Summary Proceedings

Fifth Annual
World Congress on
Industrial Biotechnology and Bioprocessing

Linking Biotechnology, Chemistry and Agriculture
to Create New Value Chains

Chicago, April 27–30, 2008

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Foreword and Acknowledgments

The Fifth Annual World Congress on Industrial Biotechnology and Bioprocessing convened in Chicago, April 27–30, 2008, organized by the Biotechnology Industry Organization (BIO), the American Chemical Society (ACS), the National Agricultural Biotechnology Council (NABC), the US Department of Energy (DOE) and the DOE's Genomics:GTL Program. Some 170 presentations were made in six plenary and ten "break-out" sessions (each with five parallel tracks); fifty-five presentations were made as posters and six workshops were held. This was the best-attended World Conference to date, with 1,100 delegates.

The organizers are grateful to BIOTECanada and EuropaBio as supporting organizations, to Industrial Biotechnology as the media sponsor, and to the many conference sponsors. Particular thanks are due the program committee for their hard work and dedication in screening submissions, locating speakers and in organizing a very dynamic program: George Anderl, Roland Andersson, Rich Burlingame, Bruce Dale, Allen Dines, Larry Drumm, Raymond Drymalski, John Finley, John Frost, Richard Gross, Jack Grushcow, Ralph Hardy, Jack Huttner, James Iademarco, James Lalonde, Sophie Laury, Birgit Kamm, Mike Ladisch, David Lee, Eric Mathur, Jim Millis, Ramani Narayan, Glenn Nedwin, Erin O'Driscoll, Adrien Pilon, William Provine, John Ranieri, Maria Rapoza, Anna Rath, Manfred Ringpfeil, Gerson Santos, Philip Schwab, Garrett Screws, James Seiber, James Stoppert, Mark Stowers, Larry Walker, David Ward, Roger Wyse, James Zhang and Paul Zorner.

The indefatigable efforts behind the scenes and careful organizational oversight of Matthew Carr, Amy Ehlers, Jocelyn Modine, Courtney Murray and Kimberly Scherr (BIO) were vital to the seamless success of the conference.

This Summary Proceedings provides broad coverage of the plenary presentations and brief highlights from the breakout sessions. It is largely the product of first-rate work by Marie Donnelly, Ben Heavner, Navaneetha Santhanam (Cornell University) and Brandon Charles (BIO) as recorders. And thanks are due Susanne Lipari (NABC) for her excellent page-layout/design work.

Allan Eaglesham
Executive Director, NABC
Summary Proceedings Editor
August, 2008

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Welcoming Remarks

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

Mr. Erickson spoke for the assembled delegates by expressing excitement in being at Chicago for the Fifth Annual World Congress on Industrial Biotechnology and Bioprocessing. He observed that some of the attendees had been present at the first World Congress, adding that this one, just four years later, was three times larger. He expressed pleasure in welcoming the delegates and hoped that they would take the opportunity to visit the exhibit hall. A robust and interesting program was in store: plenary presentations, breakout sessions, workshops and poster presentations.

Erickson also expressed pleasure in launching the George Washington Carver Award for Innovation in Industrial Biotechnology. BIO chose to honor George Washington Carver as a founding father of modern industrial biotechnology. Erickson presented the first annual George Washington Carver Award to Patrick Gruber, CEO of Gevo, Inc., recognