and deconstruction of plant cell walls to the genomics of the plants and associated soil microbial communities. It is also necessary to expand our understanding of microbial systems involved in biomass saccharification and fermentation. DOE claims that the incorporation of genomics and systems biology into research on the complex biological systems associated with biofuels production can facilitate the transition from predictive to descriptive biology and thus advance the biofuels industry.■

Enzymes for Cellulosic Ethanol

Glen Nedwin (Dyadic International)
Lambert van Orsouw (DIREVO Biotech)
Hal Alper (Massachusetts Institute of Technology)
Marco Baez (Dyadic International)

The time has come for the world to transition from a petroleum-based economy to a carbohydrate-based economy. Abundant feedstocks are available, some (like corn) with costs and others (such as agricultural residues) with negligible costs. Corn is considered to be a first-generation fuel crop and on its own cannot provide enough feedstock for a biofuels economy to develop. In order to produce more biofuels, we need to use second-generation fuel crops that produce abundant lignocellulosic biomass. The development of enzyme technology is the key to creating fuels and bioproducts from available lignocellulosic biomass. The ultimate economic goal for enzyme technology is to have an optimal cellulase/hemicellulase mixture in the same recombinant microbial host expressed at approximately 100 grams/liter.

Direvo Biotech is using novel biological entity (NBE) and directed evolution (DE) techniques to improve industrial enzymes by optimizing parameters such as thermostability and pH tolerance. Both of these methods take advantage of high throughput protein screening techniques. Directed evolution takes Darwinian evolution to a molecular level, by putting existing enzymes through mutagenesis and recombination until an optimal enzyme is obtained. NBE technology occurs through the insertion of random DNA loops into a gene coding for a specific protein, and the formation of an artificial NBE library of variants of the protein of interest. The NBE variants in the library are then screened and tested. NBE has been used so far mostly for proteases, but it is applicable to other enzymes. Direvo hopes to use these technologies to improve biofuel production processes and help overcome challenges in making this industry commercially successful and environmentally friendly.

There are many opportunities and challenges in creating a cellular biorefinery for bioethanol production from lignocellulosic ethanol. The enzymes involved in the process have complex phenotypes not regulated by one single gene, which makes accessing them challenging. Global transcription machinery engineering (gTME) is a tool used to reprogram transcription by accessing latent cellular phenotypes controlled by a multitude of genes. Iterative directed application of gTME was successfully employed to improve the ethanol tolerance of *Escherichia coli* and yeast. This tool proved to be more powerful at improving ethanol tolerance than traditional methods. Significant improvements were realized in weeks versus the decades it used to take to make such changes. gTME combined with traditional methods can help realize cellular, and therefore enzymatic, potential for biorefineries.

In order to find a cost-effective system for lignocellulosic ethanol production, it is necessary to understand the dynamic relationship between feedstock, pretreatment and the enzyme mixture. The various components of biomass may need to be degraded by different enzyme mixtures. One goal of Dyadic International is to construct highly efficient compositions of cellulases for the enzymatic hydrolysis of cellulose. They have identified a wide spectrum of crude mixtures of cellulolytic enzymes and key individual components for efficient saccharification of cellulosic feedstocks for bioethanol production. They have also identified key enzymes that may be used in mixtures tailored to specific pretreatments and types of biomass.■

Biomass to Fuel

Michael Ott (BIOWA)
Georg Anderl (Genencor)
Colin South (Mascoma)
Dimitre Karamanev (University of Western Ontario)

In the United States, Iowa is the leading producer of corn, soybeans and ethanol, and BIOWA hopes to build biorefineries in Iowa in the near future. A biorefinery will break down biomass feedstocks to a common source and then produce fuel (such as ethanol) as the main product as well as by-products such as commodity chemicals, natural fibers, and electricity. Biorefineries will use lignocellulosic feedstocks such as dry distiller grains, corn stover, bagasse, switch grass, and straw. Methods of feedstock collection, transport and storage will be key factors in making biorefineries sustainable. Even though there is still great need for technological advances, biobased products are here to stay and are fundamentally better than petroleum-based products.

Ethanol-market drivers are carbon constraints, positive energy balance, ecological efficiency, and sustainability. In 2005, the ethanol that replaced gasoline reduced greenhouse-gas emissions by 8 million tons, equivalent to the annual emissions of 1.2 million automobiles. But ethanol currently makes up only 2% of our transportation fuel. In order for it to become a major transportation fuel, cellulosic ethanol production will have to be commercialized. In 2000, the major cost-bottleneck for cellulosic ethanol production was enzyme price, but there have been recent advances in enzyme production. Genencor has achieved a 30-fold cost reduction for cellulases, resulting from improvements in enzyme effectiveness and yields. Technical strategies include engineering and recruiting improved cellulase components, developing strains of the filamentous fungus *Trichoderma reesei* with all key activities in one host, and improving efficiency of the manufacturing process. Biorefinery issues that the industry still faces include the feedstock supply chain, the ethanol demand chain, investment strategies, and obtaining government derisking funds.

Mascoma is dedicated to advancing and deploying technology to aid commercializing cellulosic ethanol. Wide-scale bioethanol production will have to utilize a range of feedstocks and will need to take into consideration the interaction of pretreatment, hydrolysis, and fermentation systems. It is expected that the substrate, regional, and scale effects at different production sites will require a range of effective solutions for the processing of cellulosic feedstocks. Mascoma plans early deployment of commercially relevant cellulosic ethanol plants with continuing development of the operation platform as new technologies are developed. Mascoma is developing a microbe that ferments all biomass sugars with temperature and pH optima matched to those of commercial cellulases. Such an organism may further the achievement of ethanol production in one reactor—a consolidated bioethanol process (CBP).

A hydrogen economy that utilizes fuel cells may be a major solution to climate change due to the accumulation of greenhouse gases in the atmosphere. Important requirements for a successful hydrogen economy include more-efficient fuel cells that use much less or no platinum at much lower costs than existing fuel cells. One approach is a hydrogen fuel cell that uses a microorganism as a (bio)catalyst of the oxygen-reduction reaction, while providing a direct electron transfer between the microbial cell wall and the cathode. *Acidithiobacillus ferrooxidans* uses the oxidation of ferrous iron as an energy source and fixes CO_2. A novel electrobioreactor was set up to investigate if ferrous iron could be replaced by a cathode as an energy source for *A. ferrooxidans*. Viable organisms grew for 10 days in such an electrobioreactor—the first record of an organism growing on electrical energy. The bioreactor was then successfully adapted to function as a 320-watt hydrogen biofuel cell that is twice as efficient and uses a quarter of the platinum compared to existing fuel cells.■

Building Infrastructure for the Biodiesel Industry

Timothy Conner (Monsanto)
Christopher Schroeder (Centrec Consulting)
Morten Wurtz Christensen (Novozymes)

Biodiesel has similar expected benefits to those of bioethanol including enhanced energy security, opportunity for farmers, and the fact that it is renewable. Feedstocks available in North America are soybean and canola/rapeseed, which make up 85% of the cost of biodiesel production. Monsanto is using breeding and biotechnology in parallel to increase productivity and quality of feedstocks. They are looking at new technologies such as MRI (magnetic resonance infrared) and NIR (near infrared) imaging for compositional analysis of feedstocks, as well as genomics and germplasm exchanges. Monsanto is engineering fuel crops with innovative techniques for insect protection, herbicide resistance, and improved yields, as well as for nitrogen-use efficiency and oil content. They are committed to improve the availability of fuel crops.

Increases in feedstock prices could limit biodiesel-production growth. A feedstock model can be used to rationalize a combination of feedstocks to produce biodiesel under various future scenarios. Feedstocks used in the model include oil seeds such as canola, imported oils such as palm, and animal fats such as tallow. The model predicts how much biodiesel will be produced given assumptions about the market and what will happen to feedstock prices if biodiesel production increases. Key variables of the model are break-even prices (of petroleum, production/distribution costs, *etc.*), tax incentives and baseline prices. The model shows that maintaining tax incentives is essential and that the two primary variables governing biodiesel production are diesel and feedstock-oil prices: growth in biodiesel production will be driven largely by economics.

Vegetable oil from rapeseed, sunflower and soy are the main feedstocks for biodiesel today, which may not be sustainable in the future if demand continues to grow. Increasing demands for biodiesel crops may be addressed by increasing oil yields per unit area, growing more rape and soy, and utilizing other sources such as nonfood oil crops like *Jatropha* and castor as well as waste oils. Utilization of new feedstocks will require modification of production technologies. Novozymes is developing a simple, cost-effective process that is feedstock-flexible. It is enzyme-catalyzed and converts both free fatty acids (FFAs) and triglycerides into fatty acid methyl esters (FAMEs) in one process, using aqueous lipases instead of the non-aqueous lipases that have been used until now. Pilot-scale production has been successful. Future developments of the process include the use of multiple enzymes to maximize cost-performance on various feedstocks, the incorporation of high-shear mixers to increase initial reaction rates, and the combination of esterification and enzyme-degumming in one process.■

Communicating Progress: What Investors, Reporters, and the Public Want to Hear

Jason Rubin (The Redstone Group)
Daniel Robin (Integrated Investments)
Florence Lindhaus (DIREVO Biotech)
Holly Mitchell (Mitchell & Associates)
Ellyn Kerr (Industrial Biotechnology)

Key emerging biotech areas such as biofuels, biorefining and advanced renewable materials are becoming more mainstream, but bridging the communication gap between scientists and inventors to investors is essential. The first step in getting a new technology off the ground is to have a good management team to effectively communicate the benefits as well as be candid about risks associated with the venture. People would rather invest in a solid team that they trust with an unknown technology than the converse.

Germany is a world leader in biodiesel use and production, and government funding along with tax incentives are helping to increase use and production. Although political and public support for industrial biotechnology (white biotech) in Europe is large, communication with the public about biotechnology is essential. Lessons learned from the green biotech debate in Europe are to discuss problematic issues candidly, to speak more about products than technology, to communicate solutions provided by white biotech, to conduct discussions with environmental interest groups early, and not to argue publicly with other biotech companies.

Communicating emerging biotechnologies with a skeptical public needs inclusion of education and trust building. Western populations are wealthier and live longer, and are, therefore, more concerned with long-term risks. Today's public is also more aware of new technologies due to information readily available via the Internet, but—ironically—this has exacerbated public ambivalence and confusion. Scientists and engineers need to engage the public in clear non-technical language, with balanced coverage of risks and benefits, along with discussions that include input from credible experts and the citizenry. Such discussions should help inform people with balanced information and to help fill the information vacuum before public opinion is polarized, as well as involve citizens in public decision-making.

Innovators of new technologies need to foster good relations with the media since they strongly affect public opinion. Often, scientists are poor communicators, and new biotechnology businesses may want to employ media communications experts. It is important to remember that the media can be a conduit to investors, customers, and collaborators. When utilizing the media to disseminate information, it is important to know who the audience is and communicate accordingly. Being candid with the media is critical; when journalists suspect that industry is being secretive, they tend to "dig deeper" for problematic issues.■

Biofuels and Biobased Business in Asia

Matthew Chervenak (General Biologic)
Pogaku Ravindra (University of Malaysia)
Roger Wyse (Burrill)

In 2005, industrial biotechnology came of age and was driven by a "perfect storm" of supportive policies, technological advances, and market pull. High crude-oil prices and recognition that the world's energy supply is vulnerable increased global interest in biobased fuels. China, with its growing demand for energy, is aggressively developing ethanol plants for the conversion of corn, cassava, and sugar cane, and has set a goal of 10% of renewable energy by 2010. Major investments in biodiesel have been made in India with huge jatropha plantations. Malaysia has set a national goal of 5% biodiesel from palm oil by 2007, and plans to develop a 10% global market share. The special challenges for Asian ventures are that there are few sophisticated bio-investors and few experienced entrepreneurs.

Biodiesel could be incorporated into the current transportation fuel infrastructure in Malaysia and allow easy integration and transition from fossil fuels. It is mostly cleaner burning than fossil fuels (biodiesel has higher NOx emissions, offset by lack of lead, sulfur and mercury). Local potential feedstocks include palm, jatropha, coconut, peanut and soy. Palm is a key feedstock for the development of a viable biodiesel industry in Malaysia is due to its high yields of oil per acre. In India the interest in growing jatropha for energy is due to easy propagation, minimal input requirements and adaptability to drought conditions.

China may be the key to the success of globalization of biotechnology, with vast opportunities for investment. China already dominates in "traditional" biobased products like penicillin and vitamin C; however, these high-volume products suffer from intense competition and over-capacity, and require consolidation. White biotech business is growing rapidly there due to growth in energy demands, severe environmental impacts due to domestic industrialization, and demands for raw materials for domestic and export purposes. New government policies are providing loans and other financial incentives to encourage biofuel ventures. There are fifty mid-sized enzyme companies, including Novozymes and Genencor-Denesco, in China, which serve mostly the international market. China is the third largest bioethanol producer in the world at 650 million gallons per year. The major growth challenge is high feedstock cost. The Chinese biodiesel market is just starting, but is increasing and investments are being made for both domestic and export markets. Biopolymers are another area of potential investment in China with pilot plants for polylactic acid and polyhydroxyalkanoates already in place.■

Issues in Enzymatic Hydrolysis of Lignocellulosic Biomass

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Bin Yang (University of California at Riverside)
Kevin Gray (Diversa)
K.C. McFarland (Novozymes)

According the billion-ton report, 1.3 billion tons per year of biomass could be available for fuel production. The major barrier for a large-scale lignocellulosic biofuel industry is overcoming the recalcitrance of biomass. Biotechnology is dramatically improving cost effectiveness, but pretreatment/enzymatic hydrolysis (*i.e.* the production of fermentable sugars) is still a key roadblock to success. Cellulase efficiencies need to be improved and the synergy of different pretreatments, different enzyme cocktails, and different substrates needs to be understood better.

The factors that affect enzymatic susceptibility of lignocellulosics can be divided into those that are enzyme-related and those that are substrate-related. Pretreatment has a large impact on substrate-related factors, while the enzymes affect enzyme-related factors and substrate factors. Various strategies have been used to increase enzyme efficiency and reduce cost, including molecular biology techniques that increase enzyme activity, creation of better enzyme mixtures with improved synergy, variations in reactor design, improvement in substrate/enzyme interactions by blocking non-specific binding to substrate, and enhanced cellulose reactivity through pretreatment. A study was conducted to better understand why the rate of enzymatic hydrolysis of cellulose drops off with time. "Restart" involves periodic removal of cellulases, washing substrate, and restarting the hydrolysis with the same initial conditions. When "restart" of enzymatic hydrolysis of cellulose was conducted every hour for four hours, the hydrolysis rate did not drop off as during an uninterrupted four-hour hydrolysis. During interrupted hydrolysis, substrate reactivity remained constant or increased with conversion. Slowdown during uninterrupted hydrolysis may result less from loss in cellulose reactivity than from enzyme activity being slowed either by obstacles that interfere with their path, or loss in activity or processivity making them less effective.

In order to expand bioethanol production according to EPA goals, it will be necessary to develop alternative feedstocks to corn, such as lignocellulosic biomass. Due to its complexity, production of ethanol from lignocellulosic biomass requires integration of all of the steps involved in the process. Feedstock production, harvest, and storage can affect pretreatment requirements and impact the success of enzymatic hydrolysis and fermentation of sugar monomers to ethanol. Differences in feedstocks, such as variations in lignin and hemicellulose structure and content between hardwoods and softwoods, may require different pretreatments or different enzyme cocktails. Different pretreatments—alkali, acid, organosolvents, *etc*.—define what type of substrate enzymes will encounter. One goal of Diversa is to construct a suite of enzymes (tightly coordinated with feedstock and pretreatment technologies) with a high synergistic rate of hydrolysis that will allow reduction in total protein requirements.

Currently, there is no market for biomass-to-ethanol enzymes and there are significant technical and economic challenges to the commercialization of biomass ethanol. Major technological challenges include high capital cost for pretreatment, low solids concentrations during enzymatic hydrolysis, washing biomass and wastewater, and enzyme production capital costs. The prediction of rapid growth in the cellulosic ethanol industry will require large increases in enzyme efficiency and productivity. Enzyme activity may be increased by raising specific activities of individual enzymes, improving enzyme mixtures, or producing more thermoactive enzymes. Enzyme production costs may be improved by increasing fermentation yield, simplified enzyme recovery, and on-site production. Overall process costs may be reduced by increased solids loading for enzymatic hydrolysis and reduced pretreatment requirements. Novozymes, committed to improving biomass-degrading enzymes, has been working with enzymes involved degradation of arabinoxylan (carbohydrate present in biomass). They have tailored an enzyme mixture for different arabinoxylan substrates that reduced enzyme loading by 14–27-fold compared to the best currently available products. ■

Bioenergy Production from Wet Crops and Organic Wastes

Manfred Ringpfeil (Biopract)
Christian Weigel (Arthur D. Little Austria)
Matthias Gerhardt (Biopract)

Microbial biomass conversion under anaerobic conditions produces methane. A consortium of microorganisms and free enzymes are needed to convert biomass into fatty acids, carbohydrates, and protein. It has been shown that the enzymatic step is limiting and the addition of free enzymes can improve biogas production. Such a process can also produce ethanol and methane, but a separate reactor is required for ethanol production while ethanol byproducts can be converted to methane to power the ethanol producing plant. Separation of methane from the liquid phase and from CO_2 is not problematic; this is a low-cost way to make methane.

European demand for natural gas is on the rise, whereas natural gas production is declining. A supply gap of 120 billion m^3 is expected in Europe by 2020, and natural gas price increases are positively correlated with rising crude oil prices. Biogas production potential in Europe is estimated to be 35 billion m^3, which is 7% of European natural gas demand. 80% of the biomass needed to produce this amount of biogas could be based in agriculture, but currently only 16% of this potential is realized. Reasons for this gap include scattered supply, missing infrastructure, and high production costs. Current methane production costs in Europe are much higher than of imported liquid natural gas (LNG). But technological improvements should reduce gas-production costs while LNG prices are expected to rise. Biogas-production costs are expected to equal LNG prices by 2030 as the number of companies and investment volumes are increasing for European biogas plants.

Biogas production began in Germany in the 1980s as a waste-reduction strategy. The Renewable Energy Act forced the development of biogas production from agricultural crops, and the industry has been growing at a rate of 20–30% since the year 2000. Enzymes are playing a role in improving biogas yields. Non-starch polysaccharides, which make up approximately 51% of the biomass used for this process, require enzymatic hydrolysis to oligomers of 2–8 carbons, and this hydrolysis was improved by the addition of free enzymes. In field trials conducted at thirty biogas plants, 100 grams of enzymes per ton of dry biomass increased biogas production by 3 to 35%, and increased the net profits of the biogas facilities.■

Feedstocks for Bioprocessing and Biomaterials

The Importance of Agricultural Feedstock for Bioenergy Production

Maurice Hladik (Iogen)
Joe Bouton (Samuel Roberts Noble Foundation)
Anna Rath (Ceres)

In the future, biorefineries will require huge amounts of biomass. A cellulosic ethanol facility that produces 50 million gallons per year will need 2,000 tons of biomass per day in order to operate at full capacity. Biomass storage is one part of the supply chain that needs development since simple baling is a cumbersome way to store and bring biomass to a refinery. Agricultural residues will likely kick-start the biorefinery industry, but establishing dedicated perennial biomass crops will be ncessary if the industry is to grow beyond a few dozen biomass plants. Critical industry needs include obtaining secure and sustainable feedstock sources, dealing with a limited number of suppliers, secure knowledge of the agriculture residue including grower intentions to contract, and demonstrated grower ability to reliably produce perennial biomass crops. Farmer confidence in being able to meet contract terms with perennials is currently a major issue; government policies may be needed to assist farmers as they learn to grow these crops.

The Noble Foundation, in partnership with Ceres, is trying to improve switchgrass as a dedicated biomass crop. Switchgrass was selected for development since it is a seeded C_4 warm-season perennial grass that is found throughout North America. It will yield 80–100 gallons of ethanol per ton and, therefore, 800–1000 gallons of ethanol per acre if 10 ton/acre biomass yields are achieved. This yield target is important since transportation costs decrease as yields increase, and yields around 10 tons/acre are needed to keep transportation costs feasible. A 1990s DOE program on herbaceous biofuels found lowland switch grass types had greater yields than upland counterparts; Ceres and the Noble Foundation are hoping to developing high-yielding, commercial varieties of switchgrass. They have created a synthetic cultivar that demonstrates a 26% improvement over currently available varieties, which they hope to commercialize. Development of elite bioenergy cultivars will continue.

Several issues, among them energy security and environmental sustainability, have recently come together to create the "perfect storm" driving biofuel development. Despite the momentum, several perceived problems are commonly cited:

- not enough biomass exists to make a difference;
- not enough land is available to grow biomass for energy uses;
- farm economics will not drive farmers to grow energy crops; and
- the cost of cellulosic ethanol is too high.

However, not all of these problems hold up under scrutiny and dedicated biomass crops may help overcome these objections. Yields are expected to increase from 5 tons/acre today to 10–15 tons/acre and conversion technology will improve from 60 gallons/ton to 80 gallons/ton. Also, with yield increases, 100 million acres could produce more than 50% of the US gasoline demand by 2015. Future ethanol yields from biomass could exceed those from corn, driving farmers to grow biomass crops. Finally, plant biotechnology may contribute to coproduct development, to help overall process economics.■

Production of Novel Molecules in Plant-Based Systems

Eric Seewald (Bayer CropScience)
Bruce Ferguson (Edenspace Systems)
Ann Bublitz (Biotech Decisions)

Prior to the petroleum era, society obtained around 95% of its materials from plant-based sources. Cheap, available petroleum drastically reduced usage of plants for raw materials, but this is changing as the price of oil rises and becomes increasingly unpredictable. In general, the technology toolbox for producing materials in plants is already available, simply requiring the pieces to be put together. Plant biotechnology has undergone two developmental generations: the first consisted of input traits such as herbicide tolerance, insect resistance, and improved yields; the second generation has focused more on output traits such as higher expression, functionalization, and new materials. For those considering using a plant as a biofactory, several selection criteria are to be considered, including the plant's germplasm, genomics for transformation, seed scalability, harvests per year, stewardship required, identity preservation, yield, replanting rate, and oil in the seed or fruit. Separation and purification costs must be managed carefully.

Since a cheaper supply of medicines would be desirable for the pharmaceutical industry, plants offer an attractive production platform due to their lower capital costs, fixed operating costs and scalability. However, there are novel risks and many important considerations when using plant-based production systems. Plants can be grown in open fields or in confined facilities, but when using an open field, sterility-control mechanisms are vital to control gene flow and avoid major regulatory problems. Also, choice of plant platform has been historically based on what the scientist is familiar with rather than what is optimal for pharma and industrial proteins. However, this constraint is changing as information is now available for several species. Currently, plants including corn, rice, barley, alfalfa, soybean, safflower, tobacco and others can be used for industrial or pharma applications. However, differences exist between plant species in terms of technical feasibility, ease of containment, intellectual property issues, protein production and modification characteristics, and ease of purification. Good choices are made when carefully weighing these issues,keeping in mind that a "perfect" platform does not exist.

In addition to pharmaceuticals, plants also have great potential for fuel production. Edenspace is trying to integrate enhanced crops with today's production facilities, while paying due attention to downstream processing costs. Their efforts have focused on using corn as a biomass crop by engineering the endoglucanase E1 and a bioconfinement gene into the plant. They are also targeting biomass traits and the cellulose/lignin ratio in corn for optimization. Producing the enzyme in the plant reduces exogenous enzyme costs and mixing difficulties, but intellectual property and regulatory challenges exist. By optimizing crops for energy production rather than food, they have demonstrated a 50% increase in biomass yield. However, current intellectual property practices along with existing industry and regulatory structures are delaying production and development; adoption of an approach modeled after IBM's *Ventures in Collaboration* program would greatly benefit agriculture biotechnology.■

Independent Assessment of the Billion-Ton Report and Feedstock Availability

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Kenneth Muehlenfield (Auburn University)
Melvin Askey (Central Science Laboratory)

The USDA and DOE recently issued what is known as the "billion-ton report," projecting a scenario where the United States produces 1.36 billion tons of biomass annually for energy production, which in turn reduces petroleum consumption by approximately 30%. Notably, the report focuses on total petroleum consumption rather than gasoline consumption; therefore, 30% represents about 95.5 billion gallons of petroleum displacement. The 1.36 billion tons are derived from forest resources (368 million tons) and agricultural resources (998 million tons). Perennial biomass crops are expected to provide 377 million tons annually with a yield of 6.7 dry tons/acre. Switch grass is the most widely discussed perennial biomass crop and already has yields of 7 dry tons/acre on commercial plots in the southeastern United States, although the great plains and midwest have not yet reached this target. In general, the report used reasonable yield numbers, and other crops such as *Miscanthus*, energy cane, and giant reed may have potential with demonstrated yields already higher than those of switchgrass. One important question, however, is whether or not conversion technologies will pay growers $50/dry ton, which is an estimate for what they would actually need or want to do the work.

The billion-ton report also projects crop residues playing an important role in energy production and uses several assumed values in estimating what contributions will come from these sources of biomass. For instance, average corn yield is projected to be 278 bushels/acre annually in 30 or 40 years, representing an increase of 50% from the current level. Also, the soybean residue/grain ratio is expected to rise from a current 1.5:1 to 2.0:1 with the development of a large-biomass soybean. Additional assumptions include an ability to recover 75% of the stover and universal adoption of no-till farming practices. Some of the assumptions are questionable: dryland corn has experienced a linear yield increase over the last 20 years; extrapolating this to 2043 results in around 250 bushels/acre, well below the 278 bushels/acre the report assumes. For the proposed large biomass, soy grain yield and grain harvest efficiency are unproven. Finally, machinery will very likely be capable of recovering 75% of the stover, but widespread adoption of no-till farming is highly questionable.

In addition to agriculture, forest-derived resources are expected to make significant biomass contributions to the billion-ton goal. An important constraint in using forest-derived biomass is that the use must be sustainable and not displace current needs. Some assumptions used in the report were 41 million dry tons from recovery of logging residues, 60 million dry tons currently from fuel-treatment thinnings, 28 million dry tons from consumer waste streams, and 8 million dry tons from mill residues. In general, 279 million dry tons of forest-derived biomass were estimated to be currently available with growth adding an additional 89 million dry tons by 2030. Overall, these estimates are reasonable, although some challenges include how to utilize contaminated consumer waste streams and the difficulty in estimating the availability from fuel-treatment thinnings. Additional factors include potential export of woody biomass to other countries for energy, and additional biomass contributions that could come from public lands.

Finally, some general observations about biorefining suggest everyone knows everything about it, but nobody knows how to achieve it. There is poor understanding of the system as a whole, from the supply chain and its needs to the manufacturers and their needs. The manufacturing sector is unaware of what the land-based sector can offer and land-based sector is unaware of manufacturing needs. Potentially, there are many types of biorefining, and co-products, along with ethanol, require attention. A wheat field can be thought of in terms of wheat grain alone or as a whole range of opportunities, such as the colloidal silica in the straw, which, when refined has a value of $18,000/ton to the water industry as a filtration agent. Oilseed rape is another under-utilized plant in that its biomass isn't used and solvents could likely be produced from the oil. Meal, an animal feedstock, is virtually unexploited, but could be used for producing a range of materials including a residual fuel feedstock. Many opportunities exist; better organization and prioritization will be needed if they are to be realized.■

Biomass Harvesting and Soil Nutrients

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If cellulosic ethanol technology becomes widespread and provides a significant fraction of total transportation fuel needs, large amounts of biomass from many sources will be required. Eventually, these biomass requirements are expected to be met by a combination of dedicated energy crops as well as leftover residues from traditional agriculture. However, the impact of residue removal must be carefully considered. A field of stover might be seen as "going to waste" by an entrepreneur, but stover does provide certain ecological services such as erosion control, feed, bedding, and importantly, a source of organic matter for the soil. In particular, soil organic carbon (SOC) must be carefully monitored; current soils already have lost 50% SOC since pre-cultivation times, and if biomass is simply harvested without careful management, SOC will likely undergo another significant decline over time. Residue return and reduced tillage are the only currently known ways to increase SOC, so an important question is how can soils be managed to at least mitigate any further decline? An objective of the renewable energy assessment project (REAP) of ARS is to develop tools and algorithms to predict SOC changes and aid in management.

Nutrient recycling in biomass-to-energy scenarios will be an important part of ensuring that these systems are sustainable. Importantly, plant nutrients should not be thought of as contaminants in biofuel production since artificial fertilizer inputs are energetically intensive and costly. By linking feedstock production with biomass processing in a manner analogous to integrated plant-animal production systems that were developed in Europe during the 1500s, significant gains may be possible. The goal of linking fields and biorefineries through nutrient recycling is to minimize fertilizer inputs and nutrient loss. Unused residue from the enzymatic process could be gasified, which would allow recovery and recycling of ash and ammonia. Potentially, in a system with a 120 kg N/ha input, the potential recovery is 75 kg/ha, or about 66%. Work on optimizing this system, including optimizing feedstock rotations and studying impacts on soil properties, remains to be done.

While biomass-to-energy systems will need to be carefully managed through good soil-use practices and nutrient recycling, designers will also need to realize the competing demands that will be placed on biomass. Currently, the interest in biomass is focused mainly on cellulosic ethanol, however as carbon taxes or credits loom in the future, biomass will have added value to those industries facing carbon constraints. Already, biomass for power and heat generation has surpassed hydropower as an energy source in the United States; the potential conflict in demand between biopower, biofuels, and bioproducts suggests that the pricing structure of biomass could change dramatically. Many technologies exist for biomass conversion to power and heat; those that provide the best quality ash in terms of carbon and minerals will have an advantage. Additionally, to lower energy requirements of the total production system, alternative ways of fixing nitrogen into fertilizers, such as biochemical fixing, anaerobic digestion, and electrochemical synthesis of urea from flue gas NOx should be a research priority.■

Enhanced Utilization of Corn

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Coproducts from corn-ethanol production have stimulated several new and unique research interests, as well as advances in nutrition principles that are now widely used in animal production systems in order to increase animal productivity. Historically, ethanol plants were built and optimized for ethanol production and the resulting coproducts were more of an afterthought as they were simply placed on the market, which then determined the value of the coproduct. Letting the market decide, however, may not be the best option: corn ethanol syrup value has a similar amino acid profile to milk, but has markedly less value. A more sophisticated approach needs to be adopted that systematically looks at products and determines the best value proposition and identifies market opportunities where nutritional attributes fit with market needs. For example, front-end fractionation separating the bran and germ results in higher starch levels for fermentation and higher protein DDGs, which should be targeted to dairy markets. Product variability, though, is a major hurdle that must be overcome as coproduct users have tight tolerance requirements where even 6–8% truck-to-truck variation is too extreme. Therefore, stringent product controls need to be adopted.

China is using corncobs, stover, and wheat straw as raw materials for their nascent biorefinery industry. As in the United States, Chinese plants are struggling to bring costs down for cellulosic processes so that the resulting ethanol is competitively priced with traditional starch-produced ethanol. A cellulosic biorefinery that produces several coproducts as well as ethanol may offer the best chance of economic viability. Some plants in China have used the higher hemicellulose content of corncobs for production of various coproducts such as xylose, xylitol, furfural and related products. The resulting waste residue has a cellulose content of 56–60%, which could be hydrolyzed into its constituent sugars by cellulases and then fermented into ethanol. The long-term objective in China is to establish a 50,000 tons of ethanol/year corncob biorefinery and expand raw material and coproduct use as well as establish a set of demonstration facilities that will refine the whole corn crop into various products.

In ethanol dry-mill plants, DDGs and ethanol are the primary products. As more ethanol plants come online, new outlets for DDGs will need to be found. Upstream fractionation processes result in less DDGs production, but low margin feed products are produced and corn requirements are increased. Nutritionally rich corn, produced through biotechnology, may be used in a novel processing technology that allows better fraction separation and higher-value coproducts. The biotech corn has elevated levels of protein, oil, and lysine compared to currently available varieties. The process technology is a dry separation system resulting in highly fermentable and high-oil fractions. The fermentable fraction yields ethanol and high-protein, low-fiber DDGs. The high-oil fraction undergoes further processing into corn oil and a nutrient-rich meal. Rennessen's technologies are designed to maximize oil and nutrient recovery rates, and the oil-extraction technology has a lower cost and results in higher value meal than current alternatives. The overall system mimics wet ethanol corn facilities, resulting in significant savings.■

Feedstock Pretreatment and Hydrolysis

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There have been significant advances in cellulosic ethanol production in the last several years, but it is still far from being a mature technology. One developing area is pretreatment technology. Currently, the leading technologies are dilute-acid pretreatment and ammonia fiber expansion (AFEX). Both result in degradation products, but dilute-acid pretreatment results in several, with furfural, formic acid, hydroxymethylfurfural, and levulinic acid most prominent. AFEX pretreatment results in a substantial increase in phenolics and lactic acid as well as some acetic acid and 4-hydroxybenzaldehyde; however, it causes less enzyme inhibition than does dilute-acid pretreatment. Importantly, scanning electron microscope analysis showed that while AFEX also yielded many types of aliphatic acids and sugar degradation products, there were no furfural or furfural-based products.

Within the cell-wall structure, cellulose forms microfibril aggregates, which have significant potential as a reinforcing material in biocomposites. However, current extraction, purification, and processing methods face certain challenges—*e.g.* high energy requirements—which present obstacles in expanding commercial use. An enzymatic pretreatment approach is being investigated for the production process as a way of reducing overall energy requirements. For instance, expansin weakens hydrogen bonding between cellulosic microfibrils and the hemicellulose chains that they are effectively tethered to; this weakening is reversible and allows easier separation and purification. Treatment with this enzyme (either directly or through application of a fungal species producing the enzyme) results in shifting microfibril-diameter distribution to the lower diameters. Hopefully, enzymatic pretreatment routes will allow elimination of cryocrushing and fewer passes in high pressure homogenization, which are energetically intensive processing steps.

Aside from cellulose, other components of the plant cell wall merit attention for industrial processing and offer opportunities for creation of value-added products. Xylitol offers many health advantages over traditional sugar since it combats dental cavities, inhibits plaque, is safe for diabetics, and has pharmacological value. Currently, about 40,000 tons of xylitol are produced annually, with growth rates of 5–8% *per annum*. Commercial production relies on chemical synthesis; however, xylitol could be produced from plants using biotechnology. In essence, after acid pretreatment of a cellulose feedstock, microbes could ferment xylose into xylitol. However, this process needs to be optimized. Current work is focused on three factors: hydrochloric acid concentration in the pretreatment step, differing solid:liquid ratios, and varying exposure time in order to maximize xylose production and minimize glucose yield. Total volume and overall process time were found to be important factors in optimization.

Another plant residue that could undergo further processing is flax straw, of which approximately a million tons are currently left on the field and considered an agricultural waste. This straw could be used to produce a natural fiber that is 2–3 times as strong as cotton and could have commercial use in composite materials. However, process conditions for decortication and enzyme retting need further development for commercial viability. Therefore, work has been focused on developing a new enzyme-retting process using both novel and engineered pectinases and cutinases. Targets of this work involve finding biocatalysts with pH, thermal, and substrate stability. Results have shown that after use of the enzyme cocktail, separation of fiber bundles from epidermis and shive occurred. Refinement of this enzyme-retting technology continues, with attention focused on selection of biocatalysts and process conditions.■

Potential of Energy Crops

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Several characteristics are needed in a good biomass crop: efficient C_4 photosynthesis, rapid nutrient transport from roots to shoot during the growing season, and nutrient return to roots as the growing season ends. Although switchgrass, unlike maize and short-rotation coppice, generally meets these characteristics, it is not the only species that could feasibly be harvested for its energy content. In Europe, for example, a major energy crop under development is *Miscanthus giganteus.* Typically, this species yields more than does switchgrass, and current work at the University of Illinois has focused on whether it can grow in the mid-west and yield comparisons with switchgrass. During the 4-year trial, yields as high as 60 t/ha were recorded, although they tended to decline over time. However, even with the decline, yields—20–40 t/ha—were still higher than of switchgrass. Drawbacks to this species included no genetic diversity, lack of propagation stock, an inefficient and costly planting technique, and a general lack of familiarity.

Sugar cane and energy cane represent additional energy-crop possibilities in the United States. One advantage of working with canes, sweet sorghum, *Miscanthus* spp. and *Erianthus* spp. is that knowledge and technology needed to grow and harvest them are already available. Some important objectives for using these plants as energy crops involve integration of feedstocks for continuous delivery to biorefineries as well as ensuring that good cropping-system management information is available to maximize yields. Sugar cane would be harvested in November or December whereas energy cane, which is more cold tolerant, would be harvested around April. In general, yields for sugar cane fall from 30 Mg/ha to 13 Mg/ha by the third-year harvest; energy cane avoids these declines and typically has a biomass yield of 25–30 Mg/ha. Preliminary work has focused on developing hybrids between sugar cane and *Miscanthus* or *Erianthus*. Further development of these varieties is needed, including biomass characterization, development of best agronomic practices, and impact studies.

An additional species that may have use as an energy feedstock is *Arundo donax*, commonly known as giant reed. This C_3 species requires well aerated soils, does not tolerate waterlogging, and is used mostly for instrument reeds and as an ornamental plant. Additionally, giant reed must be vegetatively propagated similar to sugarcane since it does not produce viable seed. Research plots in Alabama showed biomass increases from 8.8 to over 20 tons/acre over a 6-year period; commercial plots would likely show lower yields of 15 tons/acre by year six. Interestingly, the plant showed no response to nitrogen fertilizer once it was established, indicating a need for further research.■

America's Energy Future: Role of the Agricultural and Forestry Community

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William Richards (Agri-Business Consultants)
William Horan (Horan Brothers)
Peter Nelson (Biodimensions)

The 25×'25 initiative is a renewable energy effort that originated with the agricultural and forestry sectors. The goal is to have 25% of the energy consumed in the United States come from forests, farms, and ranches by 2025 without negatively affecting production of food, feed, and fiber. Wind, solar, and capture of biogas are all envisioned as important sources of energy in this initiative. 25×'25 started in 2004 when the draft vision was developed. Currently, it is in its third phase: bringing the vision to life. This has involved creating a broad national alliance supporting 25×'25 as a national goal as well as creation of individual state alliances. Currently, over 200 organizations, eleven governors, four state legislatures, and many members of Congress have endorsed the goal. They hope to recruit over 50% of Congressional members as supporters, with over twenty state alliances operational by late 2006. The Implementation Plan is currently under development and is guided by the "yes-if" principle to specify exactly how the goals will be achieved.

For 25×'25 and other large-scale biomass initiatives to be successful, no-till agronomic practices will need to be widely adopted. While changing farming practices on this scale is a formidable challenge, Bill Richards has been successfully using no-till farming for over 30 years. In general, yields and soil quality have improved and planting is easier. In the United States, adoption of no-till practices has lagged behind the rest of the world, but high energy prices may encourage acceptance. However, crop stover should not be regarded as waste, nor should it be regarded as free since it performs valuable ecological services to sustain no-till; therefore, dedicated energy crops will be best suited for providing energy and raw materials. Development of nitrogen-fixing crop varieties would be a major boon for farmers and no-till. Since energy crops and no-till farming will represent the largest land-use change since the dust-bowl era, research is needed on what effects these changes will have on wildlife.

In addition to getting large-scale biomass initiatives operational, establishment of biomass-processing facilities represents another significant challenge. The Western Iowa Energy Project has first-hand experience of the difficulties faced when starting up a biodiesel plant. Their first project required 55% equity to secure 45% debt, but as interest in alternative fuels has increased, they have seen the required equity decrease to 45%. This interest also allowed recent completion of an equity drive that raised over $22 million in 11 weekdays, which caught them by surprise. Their model involves first obtaining a property option on the plant site, establishing an LLC, and commissioning a site-specific feasibility study. The board of directors is established with six local community members from various career paths. An invitation-only seed-capital meeting is organized with thirty-five non-qualified investors to raise the initial $3 million to begin building equipment, lock in steel prices, and start permitting processes and site-specific engineering. The business plan, lender negotiations, prospectus drafting, and equity drive will follow.

When considering the overall biomass supply chain, the role of the farmer should be given careful thought. The total number of farms has decreased markedly over the past 30 years, and the skew of farm-subsidy payments to the top 20% hasn't helped this trend. As use of biomass expands, farmers could have three roles:

- a farm-gate scenario where biomass is sold as a commodity,
- participation in residue-removal programs if sufficient incentives exist ($28–43/acre, depending on the crop), and
- a role in a vertically integrated model where the farmer owns part of the factory.

The farmers are positioned as stewards of photosynthesis into energy. The main growth for farm-based businesses will be in two areas: biobased products/environmental services and in new crops. Biobased products are continually expanding and environmental services involve land remediation and carbon credits for no-till farming. New crops will come from development of the many plants that have commercial applications. Research extension services will also change to focus on cyclical communication, niche deployment, and applied adoption.■

Oil-Seed Technologies to Decrease Petroleum Dependence

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Mark Smith (National Research Council of Canada)

In many cases, vegetable oils can directly replace petroleum-based lubricants. Vegetable oils have the advantages of low volatility, good boundary lubrication properties, good viscosity-temperature characteristics, biodegradability, and are compatible for blending with mineral oils. Their main disadvantages are poor oxidative stability, poor fluidity at low temperatures, and hydrolytic instability. These problems are usually overcome through chemical or genetic modification of the oil, with additives or through blending. Recent work has focused on testing some commonly used antioxidant and antiwear additives and a pour point depressant. Synergism was observed between some antioxidants and antiwear additives. Many of the formulated vegetable oils performed better in tests than did commercially available vegetable-oil varieties. Overall, results suggested vegetable oils could be used in many industrial applications, with performance characteristics similar to those of petroleum counterparts.

There are numerous reasons why vegetable oils may increasingly displace petroleum:

- higher petroleum costs,
- biotechnology advances, and
- plants hold the key to closing the CO_2 cycle.

Ricinoleic acid, or castor oil, is of particular interest because it is a hydroxy fatty acid (HFA) capable of numerous, unique oleochemical conversions. However, castor bean contains toxic ricin, and the castor-oil supply is limited and variable. Therefore, an HFA with a stable source and non-toxic by-products is desirable. Efforts are currently underway to produce such an acid by engineering the hydroxylase gene from castor into *Arabidopsis*, and then into another oilseed species. In *Brassica napus*, 16% HFA has been achieved, with most of the other oils composed of C18:1 oleic and C18:2 linoleic fatty acids. This approach yields a product with numerous potential industrial applications, including automobile lubricants, in which HFAs are a necessary component.

While many potential industrial applications exist for plant-produced oils, significant challenges remain. Homogeneity, oil content, available fatty acids, and co-products are all areas needing improvement. Homogeneity has been significantly improved by the development of high-oleate varieties where more than 80% of the fatty acids are 18:1. High erucic (22:1) varieties have also been developed, albeit at lower levels (64%). On the other hand, plants produce a variety of novel fatty acids, although expression levels greater than 20% have not yet been achieved.■

Genetic Improvement of Energy Crops and Associated Benefits

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Plant hardiness zones and ecoregions (areas defined by their thermal and moisture profiles) determine the dominant plant populations in a given geographic area. In the United States, there are ninety-eight plant-adaption regions. Switchgrass, which can grow in many regions, although not equally well in all, does not seem particularly well suited to deep southern areas. Therefore, when grown for energy content, several varieties will be needed in order to obtain maximal yields in each region. Switchgrass has octa- and tetra-ploidy levels, is difficult to breed since it is self-incompatible and hand-crossing is problematical, and each generation grows for 3–5 years. Efforts to increase switchgrass yields will have to factor in even small lignin changes, which can have large impacts on ethanol yields from cellulose. Information on lignocellulosic biomass traits and their economic value is needed and on basic genetics and biochemistry of switchgrass, as well as structure, anatomy, and composition. Conventional and molecular techniques will be needed.

Ceres is using high-throughput genetics technology and related methods to accelerate plant breeding and rapidly develop switchgrass energy-crop varieties. Yield is especially important in biomass-crop scenarios. Many traits, including stand establishment, photosynthesis, nitrogen economy, stress tolerance, hormone biology, reproduction, plant architecture, and processing readiness need to be improved or conserved simultaneously as energy-crop varieties are developed. Marker assisted breeding (MAB) can accelerate the development curve and MAB plus transgene introduction can accelerate the curve even further. Genes that influence tons per acre and ethanol yield in gallons per ton have been identified. The first full-scale seed release is projected for 2009.

In addition to switchgrass, hybrid poplars and willows have potential as biomass crops. They offer the advantages of rapid growth, straight stems for efficient processing, male and female plants, and easy vegetative propagation. However, one major issue is large stem size, which can require separate handling when harvested. For energy-crop purposes, multiple stems need to be harvested, and work on systems capable of such production is underway. Poplar 4–6 years in age typically yields 9–13 dry tons/acre/year, and 3 year-old willow typically yields 4–7 dry tons/acre/

year. If woody biomass is chipped, the mixture of wood and bark represents an important quality consideration; debarking and breeding to control bark thickness and chemical composition are possible. Typically, hybrid trees have higher cellulose and lignin contents as well as lower hemicellulose content than herbaceous crops. However, energy inputs are low, planting and harvesting is not required annually, and biomass storage facilities are not needed.■

Biochemicals

Biorefineries: Building the Future on Dirt-Cheap Sugars and High-Value Co-Products

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Gustavo Valença (UNICAMP)
Steve Lewis (Broin & Associates)
Norris Bond (Applied Milling Systems)

The ideal feedstock for biorefineries is cheap sugars, and the best way to produce them is with an inexpensive source of fiber. There are two major industry trends. The first is the implementation of dry-mill fractionation, the process of breaking the corn kernel into its components. The exterior layer, the corn bran or pericarp, and the endosperm, the majority of the kernel, are used for ethanol and feed production. The third component, the germ, is used primarily for production of edible oils or biodiesel. A potential process flow could start with the corn fiber fraction that would undergo destarching with α-amylase to produce starch dextrin for ethanol fermentation. The remaining fiber fraction would then be milled and pretreated, and the cellulose and hemicellulose fractions would be reduced to sugars with cellulases and hemicellulases. The resultant sugars would then be the feedstock for ethanol fermentation. A second key industry trend has been enzyme improvement; enzyme dosage has been decreased dramatically. The potential benefits from these trends will be increased ethanol yields per bushel of corn and higher-quality co-products.

As a result of the 1973 OPEC crisis, Brazil initiated a program to produce ethanol from sugar cane to reduce dependency on foreign oil. Although ethanol cars were available by 1975, it took five more years to establish the necessary infrastructure. Initially ethanol was heavily subsidized and research was conducted to increase both sugar content of the crop and expand the area on which it was grown. Subsidies were reduced to 3% between 2002 and 2004 and now ethanol is no longer subsidized. In addition to ethanol and sugar, by-products include vinasse, which can be used as fertilizer, and bagasse, which is burned as an energy source at the mills and excess energy is sold to the grid. A variety of chemicals can be derived from sugar cane including acetic, citric and lactic acids, glutamate, lysine and polyhydroxybutyrate. Innovation could include enzymatic hydrolysis of bagasse to produce ethanol and the conversion of vinasse to biogas. In the future, a complete biorefinery could be built including the present innovation strategies and production of polyhydroxyalkanoate polymers.

Building upon very high gravity (VHG) fermentation technology, two proprietary processes, BPX™ and BFRAC™, have been developed. BPX™ is a type of "no cook" ethanol production, where lower temperatures are used instead of a high-temperature liquefaction stage. Commercialization of the process by Broin required key innovations such as engineering enzymes that could function at low pH, and at low calcium and high temperatures. Improvements on the conventional process meant BPX™ in the raw starch process, to screen for potential enzymes. Investigation revealed that Novozymes had the best α-amylase, so the two companies collaborated to improve the process. Through their combined efforts, the enzyme dosage has been decreased significantly. BFRAC™ is the Broin fractionation of corn into fiber, germ and endosperm streams, based on the Satake Patented Milling Process. BFRAC™ benefits include increased fermentable solids in fermenters, higher value co-products, differentiated DDGs, and lower net cost to produce a gallon of ethanol. The endosperm stream created by BFRAC™ is fermented to ethanol using BPX™. With conventional high temperature liquefaction, there is loss of free amino nitrogen (FAN), which is a requirement for yeast nutrition. The BPX™ technology eliminates the high-temperature stage, so more FAN is maintained. The combined processes of BPX™ and BFRAC™ have resulted in a 5–20% reduction in net cost of ethanol production.

The fractionation process is important to the ethanol industry because it increases the accessibility of starch for conversion to alcohol with traditional fermentation. If the bran and germ are properly removed prior to fermentation, the following benefits accrue: more-efficient utilization of fermentation vessels, more-efficient starch-to-glucose conversion, energy savings in drying of co-products, conversion of bran and germ to more-valuable co-products, and increased protein content of DDGs. Different priorities apply for fractionation for food and for fermentation. The highest priorities for food are endosperm purity of the endosperm, yield of the endosperm products, and the total yield. For fermentation, the top priorities are purity of the germ (ease of oil extraction), yield of the germ, and purity of the bran. A proposed fractionation-for-fermentation process design would include cleaning and removal of unfermentable materials, moisture conditioning, mechanical dislodging of bran and germ, and purification of fractions, which would be shorter than for normal food milling. The best systems will allow for operator bias of any two fractions, and the third fraction will be a function of the other two.■

Biorenewable Industrial Chemicals

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The challenge of biorenewable chemicals is that we are changing the feedstocks for industrial chemicals. Industry has been converting hydrocarbons to chemicals, but a shift is in progress to a system wherein CO_2 is consumed by plants that are harvested and processed by fermentation and catalysis. To make this shift feasible, the ability to predict metabolic biology is required. Given the premise that biology obeys the laws of physics and chemistry, then biology should be as predictable as physics and chemistry. Predictive biology will develop from empirical knowledge of gene function, genome-expression profiling, *etc.*, and deductive knowledge, computational / mathematical analysis that is yet to come.

Metabolic biology is intellectually similar to organic synthesis, and predictive biology is the most immediate implication of metabolic biology. Much genomic work remains to be done. Although many genomes have been sequenced, 25% have unknown functions. We need to fill this gap in order to be predictive. Further collaborative integration will be crucial to reaching predictive-biology goals.

In the 2004 report from the DOE biomass program, succinate was listed as one of the top-twelve value-added chemicals that could be produced in biorefineries. The two major challenges for succinate production are increasing productivity and molar yields, and increasing product purity. NADH is the limiting factor for a high-yield succinic acid production system. Approaches to alleviate the NADH limitations include the addition of new pathway(s) to increase the NADH produced from glucose. Steps to creating this pathway include knocking out the competing lactate and ethanol pathways, deletion of *iclR* to relieve repression of the glyoxylate pathway, deletion of *ack-pta* to conserve acetyl-CoA and channel it through the glyoxylate pathway, heterologous over-expression of pyruvate carboxylase to redirect pyruvate to oxaloacetic acid (OAA) and heterologous over-expression of *B. subtilis* citrate synthase to increase citrate availability. The resulting theoretical yield is 2 mol succinate/mol NADH. The experimental succinate yield was 1.6 mol/mol. The succinate yield increased from 93 mol succinate/100 mol glucose in the host strain to 175 mol succinate/100 mol glucose in an engineered strain. A stoichiometric model was developed to determine the optimal split ratio at the OAA node. For the maximum model yield, 66% of the OAA went through the fermentative pathway to malate and 33% through the glyoxylate pathway to citrate; the experimental results showed a 31% split to the glyoxylate pathway.

The challenges of developing new chemical catalysts and biocatalysts include developing selectivity with multiple functional groups, determining the configuration effects of many chiral carbons, evaluating the conformation effects of catalyst/polyol interactions and understanding the overall effect of water in aqueous-phase reactions. The conversion of glycerol to propylene glycol and ethylene glycol involves the retro-aldol mechanism, which cleaves a carbon group from glycerol following a catalytic hydrogenation / dehydrogenation reaction. Experiments with this reaction system showed that there was no correlation of polyol length with reaction rate and the strength of adsorption of sugars onto metals at high temperatures was a function of the configuration of cyclic sugars. Conformation is another concern; at ambient temperatures polyols have a zigzag conformation in solution. In the case of polyols with alternating alcohol groups, all groups will be facing the same direction. A comparison of the reaction rate and the number of inversions shows that increased inversions resulted in a decreased reaction rate. This was not predicted by complexation results. If the retro-aldol mechanism is involved in the production of tetritols from pentitols, then an interior alcohol group must be cleaved; however, the product distribution does not fit with this proposed mechanism. The decarboxylation reaction, which removes a carbon from the end of a molecule, does explain the experimental distribution of tetritol molecules. Selectivity can be achieved by adding sulfur in a stoichiometric amount to the catalyst to suppress decarboxylation at the ends, forcing a focus on the interior carbons.

New opportunities have developed in the $5 billion global wax market making it attractive for bio-based production. The global market is 91% paraffin wax, a co-product of the petroleum refining process. Recent changes in lubricant regulations have forced refineries to switch from a solvent-based to a catalytic dewaxing process. The solvent process produced 20% wax and the new catalytic process produces no wax. Few alternatives to paraffin wax currently exist, and there will be a shortfall of 1 billion lb over the next few years. Candle producers are looking for substitutes; however, there are limitations to vegetable-based waxes. The flexibility, aesthetics (fragrance and color holding) and the melting-point range are not comparable to paraffin waxes. A solution is vegetable oil metathesis, a rearrangement of unsaturated carbon bonds, typically only used with molecules that lack important functional groups, but the use of "Grubs catalysts" opens up this chemistry to apply to vegetable oils. In September 2003, Cargill initiated collaborative research with Materia, Inc. Metathesis-modified soybean oil shows better flexibility, ductility, and aesthetics. Soy wax / paraffin blends have high fragrance capacity, but lack compressibility required for candle formation. Alternatively, metathesis-modified waxes are compressible and have enhanced fragrance capacity.

The majority of the chemical products today are produced from fossil feedstocks using "chemoconversion' (*e.g.* MTBE), however some chemical products are converted to other compounds by "bioconversion" processes (*e.g.* chiral alcohols). In addition to the biocatalysis of chemical compounds from fossil feedstocks, "biorenewables" are also processed through biocatalysis (*e.g.* L-amino acids and cosmetic esters). White biotechnology products have increased functionality and value when compared to green biotechnology products. The majority of white biotechnology today is focused on feedstock-conversion and intermediates, such as amino acids and specialty enzymes. Ethanol can be used as both a fuel and a platform chemical. Ethanol may be a building block for products such as ethyl-tert-butyl-ether (ETBE, an alternative to MTBE). There is potential to create other products using chemoconversion, *e.g.* lactic acid to polylactic acid. In 2002, Cargill built a polylactic acid plant, a future goal for which will be the chemoconversion of acrylic acid to polyacrylic acid. The chemical industry will continue to grow, and the development of pulping enzymes will produce new microbial intermediates from feedstocks and the intermediates will then produce chemical products. In the future, Degussa expects to expand white-biotech projects to include more feedstock development with enzyme innovations at the lower bulk end of the value chain and at the higher value end with intermediate differentiation and development of components for consumer products.■

Pharmaceutical Manufacturing in Biological Cell-Factories

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The typical path of biopharmaceutical development begins with gene identification, followed by plasmid insertion, selection of a transformed clone, fermentation, purification of the product and, lastly, clinical trials. This developmental process has become a victim of its own success in that *E. coli* as the cloning vehicle is essentially treated as a black box that makes the desired product. It works well for some, but not all products. The solution to this lack of understanding means dealing with fundamental systems biology—genomics, proteomics, and metabolomics—to take full advantage of biotechnology's potential.

In "traditional" collaborations, biologists identify biomolecules and develop expression vectors and bioengineers develop cultivation methods and downstream processing. This often results in miscommunication and conflict, underscoring the need for an integrated approach. For maximum protein yield, it is necessary to achieve high cell density and high gene expression. These goals can be difficult to achieve simultaneously and are dependent on the constructed host/vector system. The strategy is to identify the limiting steps and remove bottlenecks. For strain improvement, the bottlenecks may include protein formation, cellular physiology, and efficiency of gene expression. At the bioprocessing stage, bottlenecks may include cultivation conditions, meeting current good-manufacturing practice (cGMP) criteria, and chromatography methods. Another major challenge to bioprocess improvement is creating an efficient system while still meeting the dual demands of "proof of concept" and bringing a product to market as quickly as possible. Even when a strain is well developed, bioprocess improvements can still be made.

The tremendous growth in the global pharmaceutical industry means that lack of staff with appropriate engineering, business and legal skills is becoming critical. These three major skill sets are all required for success in downstream processing. Basic knowledge of biological, biochemical and engineering principles is crucial. Technical personnel need to understand the basic biology and the engineering constraints of scale up and a team approach is required to speed the process. A good understanding of regulations and of cGMPs is also required; ignorance may lead to liabilities and civil litigation. Non-technical project-management skills including writing, presentation and communication abilities, and understanding of basic business principles are also necessary. To find staff meeting these criteria, academic programs and facilities need to be developed with input and pilot-plant funding from industry.■

Driving Innovation and Growth of the Ontario Chemistry Industry

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France Rochette (DuPont Canada)

The Ontario Chemistry Value Chain Initiative (OCVCI) includes companies, associations, and individuals from R&D, manufacturing, and products and services. The goal of the organization is to create synergies across industries and bridge the gap between the chemical and biotech industries. There is a need to create new value-added products. To do this, all members of the value-chain need to participate and identify the end-users' needs as well as devise methods to use biobased feedstocks in tandem with current petrochemical processes. The process will involve building non-traditional alliances, *e.g.* with universities and government, which will foster improved alignment between research and market opportunities. The process will also require identification and reduction of constraints to commercialization of innovative technology. OCVCI aims include development of sustainable technologies, nurturing corporate responsibility, and developing shareholder value and community. The OCVCI activities include the development of human-resources plans, creation of a business model, promotion of the initiative to investors and identification of innovative technologies to commercialize.

Bioproducts appear to be a way to create faster growth in the Canadian chemical industry, although products with the requisite low cost of production have not been commercialized. One explanation is that investors are uncomfortable with the industry. However, this is a myth; investors are actually uncomfortable with proposed business models. The value capture [annualized 40% return on investment (ROI)] is the issue, not the industry. A solution is to look at investments on a portfolio basis, which will be more appealing to investors. A second investment myth is that traditional investors (*i.e.* banks) are not interested. There are two risk factors: petroleum price decreases and volatility in biomass prices. Business models have to be compelling to counter these risks—most are not, but cost contracts could be used. A key solution is building a solid portfolio of opportunities to decrease the risk of investment. OCVCI has initialized the Chemistry Innovation Fund with the targets of a 20% annualized ROI and a 3- to 5-year exit, a maximum of 35% equity share and a request for CAN$20 million first-round funding. OCVCI will facilitate communication of technology opportunities between a technology advisory panel and a business advisory board, which will then send opportunities to the Chemistry Innovation Fund to find investors. A key to the success of this strategy will be the development of alternative value models. For example, OCVCI proposes valuing opportunities based on the cost-margin between biobased and fossil fuels.

DuPont has a strong presence in the chemicals industry throughout Canada, and via Pioneer Hi-Bred it is a strong player at the input level with seeds and crop-protection chemicals. Dupont is also addressing key value drivers in agriculture, such as stand establishment, vegetative growth and grain fill and harvest. Opportunities for biofuels include a partnership with BP on biobutanol, design of an integrated corn biorefinery (ICBR) that can be adapted to wheat or other crops, and fast pyrolysis of forest wastes. Biodiesel value-chain solutions include crop innovation with oilseeds, process innovation and maximizing distribution and market presence. Pyrrolidone-solvent technologies have been developed with levulinic acid, one of the DOE's top-twelve potential chemical intermediates for biorefineries. Another new product is corn-produced 1,3-propanediol (bio-PDO™) a component in the Sorona polymer used in apparel, carpet fibers, *etc*. bio-PDO™ may be polymerized to create a polytrimethylene ether glycol (bio-PO3G). These three carbon polyols are unique to the market, because the other existing polyols have either two or four carbons. PO3G has compact and random chains, a low viscosity and melting point and is easy to process. Applicable markets include injection molding, automotive finishes, polyurethanes, copolymers and cast elastomers.■

Value Creation through White Biotechnology

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Jens Riese (McKinsey & Company)
Volkert Claassen (DSM)
Martin Austin (TransformRx)
Iain Gillespie (OECD)

McKinsey & Company recently conducted a quantitative survey of twenty-seven companies and found that more than 60% did not have good returns on R&D investments and only a fifth of the remaining 40% actually achieve worthy returns. Luck is not involved; those that had high returns on R&D also had strong innovation practices. A system approach begins with opening the solution space, by creating a holistic map of broadly defined future applications, then opportunities are scanned for fit, timing and risk and ten possible opportunities are short-listed. Business plans are then built for the short-listed opportunities, and two or three are selected for investment. Rigor is also key. In one particular case, McKinsey helped a company scan for cost-reduction innovations to deal with low-cost competitors. The company used this information and developed competing technologies to maintain their market position. Focus is important as well; no single company can do everything. Leverage is another factor. Companies will need to connect with externals to fill in biotech capabilities. There are several examples of this strategy. The "spider in web" model is exemplified by Cargill and its small group of partners. DuPont uses coordinated outsourcing, wherein portions of a project are divested to partners while DuPont maintains leadership. Shell has gone with a minority stakes strategy by making next-generation technology investments. Degussa sets up corporate project houses to focus on a particular project for ~3 years. DSM tends to use acquisition, buying out companies and external scouting, but also has formed an alliance with Novozymes. The last tool for successful innovation in white biotechnology is to be business-minded, defining the go-to-market strategy.

DSM has identified four emerging business areas (EBAs) that will likely grow: special packaging, personal nutrition, pharmaceuticals, and white biotechnology. Currently the focus is on "biospecialties": food ingredients, pharmaceuticals, and fine chemicals. Biomaterials and base chemicals will be part of the picture in the future. The white biotech business model requires a two-track approach: cost leadership and product leadership. Cost leadership will be gained with low-cost-production processes as well as low-cost feedstocks. An example of such a production process is the simulated moving bed reactor within the ZOR-f fermentative process, which is used to produce 7-ADCA (a non-penicillin antibiotic intermediate) from sugar. A more cost-effective method is being developed to produce riboflavin, using fermentation with *Bacillus subtilis.* In the nutritional product category, PeptoPro® is a supplement that stimulates insulin to transport glucose to muscle tissue, decreasing recovery time for athletes. DSM is also testing its protease collection with various peptide sources, such as milk, whey, and vegetable protein, to develop a group of products.

From the investor's view, the economics of the white-biotech space are daunting compared to those of red biotech. The variety of possible products is huge, including energy, refining, food, textiles, and remediation. The scale is also large, ranging from investment in applications and products, to processes. The scope is broad as well: yield, temperature and environment all have economic value. White-biotech companies have different business models, so comparables are hard to find. This makes valuations and fund allocations difficult; fund managers simply do not know what to do with white biotech. Points to consider are whether the investment opportunity lies in a single product or a portfolio, a platform or an application, and whether the customers are end-users or whether it is a business-to-business situation. For project funding, investors need to redefine the benchmark. Instead of equity alone, it is possible to use cash flows on particular projects. For success, it is important to set defined milestones and maintain highly focused investment. In white biotech, the investment model must also provide space for innovation, which requires risk capital and a feasible exit strategy. The investment models will be different for each opportunity. The products that will draw investment include replacement products and extension products.

Significant market potential exists in biotech manufacturing, which provides ~20% of the GDPs of the member countries of the Organization for Economic Cooperation and Development (OECD). More-exact estimates of biotech's potential are required, and to facilitate this, OECD conducted surveys in twenty-three countries. The primary objectives that those questioned felt industrial biotechnology could address are job creation, environmental impact, and energy security. Key policy goals are to deliver growth, jobs and environmental gains, secure supplies of energy and raw materials, agricultural market diversification and to sustain rural communities. When member countries were polled, overall they had high opinions regarding their R&D capabilities, but low opinions of their industrial capabilities and the supportive policies currently in place. There are many recognized barriers to biotechnology adoption, including finance and human-resource limitations, IP rights problems, low feedstock availability, and the difficulty of integrating new technologies. Perhaps new measures can be taken to address these issues, such as establishing IP platforms, increasing capital availability, creating more strategic alliances and merger business plans. Biotech venture-capital investment in Europe lags behind North American investments, however it is encouraging to see that the number of alliances has been increasing, which may boost investment. The linear model value chain needs to be replaced by a value cycle that is derived from life-cycle analysis. OECD proposes that the next international actions to take include measuring growth and environmental impacts, creating secure transfers to allow globalization of R&D and developing models to benefit rural communities.■

Industrial Production of Chiral Entities

Joe Jump (Novozymes)
Ramesh Patel (Bristol-Myers Squibb)
Alexander Pokora (BioCatalytics)

Enzyme-driven biocatalyic reactions are generally cleaner and occur at lower temperatures than for chemical catalysis, which means that there are fewer side reactions and stronger substrate specificity. When using enzyme technology, several factors need to be taken into consideration including selection of the desired transformation, availability of the enzyme, the required reaction temperature, and the optimal pH. The economics of scale-up are also a major consideration. Enzyme immobilization and recycling, throughput, substrate concentration, operation mode (batch vs. continuous) and the potential for enzyme inactivation by metal ions are all factors to consider for scale-up. Novozymes uses multiple methods for production of chiral (non-racemic) compounds. These methods include resolution via diastereomeric salt formation, chiral chromatography, asymmetric synthesis, "chiral pool" when synthesis is started with natural compounds that have chirality, and dynamic kinetic resolution of enantiomers with chiral catalysts, *i.e.* enzymes and whole cells. This method is called dynamic, because 50% of the products are recycled to form the other products. The unifying theme of these processes is that all of them source chirality from nature and all of the sources are derived from biochemical reactions. Customers that require chiral compounds include the pharmaceutical industry where optically pure forms are necessary for synthesis of activer pharmaceutical ingredients (APIs). Agricultural companies use these compounds for pesticide synthesis, and the flavors, fragrances and polymers industries also require them.

Bristol-Myers Squibb has several drugs produced via enzymatic reactions. An antidiabetic drug is a DPP4 inhibitor, which allows control of insulin production in the cell. It has an amino acid component that is produced from a keto acid that is modified by phenylalanine dehydrogenase (PDH) and formate dehydrogenase (FDH) with NH_3 and NADH as cofactors. *Escherichia coli* fermentation is used to produce PDH and FDH. Their antiviral drug is a novel HIV-1 protease inhibitor, Atazanavir, that has three chiral centers. Ketone 1 is converted by microbial reduction to (1S, 2R)-alcohol 2, using the microorganism *Rhodococcus erythropolis* SC 13845. The yield of the alcohol from this organism is 98% and the product has 98.9% diastereomeric purity. Reductive amination of ketoacid 1 is performed by leucine dehydrogenase and formate dehydrogenase; both enzymes are cloned in *E. coli* and the keto acid is converted to (S)-tert-leucine. Bristol-Myers Squibb also produces an anticancer drug that is an orally active taxane. A similar product, Paclitaxel, was produced using bark from mature yew trees and then sapling farms. However, the drug was not water-soluble, so the side chain was altered. Specifically if the C4 acetate is converted to a C4 carbamate, oral absorption is possible. Lipases are used for the hydrolysis reaction. *Pseudomonas cepacia* lipase PS-30 and *Pseudomonas* sp. SC 13856 BMS lipase are immobilized on polypropylene and can be used for ten cycles of this reaction resulting in a 49% yield with diastereomeric purity of 99.8% and an overall yield of 95%. An anti-Alzheimer's drug is created by cloning glucose-6-phosphate-dehydrogenase and oxidoreductase from baker's yeast engineered into *E. coli*. When supplied with glucose, the cells convert it to glucose-6-phosphate using NADPH as a cofactor.

BioCatalytics Inc. produces enzymes for drug intermediates and metabolites. Based on the premise that enzymes are essential to the synthetic chemist's toolbox, the company's goal is to make biocatalysis simpler. Wide ranges of chemical reaction types are possible and services are also offered in screening, development and optimization, and production and directed evolution of enzymes. The specialty enzymes include ketoreductases, ene reductases (EREDs), the first enzymes to reduce alkenes in the presence of carbonyls, and amino acid dehydrogenases, including the only existing D-amino acid dehydrogenase. The main strength of the company is in enzyme prospecting. Gene libraries are screened for motifs and then the public domain sequences are analyzed. With this method, the manufactured enzymes are 95% active, and there is a 60% success rate in identifying the R or S form from a particular motif. Creation of tailor-made enzymes is possible, to fit customers' particular needs.■

Bioplastics: Understanding Manufacturing Applications

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Rahumathulla Marikkar (InterfaceFlor)

Earth Pledge's focus is on development of sustainable solutions to environmental challenges, including energy use in buildings and anaerobic digesters. To create solutions value chain partners are sought out, specifically large companies that can provide the necessary feedstocks. Two drivers will force the development of circular product cycles as opposed to the current linear type. If a carbon tax is established on fuel, it will essentially eliminate fossil feedstocks and as land scarcity grows, tipping fees for US landfills will rise. The goal will be to go full cycle from agricultural feedstocks, although at present issues with pretreatment, engineering and scale are limiting factors. Currently, applications of plastics are the "same old" products in the "same old" fashion; Earth Pledge wishes to make plastics more appealing. Potential applications range from tractors to soy-based products, telephones, walkmen, computers and clothing. A major project is appliance development with a European company. One of the major difficulties in manufacturing is whether the product can be completely recycled. Traditional additives make this difficult, so a search is underway for alternatives. However, a more pressing issue than manufacturing is creating a product of comparable quality to that which it is replacing. The appliance project offers the opportunity to market directly to consumers, who are more concerned with better product quality and longevity rather than with how "green" it is.

Being such ubiquitous materials, the plastics industry remains strong. The production of lipids is limited to the commodities market, which means there is a race to the bottom for prices. However, value can be added if lipids are put into plastics. This will allow the marriage of the petrochemical industry to agricultural feedstocks, as long as underpinning functionality, cost, manufacturing, legislation, and education are in place. A key issue for bringing new technologies to market will be whether they can function with existing machinery. Addition of hydroxyl groups to vegetable oils requires simple, cost-competitive chemistry, but the resulting polyols have dangling chains and do not tolerate stress well, which results in limited rigidity and steric hindrance. Vegetable oil ozonolysis-reduction is an alternative. The process requires double-bond oils, making canola a good candidate. Four possible polyol structures form in this process. Saturated triacylglycerol (TAG) has only one hydroxyl group and is not useful. The diols and triols are useful, but quantification and control are needed. Currently, this process results in 85% of the theoretical yield of triols. The product distribution, whether a diol or a triol is produced, is adjustable; however, ozonolysis-reduction is not cheap. It is also important to recognize that the metrics have changed for this new product from those used for the traditional product. The hydroxyl groups increase the rigid form, therefore temperature and viscosity issues must be dealt with for pumping. The polyurethane third generation elastomer has good stress-tolerance, tensile properties and better compressibility.

Sustainability is a smart business tool increasing profits and short-term competitiveness while minimizing risks. The prototypical company of the twentieth century had a one-directional flow, to create value from process, capital and people, creating waste and depleting resources. The prototypical company of the twenty-first century (the Interface model) requires cutting off the lithosphere and switching to the biosphere, using both the natural cycle and technical cycle (reusing products rather than discarding them). For InterfaceFlor of Canada, these actions have resulted in over $13 million dollars in savings, increased exports, a zero-effluent facility, elimination of eight smoke stacks and toxic raw materials, reduced energy and material intensities, and reduced greenhouse-gas emissions. It has recycled an average of 1.8 million pounds of carpet a year and has developed the Cool Blue™ process, which can take any thermoplastic and convert it for use in flooring. Elorin is Interface's partner in natural fibers, and together they have developed a natural carpet product. Currently, the market is full of claims and there are many different environmental product labels, and to sift through the confusion, Interface is working to promote green procurement that would have many benefits including evaluation of products based on quality and performance, cleaner water and air, increased profits, improved health, and employment and validation of public policy.■

Advances in Biopolymers

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Olly Peoples (Metabolix)

Items such as animal fats, vegetable oils, soy flakes, and soy solubles / molasses can be converted to biopolymers and biosurfactants, specifically sophorolipids. Fats and oils in particular make interesting fermentation substrates because they provide structural and compositional variety in terms of chain length, saturation, and side-chains. In many cases these compounds are already required as substrates and increase yield and fermentation performance (*e.g.* as anti-foaming agents). Much work has been done with "bio-glycerol." A product of biodiesel manufacturing that is less refined than crude glycerol, it contains free fatty acids and fatty acid methyl esters, and is actually only 40% a true glycerol. Another interesting feedstock is soy molasses, a by-product of soybean processing to food products. The targeted bioproducts are polyhydroxyalkanoates (PHAs), sophorolipids (SLs) and rhamnolipids (RLs). One barrier to industrial production of rhamnolipids is that the production organism, *Pseudomonas aeruginosa*, is an opportunistic pathogen, but to minimize this hazard, a new production process requires no shaking or heating. In the development of PHA fermentation, it was important to find an organism that could synthesize medium-chain-length PHA from intact fats and oils. A wild-type strain of *P. resinovorans* and several genetically engineered strains were tested. It was found that the type of feedstock influences the composition of the polymers produced. Two of the strains could convert bio-glycerol, one produced short-chain-length PHA and one produced medium-chain-length PHA. *Candida bombicola* consumes lipid and carbohydrate substrates to produce sophorolipids. In post-production, the attachment of charged groups, specifically hydroxyl groups to oleic acid, can increase solubility of sophorolipids with gram-quantity production of hydroxylated fatty acids.

1,3-propanediol (PDO) is used in the synthesis of polytrimethylene terephthalate (PTT) and other polyester fibers. PTT is a biodegradable polyester, and has potential for carpet and textile manufacturing. The chemical synthesis routes require ethylene oxide and acrolein, which are nonrenewable. The biotechnological route uses microorganisms to convert glycerol to 1,3-propanediol. Bacteria from Chinese soil samples were screened and one strain showed glycerol production both under aerobic and anaerobic conditions. The system has been scaled up to a 50-L bioreactor; the goal is integrated production, starting from conversion of glucose to glycerol by yeast, followed by conversion of glycerol to PDO by bacteria and formation of PTT from PDO.

Polysaccharides are complex carbohydrate polymers and their properties are directly linked to their molecular structure. The "anomeric" function refers to the vast structural diversity in polymer families; the question is whether this diversity is useful for material science applications. Peptides can be robotically synthesized, but there is no comparable system for oligosaccharides. Potentially, starch has new uses (over 900 commercial products are directly derived from it); it is characterized by low cost, high purity and high molecular weight. It could be used to produce starch peracetates, which are soluble in organic solvents, but have poor elasticity properties. Various strategies are available to enhance mechanical properties, all of which relate to the plastification curve. Photochemical cross-linking of starch improved mechanical properties. In binary systems of native starch and benzoate oxidation, the proposed cross-linking mechanism was that a benzoate intermediate is grafted to a starch molecule or the intermediate can transfer a radical to chains of starch. The optimal system would use 20% sorbitol / glycerol and 3% benzoate salts with 10 minutes of UV curing producing 98% insolubles.

The success of conventional plastics is rooted in catalysis, and biocatalysis will make natural plastics successful. There are marketing issues; for example, conventional plastics do not degrade and natural plastics are biodegradable. Metabolix has created a breakthrough technology with PHA-producing engineered bacteria that can use an existing chemical plant infrastructure. The genome engineering of the proprietary organism has been in progress for 10 years, and they have monomer- and polymer-production capabilities and large-scale operation. This first platform of fermentation has been fully developed and a new strategic alliance with ADM will result in the construction of a 50,000-ton plant in Clinton, Iowa, with a 2008 start-up date. In the future, the second platform of Metabolix—co-production in green plants—will be developed. Switchgrass will be used to produce plastic, which can be extracted and the remaining biomass will be a biorefinery feedstock.■

New Tools in Biobased Chemical Production

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Jan de Bont (TNO Quality of Life)
James Millis (Cargill)

Polyhydroxybutyrate (PHB) is the most common of the polyhydroxyalkanoate (PHA) group of polyesters formed from the fermentation of several carbon sources. Strain GW2 of the aerobic Gram-negative bacterium *Methylobacterium*, isolated from Iowa groundwater, produces PHB from methanol. If valeric acid is provided along with the methanol, a hydroxybutyrate-hydroxyvalerate cross-linked polymer is produced. Although intriguing, this has no practical value, because most feedstocks are sugar-based. An alternative organism, *Alcaligenes latus*, transforms maple sap to PHA. Maple syrup is a major product in Quebec and New Brunswick and an ideal substrate for several reasons. Rich in sucrose, sterile, with optimum mixture of minerals and vitamins it is suitable for immediate growth without additives and perhaps most importantly more maple syrup is generated than can be sold. The fermentation technology and quality analysis of the PHB are areas of active research.

Fluxome Sciences uses mathematical modeling to understand the full metabolic network of an organism. Metabolic Enhancer™ is a process that determines strain construction, beginning with model simulation. This is followed by fermentation and analysis with the necessary repetition to achieve desirable results. The company has publications both on *in vivo* ^{13}C flux analysis and *in silico* work such as linear programming and genetic algorithms. The company also performs metabolome and transcription analysis. Baker's yeast is the primary model organism, because it is widely used in industry, the fermentation technology is already established, and is readily approved for food use. To improve ethanol production in yeast, 3,800 reactions were inserted into a genome-wide model. Eleven possible strategies were identified and one selected. A 5% increase in ethanol resulted, with a 31% decrease in glycerol and a 4% increase in biomass; the technology is being implemented with industrial yeast.

Aromatics have applications in polymeric compounds, but a major drawback is that they are toxic to cells. A highly tolerant strain of *Pseudomonas putida*, S12, that can grow in toluene and octanol uses a nonspecific efflux-pump mechanism to protect the organism from toxics. Using 2-D fluorescence difference in gel electrophoresis (DIGE) it was found that there is up-regulation of TCA cycle enzymes, which is logical in that the efflux system consumes electromotive force. Tyrosine phenol lyase (tpl) converts tyrosine to phenol, using transcriptomics the up- and down- regulations of this enzyme were identified and genes selected for knock out. Phenol toxicity is a serious issue. To address this problem, extractive recovery of phenol is achieved by using fed-batch fermentation with a second phase (20%) of octanol as the top layer in the vessel. Solvent-impregnated resins (SIRs) are also used. An SIR contains adsorbent and solvent molecules and pulls aromatics into the particles. The market price of phenol means that production of phenol via *P. putida* is not commercially feasible, but the organism can be used as a model to develop other products, such as p-coumarate and p-hydroxybenzoate. The pathways for these products already exist in the organism, and if the phenylalanine pathway is blocked so that the tyrosine path is favored, the yields might become economically viable.

Cargill is searching for opportunities to build on its industrial bioproducts business. The requirements for a potential investment to be desirable are that it is renewables-based, has strong IP, demonstrates a price or performance advantage, can generate more than $100 million in revenue, and will produce revenue in 3 years with positive cash flow in 5 years. It is also desirable to provide platform chemicals, a biorefinery fit and marketing and development partners. Industrial bioproducts that are currently in development are 3-hydroxypropionic (3-HP) acid, urethane polyols and renewable oil solutions (using metathesis chemistry). 3-HP is a novel platform chemical and as the propylene (C3) supply tightens, acrylic acid applications offer the best opportunities. Fermentation to 3-HP should mirror lactic acid fermentation and there are five different pathway options. The novel pathway through β-alanine was selected to convert pyruvate to 3-HP. In this pathway alanine 2,3-aminomutase is the most crucial component, and a partnership with Codexis resulted in significant improvements to the enzyme activity. Catalytic dehydration of 3-HP to acrylic acid was developed with Pacific Northwest, and >95% selectivity was achieved with no loss in activity. The soybean oil production of polyols is not an area of new activity. Currently the process is used to produce flexible and molded foam applications. Metathesis chemistry work is being done in partnership with Materia. Metathesis is the rearrangement of unsaturated carbon bonds, and with the invention of Grubbs catalysts this technology can be applied to vegetable oils. These products could all be integrated into a future oilseed biorefinery with several product platforms.■

New Products and Materials from Biobased Feedstocks

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Božena Košíková (Slovak Academy of Sciences)

Flax straw that is burned on the field could be enzymatically treated and fermented to produce various products. Potentially, decorticated straw could provide two feedstock streams: shives for extraction of biochemicals and fibers (which are bundled around the core shives. The epidermis surrounding the fiber must be broken down before either stream is accessible. A new enzyme-retting cocktail, Linase™, has been developed to degrade the epidermis; it includes polygalacturonases, which degrade pectins. This is an improvement over dew retting, which is a microbiological process requiring engineering for control. Shive processing involves pressurized extraction with low-polarity water to isolate phenolic compounds. A bioreactor with organic and aqueous phases has been developed to produce vinyl guaiacol from ferulic acid. Currently, a pilot plant is in operation to demonstrate this process. Biofiber polypropylene composites is another potential product stream for development. A coupling agent has been used to ensure compatibility between the fibers and the polymers, and additives have been found to address humidity and flammability issues. The mechanical properties of these composites in compression molding show increased tensile strength compared to polypropylene. However, the fiber retting and isolation procedures still require optimization. A sustainability-assessment tool has been developed to perform life-cycle analysis (LCA) on potential projects. For example in a flax-fiber LCA, field-burning was the default case and the results showed that there would be above zero energy use and emissions from flax harvesting and below zero energy use and emissions from use in automobile manufacturing. The use of plant fibers in the door panels of Mercedes Benz cars resulted in a remarkable 20% weight reduction, attractive to automotive and aircraft manufacturers.

Novel biodegradable lignin-based surfactants have been developed at the Slovak Academy of Sciences. Lignin sulfonate has long been used as a surface-active agent, whereas other industrial lignins require modification to improve water solubility. Glycosidation of lignin produces D-glucose and requires sodium hypochlorite. Surface-tension properties were measured and it was found that surface activity increased after this form of processing. Characteristics obtained by alkylation with lauryl bromide were identified using IR spectroscopy; it was found that the CH groups increased and the OH groups decreased and the surface-tension measurements showed results typical of good surfactants. Dispersing efficiency was tested by comparison to a commercial preparation. Calcium oleate coagulation was three times higher than with the commercial product. Oxidation of lignin with hydrogen peroxide in an acid medium was employed to create a new dispersing agent of azo-dyes for polyester-fiber dyeing. The surface-tension measurements of the dispersant and Kortanol (a commercial product) showed no difference. The effect on color deviations during high-temperature polyester fiber dyeing was evaluated for the dispersant and Kortanol. After staining at 130°C, only some of the dispersant formulas showed results that were comparable to Kortanol. However, the tension-measurement results showed that these compounds deserve consideration as surfactants and could have applications in the textile industry.■

Delivering the Biobased Economy: An Assessment of Progress

John Jaworski (Industry Canada)
Barry Fordham (CSIRO)
Ian Gillespie (OECD)
George Mallay (Sarnia-Lambton Economic Partnership)

In a biobased economy, carbon is recycled and value is increased per unit of production. In contrast, a fossil-fuel-based economy discards carbon after its use and increases value by increasing the use of limited resources. There are three dimensions that contribute to value-creation and -capture in a renewable carbon economy:

- feedstocks, in which the market and renewability will define the raw material needs,
- processing, where companies will increase both process efficiency and the value of process intermediates, and
- industrial organization that fosters consolidation and commercialization of processes.

The major challenge for a renewable carbon economy will be to decouple an increase in economic and energy use from an increase in environmental degradation.

Systems approaches have been developed to model the emerging biobased economy and to predict its impact on environmental, social, and economic sectors. A systems approach termed stocks and flows frameworks (SFFs) may be described as a mass / energy balance view of the economy. The frameworks integrate energy and material flows with capital and resource stocks to model everything in a given economy with a physical presence. This form of modeling is advantageous because it goes beyond the limitations of strict economic theory and allows better predictions of how various aspects of a biobased economy will affect the social and environmental sectors.

Industrial biotechnology has potential for a huge impact on the global economy in the near future. As a result, many countries are developing "roadmaps" and policies to dictate directions and goals for their growing biobased economies. Most countries have set policy objectives that include sustainability, economic growth, reduction of greenhouse-gas emissions, and support for rural communities. In the majority of cases, industry and government objectives coincide but the major challenge lies in developing systematic means of measuring the progress towards these objectives.

The community of Sarnia in Lambton County is a significant contributor to Ontario's chemical and refining industries. The three major industrial groups in the area, agricultural, automotive, and petrochemical / refining are working jointly with the community and the local government to move toward a biobased economy. Sarnia's strategy is to attract biotechnology plants to work with existing companies, to build education, skills, and training in the area of bioprocessing, to increase their R&D in biotechnology, and to commercialize their new technologies.■

Bioprocessing of Oleochemicals

Jaroslav Kralovec (Ocean Nutrition Canada)
Chris Dayton (Bunge)
Oliver Thum (Degussa)

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), omega-3-fatty acids found primarily in fish oil, are in high demand for their health benefits. They have been found to reduce triglycerides and to help in the control / prevention of diabetes, neurodegenerative diseases, and allergies. Enzymes (lipases) in conjunction with physical-separation techniques are being used in place of traditional chemical processes to separate and concentrate omega-3-fatty acid components (DHA / EPA) from fish oil. In addition to being cost-effective, the enzymatic process yields higher-quality products (increased levels of stable trans isomers, fewer side products) than chemical production methods.

The United States produces 9.5 million tonnes/year of vegetable oil. Traditional processing techniques for degumming oil (removal of lecithin) require the use of large quantities of phosphoric acid and caustics, providing relatively poor yields. The use of a phospholipase A1 to convert lecithin into free fatty acids (oil-soluble) and lyso-lecithin (water-soluble) for degumming is cleaner and more efficient than chemical treatment and produces usable byproducts such as the lyso-lecithin for animal feed.

The cosmetics industry is beginning to use lipases to process fatty acid esters that serve as the raw materials for health and beauty products. The final products of enzymatic reactions are odorless and have fewer contaminants than with chemical processing. A life-cycle analysis for the enzymatic production of myristyl myristate showed 62% energy savings and 76% reduction of smog-forming gases over chemical processing.■

Applications of Industrial Biotechnology in the Cosmetic Industry

Jim Thompson (Proctor & Gamble)
Dany Aubry (Millenia Hope Biopharma)
Bruce Ianni (Chemidex)

Retinoic acid improves skin tone and reduces wrinkles, but has the drawback of perturbing skin-barrier properties and causing irritation. Using microarray analysis of retinoic acid-treated skin, the gene for aquaporin 3 *(aqp3)* was determined to be involved in maintaining skin barrier properties. AQP3 is a water-pore protein that helps to prevent skin irritation and drying. After screening a library of small molecules, it was found that topical treatment with caffeine stimulates AQP3.

Phytomics (plant-cell-culture-based genomics) combined with high through-put assays are being used to identify natural products with value as anti-cancer, anti-microbial, and anti-viral agents. Compounds are identified by growing plant cultures, optimizing their growth and metabolism using bioreactor technology, extracting the extracellular compounds, and screening the purified fractions for novel functions. A natural product has been identified that strongly inhibits HIV RNase H, an enzyme critical for the incorporation of viral DNA into the host genome.

The mission of the Chemidex Company is to make it easy for formulators to find ingredients to solve problems and to enable manufacturers to advertise the benefits of their products to formulators. Towards this end, the company has developed a database of ingredients and products from various companies along with an advanced search engine (www.chemidex.com). Chemidex is a Google™ for chemists, facilitating detailed searches based on multiple criteria, including chemical of interest and applications. The database is free and permits users to request samples of ingredients directly from manufacturers.■

Industrial Biotechnology: A European Perspective

Martin Patel (Utrecht University)
Pekka Lindroos (Ministry of Trade and Industry of Finland)
Colja Laane (DSM)
Marcel Toonen (EPOBIO)

The BREW study was conducted by the European Commission to examine impacts of industrial biotechnology on production of bulk chemicals. The study included economic and environmental assessments (product life cycles). The model incorporated data from current manufacturing processes, as well as assumptions about future upper limits in production and future technological progress in downstream processing. Three scenarios were modeled with projections to the year 2050:

- LOW (bio-based chemicals unfavorable due to low oil price and high sugar price),
- MEDIUM, and
- HIGH (bio-based chemicals favorable due to high oil price and low sugar price).

The projections under the three scenarios predicted that European non-renewable energy savings in the year 2050 will be 7–10%, 20–30%, and 39–67% for each of the models, respectively.

One of the goals of the European Union is to have a competitive economy with a strong base in industrial biotechnology. The European Union is well positioned to advance industrial biotechnology because it has strong chemical- and enzyme-manufacturing industries and large public support for white biotechnology. Policy recommendations resulting from the June 20, 2006, EU Biotech Policy Roundtable include:

- creating coherent policies that integrate national and EU priorities,
- supporting innovation in industrial biotechnology,
- promoting the use of bioproducts, and
- financing small/medium businesses engaged in bio technology.

EU chemical sales account for roughly 33% of the global total, but only a fraction is derived through biobased processes. EU policy objectives are to replace traditional chemical processes with biobased methods. To move towards a more sustainable chemical industry, the EU has set certain goals for 2025 that include increasing eco-efficiency, creating more biomass-derived energy (ethanol, H_2, and biodiesel), building more biorefineries, improving technology such that at least one step in all chemical production will involve white biotechnology, and using more renewable resources for production of raw materials (green biotechnology).

Regarding economic potential of non-food crops as sustainable resources for biobased products, three main areas of study emerge: plant cell walls, plant oils, and biopolymers. The themes were chosen based on their user / consumer benefits, their scientific challenges, and their economic benefit / risk analyses. Some of the challenges facing the use of non-food crops for bioproducts were also highlighted: existing supply chains block new entrants, concerns over quality / quantity of feedstocks (plant availability varies over seasons), economic and environmental impacts, and public perception.■

Industrial Biotechnology for the Food Industry

John Finley (AM Todd)
Frank Flora (USDA)
James Seiber (USDA)

Nutrigenomics is the study of how food influences gene expression. This field is becoming more popular due to increases in obesity and concomitant diseases. Cardiovascular disease, arthritis, premature aging, and some cancers have been linked to obesity. The common theme among many of these diseases is chronic inflammation. Obesity-linked inflammation results when an increase in macronutrients raises the levels of glucose and free fatty acids in the blood, which leads to the formation of reactive oxygen species, and an inflammatory response. Compounds derived from plant sources are being screened for their ability to address conditions with clear biomarkers (diseases relating to oral health, the gastrointestinal tract, and the cardiovascular system). The overall goal is to find synergistic combinations of small molecules that reduce inflammation.

The goals of USDA-ARS are to ensure high-quality, safe food and to develop a competitive and sustainable agriculturally based economy. ARS projects include: developing functional food ingredients from grains, using proteomics / mass spectrometry to identify and reduce protein allergens in wheat, soy, and peanuts, and developing new intervention strategies for control of pathogenic bacteria in fresh produce.

One ARS project involves the use of polyphenolic compounds (caffeic acid) to stop the synthesis of aflatoxins by the fungus *Aspergillus*. Genes, *verB* / *omtB*, were identified by microarray analysis as being involved in aflatoxin biosynthesis. The practical application of this research is to reduce aflatoxins in nuts (walnuts, pistachios, and almonds), which are major US exports. Another project involves genotyping to determine the epidemiology of disease outbreaks in crops. Genotyping can be used to determine whether bacterial strains are related and/or pathogenic. This technique can be used to define bacterial strain distribution in a given geographical area and to identify the source of an outbreak of disease.■

New Technologies for Food Ingredients and Functional Foods

Mattheos Koffas (SUNY Buffalo),
Lambert van Orsouw (DIREVO Biotech

Flavenoids have anti-cancer, anti-viral, and anti-obesity properties. They are in high demand but traditional methods for extraction from plants are inadequate due to low yields and high levels of contaminants. An alternative method for producing flavenoids involves a genetically engineered strain of *Escherichia coli*. It contains plant genes for the biosynthetic pathways of various flavenoids, and current work is focused on maximizing production and finding enzymes for generating flavenoid analogs.

DIREVO novel biological entity (NBE) technology generates enzymes with new specificities. This technology involves inserting mutations into an enzyme (lipase, glycosyl hydrolase, or protease depending on the substrate) and then assaying the mutant enzymes for novel biological functions. It has been used to generate a large pool of proteases with unique specificities such as cleaving food ingredients to generate peptides that were assayed for their ability to inhibit angiotensin-converting enzyme (ACE). ACE hydrolyzes angiotensin I to angiotensin II, which, when elevated in the blood, causes hypertension.■

Industrial Biotechnology in the Auto Industry

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Jörg Müssig (Canadian Embassy, Berlin)
Andrew Daniels (Magna Closures)
Hamdy Khalil (Woodbridge Group)

The automotive industry is beginning to move towards creating renewable / recyclable cars in which most parts will be made from bioprocessed, sustainable materials. Toyota is using bioplastic derived from sweet potatoes and sugar cane and Mazda is employing corn-based polylactic acid (PLA) in component manufacture. Extensive research is in progress on short natural fibers and thermoplastics.

Natural fibers are also seeing greater usage in the European automotive industry. Natural fibers offer several advantages including light weight, low density with high stiffness, good mechanical and acoustic properties, ease of processing, stability under impact (no splintering as with glass fibers), and lower cost. Door panels and dashboards can be made of natural fibers.

Issues related to introducing biomaterials into auto-part manufacture include

- warehousing of seasonal crops for raw materials (just-in-time delivery),
- establishing of material standards for biomaterials,
- manufacture of biobased materials at a sufficient volume to meet demand,
- competing globally in auto-part manufacturing with bio based products, and
- funding of technology and development up to the large-scale-production stage.

Many auto parts composed of polyurethane could be manufactured using polyols (from enzymatically treated soybean oil), which offer the advantages of being renewable, lower in production energy, decreasing greenhouse-gas emissions, and reducing dependence on non-renewable petroleum imports.■

Industrial Biotechnology for Foods, Flavors, and Fragrance

Richard Newcomb (HortResearch)
Dragoljub Bilanovic (Bemidji State University)
Reddy Shetty Prakasham (Indian Institute of Chemical Technology)
Andrew Morgan (Danisco)

Flavors and fragrances are a $12 billion industry in the United States with consumers in the perfume, healthcare, and food sectors. Typically, flavor and fragrance compounds are chemically synthesized or extracted from plants. Germplasm sources (fruits and flowers) are being sampled for production of novel or desired volatile compounds. Gene-expression profiles are analyzed and candidate genes with putative roles in compound synthesis identified and cloned into microorganisms to test for compound production.

Xanthan is used in the dairy, produce, cosmetics, and baking industries as an emulsifier and thickener. It is produced by *Xanthomonas campestris* grown on glucose and other simple sugars. To decrease costs, *X. campestris* is being studied for its ability to produce xanthan when grown on citrus wastes, which would have the added benefit of decreasing waste produced from the citrus industry and subsequently lowering the cost of citrus-waste treatment. Estimates suggest that this approach could nearly double profits.

Acid amylase is an enzyme critical to the baking industry where it is added to bread flour to aid in gas production and starch modification during fermentation. Efforts to optimize acid-amylase production by the fungus *Aspergillus awamori* have shown that pH and the amount of soluble starch in the medium are critical factors. After optimization, *A. awamori* yielded six-fold more acid amylase.

Food-processing enzymes include xylanases, glycolipases, and hexose oxidases. The lower volume and poorer "crumb quality" of whole-wheat bread than white bread can be improved with the addition of xylanases. Also, xylanases have been designed to resist certain proteinaceous inhibitors found in flour. Glycolipases have been used for *in situ* generation of emulsifiers that improve bread volume and crumb structure. In pizza production, galactose removal from the cheese by hexose oxidases prevents browning during baking.■

Commercialization of Nutraceuticals and Functional Foods

Rickey Yada (University of Guelph / Advanced Food and Materials Network)
Ahmed El-Sohemy (University of Toronto/Advanced Food and Materials Network)
Kim Lucas (Cevena Bioproducts)
Bruce German (University of California at David and Nestlé)

The goal of the Advanced Foods and Materials Network (AFMNet) is to develop functional, healthier, high-quality food. A multidisciplinary approach is being used, linking with industry, government, and academia to achieve its goals. One example of an AFMNet program is the R2B program (research to business) where groups of venture capitalists, scientists, entrepreneurs, patent lawyers, and university / industry liaisons work together to fund the gap between bench research and final product.

The genetic basis for variation in taste and smell detection that determine food preferences is under study. The ultimate application of this research is to block or mask flavor components in healthful foods that cause them to be unappealing (*e.g.* many phytochemicals are bitter) to a given subpopulation, thereby targeting foods to specific people. One area of research is polymorphism in a gene responsible for detecting bitter compounds. One study revealed that people with a GG polymorphism were more likely to dislike grapefruit (50%) than those with a GT or a TT polymorphism (15%).

Viscofiber® is a viscous soluble fiber (oat and barley β-glucan) with health benefits including lowering cholesterol, lowering glycemic index, and increasing satiety (assists weight loss). With the soluble-fiber market predicted to grow to $470 million by 2011, Cevena is committed to moving to the forefront of the market using a strategy involving patent protection, research to prove product efficacy, marketing, and appropriate partnering.

In an attempt to improve the health value of foods, mammalian milk is being analyzed to determine the components that make it unique for postnatal nutrition. The theory behind this research is that components in milk have been evolutionarily selected for, to provide optimal nourishment for infants. Mass spectrometric analysis of human milk components identified over 200 oligosaccharides that are mostly uncharacterized. Some of these oligosaccharides were found to be prebiotics that select for the growth of the bacterium *Bifidobacterium* sp.■

The Japanese Nutraceutical Market

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Roger Wyse (Burrill)
Murooka Yoshikatsu (Osaka University)
Yoshiyuki Fujishima (Ajinomoto USA)

The rapid expansion of the Japanese functional food market—at 12%/year—has prompted the Ministry of Health, Labor, and Welfare to classify functional foods based on health claims and proven efficacy. Foods are classified as FOSHU (food of specific health use) if they have health benefits that have been scientifically proven to be both safe and efficacious. Foods can also be classified as FNFC (food with nutrient function claim) and "other" foods.

Personalized healthcare has evolved due both to public awareness that genes have a major impact on health and to a shifting of healthcare costs onto the individual. The new health and wellness market covers genotyping, supplements, functional foods and drugs, to spas, health clubs and relaxation products. New companies in the sector include Sciona (genotyping, nutritional service), Efficas (commercialization of nutraceuticals), and Phytomedics (discovery and production of bioactives).

Lactic- and propionic-acid bacteria have been shown to enhance the immune system. *Lactobacillus plantarum* (L137) is being investigated for its anti-tumor properties, and to develop an edible vaccine from the bacterium for treating mite allergies (cause of 70% of childhood asthma cases). Examples of health foods include Kurosu (rice) vinegar, which has been implicated in the decrease of inflammation and hypertension and "Natto," which has been used for over 400 years to promote longevity and as an anti-bacterial agent.

Several products of increasing popularity on the Japanese market contain polyglutamate, which aids in calcium uptake, β-glucan, which has anti-cancer properties, and diacylglyceride edible oil, which is thought to reduce body fat. Collaboration is increasing within the nutraceutical industry and with government and academia. In addition, non-food-based industries (*e.g.* Honda) are trying to enter the market.■

Bioprocessing and Novel Applications

Agricultural Nanotechnology

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Biodiesel is readily biodegradable and made from renewable resources, chiefly soybean oil and yellow grease (animal fats and used oils from restaurants). However, current production costs are significantly higher than for diesel from petroleum. Free fatty acids in the biobased feedstocks preclude processing by conventional means, and production of biodiesel would be less expensive if the neutralization and water-washing steps could be circumvented and catalysts could be recycled. A bifunctional, solid nanoporous catalytic system has been developed to economically convert soybean oil and animal fat to biodiesel. Scaled-up operations show promise. Mesoporous nanoparticles may serve as selective catalysts for other important reactions.

Cellulose production by the bacterium *Acetobacter xylinum* provides the opportunity to study fiber assembly in the absence of lignin and hemicellulose. Direct electrical current caused alignment of fibers, observed by scanning electron microscopy; similar work is in progress examining pH effects on fiber alignment. Thus, novel material architectures may be created, leading to value-added products from the forest-products industry. Biological motors, which transport intercellular "cargo" unidirectionally on microtubules, may influence cellulose orientation in the plant cell wall. Study of this process may add to the understanding of assembly of cell-wall fibers and provide a means of producing novel cellulosic materials.

The fatty acids in soybean oil are unconjugated, making the oil less useful industrially—in paints and surface coatings—than is linseed oil. A new, improved method for conjugating the double bonds in polyunsaturated fatty acids in plant oils, including soybean, employs photolysis—a 500-W bulb with maximum intensity at 520 nm—with the oil in hexane containing elemental iodine as a catalyst, at 70°C. Separation is achieved by cooling and centrifugation. The product is superior to linseed oil in its drying properties. The iodine can be removed from the hexane using a starch-water solution and the iodine and starch can then be recovered by heating the water solution.■

Breakthroughs at the Nanotech-Biotech Interface

Larry Walker (Cornell University)
Normand Voyer (Laval University)
Wankei Wan (University of Western Ontario)

New tools are needed by microbial ecologists and industrial biotechnologists for rapid characterization of microbiological communities, both natural and human-made. Nanofluidic approaches, new molecular reactions and single-molecule quantification applied to DNA and RNA analyses may help. The development of "lab-on-a-chip" technologies is expected to provide means of fully and quickly describing bacterial populations, to better understand how they function.

The importance of polypeptides in nature has been largely overlooked by biologists and chemists. They show resistance to hydrolysis, exhibit a variety of functional groups on side chains and have many functions. The potential to create new functional protein-like nanostructures now exists: a wide choice of natural and unnatural amino acids exists. Superior synthetic, purification, and characterization methods are available; 3-D structure prediction is possible from the primary sequence. Polypeptides are ideal materials for the construction of functional nanoscale devices. Accordingly, a "family" of peptide nanostructures was developed, functioning as ion channels, demonstrating the potential to design and redesign proteins in general and enzymes in particular with applications in medicine, agriculture, the food industry, energy transduction and the environment.

Bacterial cellulose—a nanofiber fermentatively synthesized by *Acetobacter xylinum*, of high mechanical strength and high water-holding capacity—promotes wound-healing. Silver has been known since the nineteenth century to have antimicrobial activity. A method has been developed to immobilize nanometer-sized silver particles on 50-nm diameter cellulose fibers. The product has antibiotic effects on Gram-positive and Gram-negative bacteria, showing much promise as the platform for antimicrobial material to prevent infection in wounds and assist healing.■

New Filamentous Fungal Bioprocesses for the Biobased Economy

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Linda Lasure (Pacific Northwest National Laboratory)

Filamentous fungi are the biosphere's major decomposers, growing on and degrading complex biomass as a result of copious secretion of organic acids and enzymes.

A multiple logistic regression model was developed to optimize pelletization in various strains of the *Rhizopus oryzae*. The pelletized form is more productive of organic acids in batch culture than is the clumped form. Conditions were optimized for pellet-excretion of lactic acid into the medium and accumulation of chitin in fungal biomass, using potatoes as feedstock, hydrolysed with exogenous amylase and amyloglucosidase. Similarly, conditions were optimized for pellet production of fumaric acid and chitin from dairy-manure pretreated with cellulase. Fungal pelletization and process optimization offer great potential for the conversion of inexpensive cellulosic materials to high-value products.

Genomics approaches are in use to facilitate fungal-strain improvement and speed enzyme discovery. *Aspergillus niger* supports high levels of protein secretion in liquid culture, *e.g.* 30 g/L of glucoamylase. Its gene sequence is in the public domain. Genes encoding major secreted proteins and contaminating activities—α-glucosidase α-amylase, carboxypeptidase, glucoamylase, pepsinogen—were disrupted in order to secrete a purer form of recombinant lactase. Enzyme activities on natural substrates cannot be predicted from sequence analysis, nor can their activities on unnatural substrates be predicted from their activities on natural substrates. A major challenge is to characterize a large number of enzymes individually and in combination for utility in a wide range of applications.

Acceleration of fungal biotechnology is needed for biorefineries of the future, for the conversion complex biomass into useful chemicals. A program is in place at the PNNL to demonstrate the genetic control of fungal morphology (pellet / clumped) for commercial processes, to maximize the utility of fungal genome-based information and tools, to demonstrate the usefulness of proteomics to accelerate development processes, to define conditions for maximum biomass conversion and maximum production using model systems, and to demonstrate direct conversion of biomass to high-value chemicals, *e.g.* glycerol, 3-hydroxypropionic acid and glutamic acid.■

The Fungal Cell Factory

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Eric Record (INRA and Provence & Mediterranean Universities)
Marco van den Berg (DSM Anti-Infectives)
Lisbeth Olsson (Technical University of Denmark)

Development of new screening and selection approaches, *e.g.* for protease deficiency, has led to improved fungal strains for protein secretion. A "suicide" approach has been found to have utility for selection of wide-domain protease mutants. In a metabolomics-based method, metabolites correlating with the process of interest are identified, those data are combined with proteomics / transcriptomics information to identify enzyme(s) / gene(s) as targets for optimization, then the gene of interest is over-expressed or deleted, or the fermentation conditions are accordingly improved.

When genes for complementary enzymes feruloyl esterase A (FAEA) and xylanase B (XYNB) were placed in close juxtaposition on the genome of *Aspergillus niger* to produce a bifunctional chimerical protein, efficiency of release of ferulic acid from destarched corn and wheat brans was improved in comparison with that obtained with free enzymes.

For the first time, a fungal enzyme—FAEA—was fused to a functional dockerin and secreted from *Aspergillus niger* to a concentration of 100 mg/L. The dockerin-FAEA chimera may be incorporated onto a *Clostridium thermocellum* cellulosome *in vitro*; such a system may, like the FAEA-XYNB chimera, have particular utility for degradation of plant biomass.

The highly successful traditional "black box" approach—classical mutagenesis—to optimizing the industrial production of penicillin is being replaced by new genomics-based, "white box" strategies. The complete genome sequence has revealed that an amplified region essential for penicillin production is common to all fungal strains used in industry, *Penicillium* and other genera. New strategies for gene-targeting are being adopted for use with *Penicillium chrysogenum*.

A genome-scale metabolic model was used to show that *Saccharomyces cerevisiae* can be engineered to over-produce succinate—an important chemical building block—by revealing right and wrong metabolic targets for manipulation. Advantages of the use of yeast include cost savings by eliminating acidification steps. *In silico*-guided gene deletions with strain evolution led to increased succinate yield, at industrially relevant levels, without inducing auxotrophy.■

United States Defense Department Non-Medical Biotechnology Programs

Jerry Warner (Defense Life Sciences)
Joseph DeFrank (Edgwood Chemical Biological Center)
Blake Sajonia (TRSG and Edgwood Chemical Biological Center)

Several entities within the Department of Defense (DoD) have interests in biotechnology. Between 2000 and 2004, federal spending on biotech increased at more than three times the rate of increase in federal spending as a whole, mainly for medical and defense purposes. Non-medical biotechnology addresses issues in the categories of threats (*e.g.* biodefense) and opportunities (*e.g.* bioenergetics, materials science, electronics and sensing, bioprocessing and manufacturing, biomimetics and biometaphors).

The US army's Research and Development and Engineering Command (RDECOM) has established a biotechnology integrated product team (Bio-IPT) to improve integration of R&D and engineering within and external to RDECOM. A primary mission is to evaluate all conceivable threats—and opportunities—posed / offered by emerging biotechnologies, thereby eliminating the possibility of technological surprise.

Many opportunities exist for biotechnology innovation through collaboration with the DoD. Technology-transfer partnerships can benefit public and private interests and help leverage US-taxpayer investments in people, infrastructure and intellectual property for applications beyond the original DoD mission. Dual-use activities develop technologies that not only have defense applications but also non-defense utility. Spin-off activities demonstrate non-defense potential for technologies (being) developed for national security purposes (*e.g.* DEFENZ™, an enzyme preparation for decontaminating hazardous chemicals marketed by Genencor, originally developed at the US Army Edgwood Chemical Biological Center). And spin-on activities demonstrate national-security utility of technologies developed outside of DoD [*e.g.* vaporized hydrogen peroxide (VHP) technology developed by STERIS].■

Recent Achievements in Marine Biotechnology

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Mark Hamann, (University of Mississippi)
Yonathan Zohar (University of Maryland)

In addition to thermostable enzyme systems, hyperthermophilic microorganisms (most of which are archea) contain stabilizers that protect proteins and membranes from heat damage. These stabilizers have applications in research and cosmetics, and, potentially, pharmaceuticals. *Escherichia coli* genetically engineered to express a small heat-shock protein from the thermophile *Methanococcus jannaschii* grew at 45°C and survived at 50°C better than its wild type, indicating the potential to manipulate industrially important organisms for improved function at elevated temperatures. Similarly, vaccines that contain live attenuated bacteria may have improved shelf-life—even without refrigeration—after being genetically engineered to express chaperones from hyperthermophiles.

Psychrophilic microorganisms and the enzymes they produce have many potential applications:

- as additives in detergents: cold-water washing
- as additives in food: cheese, meat, baking
- for bioremediation of cesspools, septic tanks, wastewater, soils
- for biotranformation of thermosensitive substrates
- in molecular biology: cloning of psychrophilic strains

Generally, cold-active enzymes are of high specific activity at low and moderate temperatures, are highly labile to elevated temperatures, and are of lower specific activity than mesophilic counterparts at their respective environmental temperatures. In order to maintain activity at low temperatures, enzymes from pychrophilic organisms display increased molecular flexibility. The determinants of cold-adaptation represent potentially powerful tools for structure / function manipulations in biotechnology.

Manzamines, complex alkaloids that have antiparasitic activity, originally isolated from marine sponges, are now known to be produced by associated *Micromonospora* bacteria. They may have particular utility in the treatment of malaria, for which relatively few drugs are available, if the bacteria can be engineered to synthesize less-toxic, more-active derivatives. Kahalalide F is a depsipeptide—a novel anti-tumor agent—isolated from the sea slug *Elysia rufescens*.

Biotechnological tools offer opportunities to address impediments to the development of sustainable aquaculture systems, which are becoming essential in view of increasing pressures on wild fishery stocks. Bioinformatics, proteomics and functional genomics will lead to the identification of genes and mechanisms for their regulation, encoding commercially important traits. For the first time, spawning was achieved recently in captive blue-fin tuna. Fast-growing genetically engineered fish must be grown in tanks to preclude the escapes that occur from offshore pens. Biotechnology also offers means of manipulating microbial communities for bioremediation of aquaculture water to permit recycling.■

Carbon Management and the Impact on Biobased Materials Markets

Chris Ryan (NatureWorks)
Luca Zullo (Cargill)
Ramani Narayan (Michigan State University)

A clear advantage of industrial biotechnology versus conventional technologies often is a reduced environmental footprint as a consequence of reduced usage of fossil fuels and decreased emission of greenhouse gases. Increasingly, environmental benefits are resonating with consumers. Polylactic acid (PLA) provides a good example of a biodegradable polymer, produced from sugar after hydrolysis of starch from corn kernels (*i.e.* a renewable feedstock). Fermentation of the sugar produces lactic acid. The polymer is marketed as pellets that are convertible to film (for packaging) or fiber (for fabric, *etc.*) forms. The combination of the renewable-resource feedstock and purchase of renewable energy credits means that PLA is the world's first greenhouse-gas-neutral polymer. An international supermarket chain is using PLA packaging, emphasizing decreased CO_2 emissions in the manufacturing process.

The Kyoto Protocol—effective since February, 2005—is a first step towards controlling greenhouse-gas (GHG) emissions. Only two countries are non-signatories: Australia and the United States. It is underwritten by governments and is governed by global legislation enacted under the aegis of the United Nations. Governments are in two general categories: developed countries, referred to as Annex 1 (who have accepted GHG-emission-reduction obligations); and developing countries, Non-Annex 1, (who have no GHG-emission-reduction obligations). Any Annex 1 entity failing to meet its Kyoto targets is subject to a fine and further penalized by having its reduction targets increased by 30%. By 2008–2012, Annex 1 countries have to reduce their GHG emissions by ~5% below their 1990 levels. "Flexible mechanisms" allow Annex 1 economies to meet their GHG targets by purchasing GHG-emission reduction credits from elsewhere. These can be bought either from financial exchanges (such as the new EU Emissions Trading Scheme) or from projects that reduce emissions in non-Annex 1 economies. Kyoto established the Bonn-based Clean Development Mechanism Executive Board to assess and approve projects (CDM Projects) in Non-Annex 1 economies prior to awarding certified emission reductions (CERs). A similar scheme, joint implementation (JI), applies in transitional economies mainly covering the former Soviet Union and Eastern Europe.

In the United States there is inaction at the federal level *vis-à-vis* Kyoto. But there is activity in some states. Voluntary action at the business level is driven mainly by public perception and liability protection. The Chicago Climate Exchange (CCX) is the world's first, and North America's only, voluntary, legally binding pilot GHG-reduction and -trading program for emission sources and offset projects in North America and elsewhere. Companies joining the CCX commit to a mission-reduction schedule to be achieved via either actual emission reduction or intra-CCX credit trading.

Cargill Environmental Services has been set up to originate, develop and market emission-reduction and renewable-energy projects around the world, within Cargill, Inc.

Carbon-14 content provides a built-in time clock for differentiating carbon in a product from contemporary sources from carbon from petroleum—providing a measure of "biobased" content—since fossil carbon contains zero ^{14}C. Having a clearly verifiable and scientifically valid consensus standard to identify and measure biobased content is critical to a growing biobased products industry to eliminate confusion, misperceptions, and misleading claims and to allow quantifiable carbon trading. Life-cycle-impact assessment is essential for all new products; even biobased products can have adverse environmental effects as a result of the manufacturing process or how they are eventually disposed of.■

Bioinformatic Approaches to Protein Engineering

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Richard Fox (Codexis)
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Stephen Van Dien (Genomatica)

Enzymes are increasingly important tools in industrial biotechnology. However, their properties are not necessarily optimal for their intended tasks. *In vitro* evolution is a set of technologies useful to address their shortcomings. "Family" shuffling starts with mutagenized genes that are homologous in the range 65%–95%, from which a library of chimeric genes is created. Screening identifies improved variants. The ProSAR (protein sequence activity relationship) approach utilizes a search algorithm predicated on the advent of high-throughput screening and modern statistical techniques, consisting of

- screening a small number of samples from a combinatorial library, associating sample activities with their sequences,
- building a statistical model that correlates sequences with measured activities,
- using the statistical model to identify residues that contribute most to improving the desired activity or fitness,
- building the next-round library by incorporating residues that contribute most to the predicted fitness, rejecting those residues predicted to confer low fitness; residues with putative neutral contributions in the current combinatorial context may be kept in the search in order to find potentially beneficial epistatic interactions in the new context, and
- iterating to achieve higher fitness.

As the search algorithm continually removes diversity from consideration, diversity-generation methods in concert with the search algorithm have enabled rapid evolution of active ketoreductases of varied specificity. This has provided significant information on structure-function relationships, providing the ability to rationally design mutations for large increases in enzyme activity.

Using ProSAR, an enzymic process was developed to replace chemical synthesis of hydroxynitrile, the key intermediate in the production of atorvastatin (Lipitor™). The volumetric production of the enzymic process was improved by a factor of ~4,000 by a fourteen-fold reduction in reaction time, a seven-fold reduction in substrate loading, a twenty-five fold reduction in enzyme use, and a 50% improvement in isolated yield; concomitant process simplification occurred, with overall reductions in energy consumption and waste production.

Advances in synthetic biology along with novel computational tools represent a powerful combination. Several proteins have been optimized directly for commercial properties using datasets of less than a hundred unique sequences. Such properties often cannot be optimized through generation of random libraries and high-throughput screening due to assay complexity. Protein-design algorithms requiring testing of only a small number of variants represent significant progress in the development of a generic resource-optimized approach for the production of biocatalysts for polymer processing.

Research in industrial biotechnology is increasingly computer-model-driven. For example, SimPheny™ ("simulating phenotypes") is an enterprise-level software platform that enables the development of predictive computer models of organisms, from bacteria to humans. With cellular metabolism at its core, SimPheny can build virtual cells from their basic molecular components, and can simulate the activity of the cell's complete reaction network. It serves as a biological knowledge-management system with predictive capabilities. High-throughput data, such as genomic-sequence, gene-expression, and proteomic information, can be integrated, analyzed and dynamically visualized. This integration provides a natural bridge between experimental and computational research efforts within an organization. In addition, SimPheny enables models to be refined and improved as more data become available, thus driving research and discovery. Each model serves as the most current representation of collective knowledge of the metabolism of a specific organism, allowing management of that knowledge in a manner consistent with the underlying biochemistry and genetics of the system. Model-driven strain design can lead to the development of superior biocatalysts for many applications.■

New Tools in Bioprocessing

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A high-throughput robotic screening (HTRS) system is under development using the filamentous fungus *Chrysosporium lucknowense*, initially to identify strains for overproduction of cellulase. This is the only technology utilizing a fungal host for functional expression, thus circumventing limitations encountered with bacteria and yeast. Although the fungal strain isolated from the wild was filamentous, evolution in the laboratory led to a distinctly nonfilamentous morphology characterized by lack of matting and low viscosity, necessary for HTRS in microtiter plates. This system will have utility for the discovery / improvement of genes and proteins other than for hydrolysis of cellulose.

A bacterial system has been developed in which cell-generation is uncoupled from product formation (*e.g.* lactic / glutamic / succinic acid, ethanol). Substrate consumption for vegetative functions is greatly reduced, leading to higher rates of conversion to products. The key aspects are high cell-density and decreased oxygen level. *Corynebacterium glutamicum*, genetically engineered to utilize xylose and arabinose as well as glucose simultaneously, shows promise for utilization of lignocellulosics in this semi-continuous process, particularly since it is relatively insensitive to growth inhibitors (aldehydes, phenols, organic acids) that are typically present after saccharification of biomass.

Feedstocks used in many industrial fermentations are of agricultural origin, harboring many microorganisms of varied type. A technology utilizing bacteriophage provides rapid and significant control of contaminating bacteria while optimizing the process to produce a preferential environment for the production organism.■

Breakthroughs in Enzymic Bioprocessing

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Even with recent reductions in cost of cellulases, additional efforts are needed to improve the economics of hydrolysis in the conversion of lignocellulosic biomass to ethanol and chemicals. Reversibly soluble polymers can change solubility with small changes in their environment (*e.g.* pH, temperature), and when enzymes are bound to such polymers, novel biocatalysts are produced. In the soluble form, the biocatalyst will be active, whereas in the insoluble form it will be precipitated and can be recovered for re-use. Cellulase conjugated to microcrystalline cellulose was effectively recycled; glucose yield from wheat straw was unaffected by seven cycles of precipitation and solubilization. This process, applicable to other biocatalytic systems, could become a core technology in biorefineries.

In the conversion of lignocellulosic material to fuel and chemicals, the effectiveness of cellulase is adversely affected by non-specific adsorption of non-cellulose components to the substrate, such as lignin. Non-catalytic proteins [*e.g.* bovin serum albumin (BSA)] and some surfactants have been shown to enhance enzymic hydrolysis of cellulose. BSA was found to only slightly affect the adsorption of cellulase to pure cellulose, but to be strongly adsorbed to lignin and to block non-specific adsorption of the enzyme to lignin. BSA had little effect on the hydrolysis of cellulose unless lignin was present. BSA treatment of lignin-containing biomass enhanced hydrolysis by 5–20%, depending on pretreatment. Other proteins and amino acids also enhanced cellulase performance.■

Emerging Issues in Industrial Biotechnology

Using Marine Biotech to Develop Industrial Applications

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Kevin Kelly (Center for Marine Microbial Ecology and Diversity)
Russell LaMotte (Beveridge and Diamond)

Since 1972, marine organisms have been systematically investigated for pharmacological activity. Sponge-produced compounds are being used as templates for commercially important antiviral agents. The process of systematic exploration of marine organisms is cumbersome and spans over 15 years or more from the structure to the drug. Marine natural products can be used as molecular tools to elucidate cellular communication processes such as signal transduction. The mechanisms and genetic machinery in flatworms, sea stars, *etc.* are rather complex but surprisingly similar even though these species are taxonomically unrelated. This is because marine invertebrates contain symbiotic bacteria that are involved in the biosynthesis of marine compounds. It can be difficult to find the right culture conditions for fermentation of symbiotic bacteria. However, the gene cluster of significance can be identified and expressed heterologously in suitable hosts.

A marriage of agriculture, medicine and environmental studies is occurring. The University of Hawaii has developed expertise in oceanography and earth science, microbiology and genetics and tropical agriculture. The Hawaiian Ocean-Time series was founded in 1988 as a technology to establish and maintain deep water hydrostations. Hawaii has many sea-going assets like the SWATH mapping system and the research vessel Ka Imikai-O-Kanaloa, which gives scientists the opportunity to explore new areas. At the Center for Marine Microbial Ecology and Diversity (CMMED) there has been extensive collaboration between separate disciplines—medicine, chemistry, oceanography and geology—due to their common interest in toxic algal blooms. International laws and regulatory frameworks are continuously changing and the industry needs to keep pace.

There is need to develop a new legal regime to facilitate or restrict the development of emerging trends and themes. Issues have recently risen to international levels regarding the need to institutionalize the arrangement for access to genetic resources and uses of such resources as well as sharing of benefits. Developing countries are worried that patent rights to their resources have been lost leading to an uncertainty in the political framework and unequal benefit sharing. The international efforts on Access and Benefit Sharing (ABS) that have been made are in the form of negotiations and new initiatives at the United Nations on marine genetic resources in areas beyond national boundaries. The Law of the Sea Convention is more focused on mineral resources, begging the question as to whether genetic resources are similar to deep-sea minerals or to living resources free to be collected by anyone and whether they should be considered the common heritage of mankind. Contrary to the view of developing countries the United States feels that they are the common resources of mankind and should come under of "flag state" jurisdiction, *i.e.* the state that conducts the experiment should have jurisdiction over the area.

The focus of the Convention on Biological Diversity(CBD) is on areas within national jurisdiction. The United States is not a party to the CBD due to reluctance to restrict R&D. Destructive fishing practices are not analogous to scientific research which requires only small samples. Other countries like Japan, France, Germany, Canada, United Kingdom want to keep the high seas accessible to research. Some of these ecosystems are transient, *e.g.* tectonic movements can change the hydrothermal vents, and must be studied as and when they appear.

The CBD urges countries to respect traditional knowledge and mandates mutually agreed terms regarding access to genetic resources and benefit sharing. Developing countries like India and Brazil wish to have the patent system as a policing mechanism for accessing genetic materials. They require identification of the origin and source of any genetic material that is in use, with prior consent from the source before doing anything. Such a requirement as a condition for allowance of patents will defeat the incentives for investment and development. Efforts are being made by the United States to include developing countries in discussions "prior to" so that the countries involved become part of the work and also assume some of the risk involved in the research.■

Intellectual Property for the Bioeconomy

Mark Sajewycz (Gowling Lafleur Henderson)
Christopher Tsang (Jones Day)
Paula deGrandis (Cargill)

Patent rights include the right to exclude, make use of, sell, offer to sell or import the patented invention, but not the right to practice it. A company must learn to manage its risks defensively. It must ensure that competitors cannot file a patent in the same field. The patent portfolio in a certain area must have a timed press release so as to affect stock prices. Many companies are passive after filing patents. They should monitor the patent's progress in the legal environment, in the patent office and know what competitors are doing. There should be internal evaluations of inventions—any breakthroughs, the platform, and the probability of obtaining patent rights.

It is important to know how intellectual property (IP) law affects technology development as it can be used to enhance the company's competitive position. IP law is significant in the form of a patent law regime or a trade secret regime. In the patent regime, although patent protection is available it is easy to be disqualified for it. Patent laws differ in different countries. Not everything is patentable, *e.g.* higher forms of life cannot be patented in some countries. Patents are important as the court can grant injunctions to block competitors from using the invention. This right will be extinguished if it is publicly disclosed. A trade secret can exist forever. It is information that yields advantage over the competitors because the information is never publicly distributed. There is a need to ensure secrecy by maintaining confidential documents with restricted access. Trade-secret protection is extinguished on public disclosure. A competitor's patent can block the practice of a company's trade secret. IP law presents an opportunity to constantly glance in the rear view mirror while competitors are probably doing the same.

IP transactions usually consist of three stages:

- "before," when the strategy is developed and the deal structure is chosen,
- "during," when the portfolio is developed, and
- "after," when the continuing obligations, royalties and the confidentiality are maintained.

The due diligence includes internal and external reviews to find gaps in terms of inventions that have not been filed and a litigation check to see if the partner has any outstanding litigation. The patent landscape must be reviewed from a geographic perspective. A strategy must be developed to ensure a supply chain that will get the product to the market. IP ownership by inventorship could have an impact on the collaboration of the parties involved and on filing strategies. Internal collaboration within the team and collaboration between partners requires good contract management. Continuing contract-management obligations must be institutionalized. Patents are granted based on "first to invent" in the United States, whereas in the rest of the world it is based on "first to file."■

Commercialization: From Research to the Market Place

John Culley (Agriculture and Agri-Food Canada)
James Iademarco (Bayer CropScience)

To build a strategy there is need for the proper tools for prospecting, planning, capturing value and getting it to the market. Current megatrends are in energy security, global warming and in sustainability, leading to increasing preference for bio-based materials. The first wave provided a tool box: biological feedstocks, biotechnological processes like fermentation, biocatalysis, and production of industrial products. The unmet needs of traditional chemistry must be determined and the question of whether plants can be used to produce new products needs to be considered. The right approach to innovation is to generate new ideas and screen them based on opportunity assessment, attractive product possibilities, customer benefits and technical feasibility. The idea-validation cycle follows—research the "proof of concept," applications testing, market analysis and business validation—and is an iterative process. The key strategic question is: how can value be captured? Looking ahead into products for the future shows that a biotechnology strategy can leverage multiple innovation platforms. Building the strategy requires a vision, an organized approach, evaluation of core assets and capabilities, and a definition of initial parameters. A complete tool box is required to develop the best solution.

Milligan BioDiesel is the only North American commercial product—purely from canola oil—that meets European standards. In 1993 Milligan met with Agriculture and Agri-Food Canada (AAFC) and conducted tests with the University of Sasketchwan funded by AAFC to examine engine wear. It was officially founded in 1996, before the energy-security question arose. Problems of oil extraction were solved in 1999, and biodiesel production has reached 30 tons/day, albeit the process is still in the pilot stage using a process developed by AAFC. However, government lawyers are uncomfortable with "partnerships." True partnerships involve indemnification of one of the partners for activities of the other. This creates business risks for which the particular government minister may not have authority to guarantee a commitment. In government you can do only whatever is authorized, whereas corporations can do whatever is not prohibited. Transparency of a relationship requires equitable access to the government's assets. AAFC's policy is thus to avoid joint ownership of IP developed under collaboration. Joint ownership creates a *de facto* partnership, without all related terms and conditions having been resolved. Canada's public-private R&D business and legal framework is different from that of the United States.■

University / Industry Collaborations

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David Gulley (University of Illinois)
Hans Blaschek (University of Illinois)

The R2B or research to business program deals with up-and-coming markets. Consumers are far more demanding of the wellness products they buy; tomorrow the environment will be added to their list. Longer and more comfortable lives require increases in food quality and safety needs. Canadian companies need to export and compete in the United States. The vision for agriculture is that by 2015 Canada will be a world leader in human / animal / environmental health through R&D and innovation with more food grown on less land with half the water. Few venture capitalists understand the food materials market and its technology dynamics, but that is changing. Foragen is a Canada-based fund focused on North America, consisting of an experienced team of professionals. The venture process involves deal-prospecting and sourcing as well as constant critiquing of potential. The main reasons why start-up companies fail are insufficient funding, lack of management experience, inability to deliver results or commercialize scientific ideas.

When considering academia / industry interactions, eight mid-west states account for about 18% of the interactions in the United States. The mid-west accounts for 20% of the US total of 16,792 inventions and for about 25% of the US total of 4,578 licenses/options with industry. There were problems with early-stage venture-capital investments and 56% of the venture-capital deals were on the east and west coasts only.The mid-west research university network was formed in 2002 and about 17% of the US totals, 462 start-up companies, were from the mid-western states. The University of Illinois model for commercialization has an office of technology management, the required start-up company due diligence and several research parks. The offices of technology management oversee the invention, its screening, assessment, its market and the license disclosure while the Illinois Venture Services provide consultation—“productization” of technology, and venture funding with pre-seed and seed monies.

Interactions between Archer Daniels Midland (ADM) and the University of Illinois at Urbana-Champaign (UIUC) are feasible because of their close proximity and common interests. ADM wanted to fund projects to increase the value of co-product streams in dry- and wet-mill biorefineries. The collaboration is focused on overcoming the limitations to making plant-based resources become a viable alternative to petroleum-based products, increase the efficiency of bioconversion of plant fibers to value-added products and increase economical and efficient extraction of high-value-added products. Meetings of UIUC faculty and ADM affiliates on a common platform help identify areas of interest with USDA/DOE projects. The goal is commercialization and co-development of technologies to enhance the value of agricultural products. Methods include an open environment for the exchange of ideas and for developing a focus on key technologies with merging of different cultures. The motivational force for connecting people from different disciplines is a demonstration that the whole is bigger than the sum of the parts. The ultimate goal is to move the results of research to the market—to publish and commercialize.■

Growing Biobased Business: Policies, Tools and Approaches

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Carl Muska (DuPont)
Ken Zarker (National Pollution Prevention Roundtable)
Barbara Lippiatt (National Institute for Standards and Technology)

The USDA’s Federal Biobased Products Preferred Procurement Program (FB4P) stipulates that the federal agency must give purchasing preference to the biobased products designated by this program. Authority for the program is included in the Farm Bill. The act defines the biobased products or renewable domestic agricultural products by specifying domestic content, which is interpreted as the content from countries with which the United States has perennial trade relations. The program requires such specifications unless the products are not reasonably available, fail to meet performance standards or are unreasonably priced. The Resource Conservation and Recovery Act’s (RCRA’s) recycled products program has priority over biobased products unless there are environmental preferences for the latter. Generic groups of products are designated as items for preferred procurement. The requirement applies in order to claim coverage, certify that biobased content is consistent with statutory definitions and meets minimum ASTM standards. Under the voluntary labeling program, qualified biobased products may gain a USDA-certified biobased-product label and logo with the authority being granted for a limited number of years.

Congress created the FB4P to spur the demand for new biobased products and stimulate economic growth of agricultural communities. “Biobased product” is one determined by the Secretary to be a commercial or industrial entity (other than food or feed) that is composed, in whole or in significant part, of biological components or renewable domestic agricultural (including plant, animal, and marine) or forestry materials. The role of USDA under Section 9002 requires it to develop guidelines for use by federal agencies to establish a voluntary “USDA Certified Biobased Product” labeling. ASTM and BIO provide much-needed forums for stakeholders to engage on these issues.

The National Pollution Prevention Roundtable believes that the path ahead requires an increasing focus on material substitution and economic competitiveness. The “low hanging” fruit has been picked. Performance-based environment management allows facilities to have flexibility. Phase I involves identifying the alternatives and Phase II involves an analysis of alternatives selected for study. Technology diffusion requires acceleration and adoption of innovation. There is a need to identify the opportunities, to identify and recruit the opinion leaders and establish demonstration sites at facilities. Key elements for a successful program are that it must be “customer driven.” Innovative P2 designs must be “test driven” to reduce the adopter uncertainty.

At the Building for Environmental and Economic Sustainability (BEES), research is translated into terms understandable to the purchasing community. The BEES 3.0 model takes a life-cycle approach based on consensus standards. It measures economic performance—using a life-cycle-costing approach for installation, maintenance, repair, replacements—to know the time value of money, and also measures the environmental performance. It then conducts a multi-attribute decision analysis that is fair and comprehensive, includes raw material / transportation / use / disposal and the entire range of environmental and other impacts. The value of FB4P is that it provides scientific integrity, permits comparability and promotes innovation, thus making the evaluation performance-based rather than prescriptive and helps manufacturers identify their weak links. The answer to the question “What makes a product green—recyclables / renewables?” lies essentially in the trade-offs involved.■

Achievements of Focused Alliances in European Industrial Biotechnology

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Jeff van der Lievense (Tate & Lyle)
Luuk van der Wielen (Delft University of Technology
Robert Hermann (Industrial Investment Council)
Peter Lednor (Shell Global Solutions)

The Kluyver Centre has strong infrastructure in academic and industrial fermentation research with the latest partner being Tate & Lyle. The economic target is to double the turnover in the next 5 years by training researchers in the chemical, pharmaceutical and food industries, developing genomics-based diagnostic tools, developing genomics-based strain improvement in non-genetically modified organisms as well as effecting strain improvement through metabolic engineering. The highlights of the program include development of an efficient process for ethanol production from xylose through the introduction of a xylose isomerase modified by adaptive evolution.

Tate & Lyle aims at building a consistent global portfolio in personal-care and animal-nutrition products. Fermentation is an attractive technology to invest in because of the feedstock driver. The open innovation model is a combination of internal R&D, partnership acquisition and external research and development. The most recent Tate & Lyle acquisition is Hycail, which produces polylactic acid (PLA) and other PLA-based polymers.

The Delft University of Technology has a tradition of involvement in industrial fermentation, providing fundamental insights into yeast physiology. Challenges to be faced include regeneration of lactic acid from lactate, polymer synthesis and the high cost of impurity separation. A possible solution is to create a new lactic acid process strategy with yeast that produces lactic acid but no ethanol, has high lactic acid tolerance and has reduced impurity production.

The Industrial Investment Council is an investment-development agency offering advice to international companies investing in ex-East Germany. It helps investors approach the right companies for availability of raw materials such as non-food crops to solve problems of bioproduct market generation to discern what other markets will be influenced and what the effects of federal policies are likely to be. Farming opportunities and available land in ex-East Germany are much greater than in ex-West Germany. The Council provides a new regulatory framework with tax exemptions for all biofuels with no requirement of benefits. Unlike the United States, Germany has a significant focus on biodiesel. There has been investment in biogas, whereas biodegradable plastics require a more supportive regulatory framework.

Shell aims at building in-house capabilities, creating a merger of upstream and downstream processes. While supplying 10% of the world's transportation fuels, it is the largest supplier of first-generation diesel from biomass. The raw-material options to produce biofuels include conversion of edible parts of food crops for Mogas alternative fuels and the non-edible parts for biodiesel. Shell markets the above products, but is now focusing on second-generation fuels produced from waste-streams. It supports the development of technology at Iogen (Canada) and has brought the Fischer Tropsch technology to Choren (Germany) using woodchips. Shell is also part of B-BASIC (biobased sustainable industrial chemistry) collaboration with nine partners, four universities, four companies and one government entity. B-BASIC's strategic location in Delft is a distinct advantage. The results of collaboration are enthusiastic participation, networking, a broadening awareness of development and good sources of recruitment.■

The Integrated Forest Biorefinery and the Future of the Pulp and Paper Industry

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Adriaan van Heiningen (University of Maine)
Stefan Muller (Tembec)
Marc Sirois (AgroTerra Biotech)

The Quebec Center (CQVB) stimulates the development and transfer of technology to enhance public awareness and to regulate the role of the life sciences. In order to capitalize on opportunities, new product streams need to be created from biomass by partnering with petrochemical and bio-materials companies. The forest-products industry is facing challenges in the form of global competition, with wood-pulp pricing falling by 1% every year and feedstock prices increasing. It needs more revenue from value-added products besides wood, pulp and paper. The ethanol market to replace gasoline is unlimited. The maximum theoretical yield of ethanol from cellulose pulp on a weight basis is about 50%. Ethanol price must be greater than \$3/gallon for economic conversion of pulp. Hemicelluloses need to be extracted before pulping and the extract could be used for biofuels and bioproducts. The hydrocarbon-conversion strategy should include producing oxygen-containing products to increase yield, to shorten the conversion path and to increase the competitiveness relative to petroleum-based products. R&D issues are those related to separation and purification. Research on selective cleavage of lignin-carbohydrate bonds is required.

Tembec is a large, diversified and integrated company marketing forest-products pulp, paper, paperboard and chemicals. It has sites in Canada, the United States, France, Chile and has chemical sales of >\$200 million. The current chemical-product segment consists of phenol-formaldehyde resins and melamine, lignosulfonates, ethanol and hydrochloric acid. The majority of the wood goes to pulp and the waste-stream liquid is fermented and distilled to make ethanol. Tembec is the second largest supplier of industrial alcohol in Canada. However ethanol from wood does not make economical sense at this time. Tembec is also the second largest manufacturer of lignosulfonates, with 55% directed at the concrete-additive market. Lignosulfonates reduce water and increase compression strength.

Novel enzymes are being developed to improve efficiency of existing chemical programs and to make them cleaner, more cost effective and capable of addressing current challenges. The assets of AgroTerra Biotech (ATB) include strategic partnerships—with Kruger and Cascade—and a unique location in the Mauricie region of Canada. The challenges to the pulp and paper industry include poor productivity, increasing cost of energy, increasing the use of recycled fibers and developing added-value products. Pectin interference with retention costs the industry \$100–300 million annually. Pectinase-1 is AgroTerra's first enzyme-based

www.chemistry.org/portal/a/c/s/1/home.html

product; it cleaves pectins, reducing their size and making them more easy to eliminate. Accelerated evolution allowed ATB to generate a pectinase with the required characteristics in terms of pH and temperature profiles and cost.■

Enzyme Applications for Lignocellulose-Based Biorefineries

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Liisa Viikari (VTT)
Bill Baum (Diversa)

Enzymes are used for biomechanical pulping of woodchips, and for control of extractives and stickies. Pectinases are used for paper-machine retention and cellulases are used for de-inking of mixed office wastes. About 10% of North American kraft pulp products are bleached with xylanases, which lowers the cost of chemical bleaching and increases throughput. Pulp bleaching is nothing but lignin removal. In a proposed forest biorefinery, the wood chips need to be hydrolysed by steaming or acid pretreatment. In enzyme-modified pentosans, the degree of polymerization is controlled by xylanase and the charge is altered by accessory enzymes. There is a huge opportunity for resource recovery. Effective effluent treatment will still be required in future biorefineries.

In total hydrolysis of cellulose, lignocellulosic materials undergo pretreatment followed by enzymatic hydrolysis. Fermentation of the sugars produced is followed by distillation for separation of solid residues from ethanol. This requires a high-temperature enzyme and high consistency of conversion. Factors affecting the efficiency and economics of enzymatic hydrolysis of lignocellulosic biomass are the composition of the substrate, properties of the enzymes, the composition of the enzyme mixture to give optimal synergy, effective production systems, hydrolysis technologies for temperature control and recycling of enzymes. The advantages of thermostable enzymes are that higher specific activity allows reduced enzyme loading and higher stability leads to extended lifetimes. Process concepts for thermophilic cellulases need to be developed for consistent bioethanol production and a sensitivity analysis needs to be done. Equal hydrolysis at 60°C as at 45°C was obtained with a four-enzyme mixture.

Iogen's enzyme manufacturing facility has a highly automated production system. In 1986 it was reported that xylanase treatment of kraft pulp decreased the amount of chlorine required for bleaching. Iogen introduced the native thermophilic enzyme in 1991; it was limited to a maximum temperature of 55°C at pH 6.5. Three amino acids were replaced to increase thermostability by 15°C and the pH by 1.5, resulting in cleaner and more-efficient pulp bleaching while operating at higher temperature and pH.

Pulp-bleaching market trends show that use of elemental chlorine as a bleaching agent has been completely eliminated. The global market for biobleaching is $1.5 billion. With kraft pulp, the application of Luminase enzyme reduces the requirement for oxidizing agents. The objective is to achieve high levels of pulp brightness with reduced chemical usage through the application of a novel xylanase. A discovery screen has been developed for finding new xylanases from varied environments using in-house ultra-high-throughput screens. Screening with different pulps gives correlations that have enabled selection to be profile-specific.■

Biorefining of Lignocellulosic Forest Feedstocks

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David Layzell (BIOCAP and Queens University)
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Pulping wood chemically requires maximizing lignin removal and maximizing carbohydrate retention. A typical kraft pulp mill will process around 630,000 tons of wood per year with 90,000 tons of hemicelluloses degraded. The caveats in the biorefinery approach are pre-extraction considerations: loss of hemicelluloses at the beginning of pulping must be limited as an α-cellulose content greater than 80% results in loss of paper-sheet strength. The post-pulping approach involves extraction of cellulose- and lignin-derived chemicals from black liquor, recovery of tall oil soap and extractives from black liquor and gasification of black liquor residuals. Fluidized bed reactors can leverage existing forest-product resources for increased productivity.

With rising fossil-fuel prices, increasing energy demands in Asia, political uncertainty in the Middle East leading to oil-price volatility, climatic changes and growing challenges to the pulp and paper industry due to declining demand for newsprint and increases in energy and wood prices there is need for facilities with novel value-added products from the current infrastructure. The advantages are that the feedstock is well known and well studied, the infrastructure is large with efficient separation of lignin from cellulose and there is good synergy and integration with other industries. The Canadian biorefinery network has several key participants: the forest industry, the research community, chemical and energy companies and the government which has an interest in preserving rural economies. The technological challenges are that the kraft process should not be changed too much and that the hemicelluloses must be exploited.

Canada has yet to define a long-term bioenergy goal. It has a green advantage with the highest forest area per person and second highest arable land per person among the G20 countries. The vision for a bioenergy future with a 6% growth/yr, includes $7.8 billion/year invested directly in farms and forestry operations providing a $30 billion/year stimulus to the national economy. There is a need to address the challenges of scale. Large distances between sites of production and use require establishment of energy corridors to integrate into the existing fuel-distribution system and the development of dedicated bioenergy-linked pipelines. The bioenergy pipeline could have smaller production systems to meet local needs, with major users at the end of pipeline and minor users near production sites. Innovative thinking and industry-government commitment will be key.

Biofuels may not decrease the price of oil, but they may stabilize it. The biorefining platforms may be biological, using structured separations, or thermochemical, using fast separation and reconstitution. In Brazil, relatively simple steps are required for ethanol production from sugar cane. In the United States, a slow hydrolysis step is required to get from starch to ethanol. Lignocelluloses are a complicated mixture of hexoses, pentoses, *etc*. Thus, there is a tradeoff between feedstock cost and process complexity. New uses for lignin need to be developed. The greatest historical increase in demand for transportation fuels is now approaching. There is no doubt that second-generation biofuels have the potential to meet future demands.■

Industry-Based Forest Biorefineries

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The Agenda 2020 Technology Alliance is a special project of the American Forest and Paper Association (AF&PA). This industry-led partnership with government and academia has the objective of reinventing the US forest-products industry through innovation in processes, materials and markets by facilitating collaborations, pre-competitive research and development to advance breakthrough technologies. The ultimate objective is the creation of integrated forest-product biorefineries (IFBPs). In some parts of the United States there is good overlap between the pulp and paper mills and petroleum refineries; such proximity allows bonus thermo-chemical applications. The objective is to implement a fully integrated agricultural and forest-products biorefinery that would use untapped wastes.

Ensyn's rapid thermal processing (RTP) method produces high yields, typically 75% by weight, of bio-oil from low-grade wood chips. Bio-oil and the other principal RTP products, charcoal and a combustible gas, can be used as fuels without further processing. However, it is more economical to first recover valuable chemicals, natural resins and resin ingredients, and novel biochemicals for nutritional and medicinal uses. A refinery in Ontario processes up to 80 dry tonnes of wood, for production of high-value commodity and specialty chemicals

The objectives of the Canadian Forest Services are to assist the industry in developing value-added applications, to develop new strategies for sustainable forest management and to identify information gaps in order to progress in the development of the bioeconomy. The Canadian forest flora is a rich source of bioactive natural products. Naturally occurring compounds are products of primary metabolism and are vital for the maintenance of living processes and of secondary metabolism with pharmaceutical value. Biochemical metabolites perform many important functions such as UV protection, have anti-viral / anti-fungal activity. Extraction procedures pose challenges and there is a need for alternative procedures such as solvent extraction. For any naturally occurring non-timber forest crop over-utilization needs to be minimized. Protocols must be laid out to ensure harvesting is ecologically sustainable.

Wood-cellulose microfibrils, after separation from lignin, show potential for use in composites. They have the advantage over petroleum-based polymers of being renewable and CO_2-neutral as well as having high strength and high aspect ratio. Challenges to their use include a high-energy requirement in their separation. Microbial and enzymatic means of separation of cellulose microfibrils are being investigated.■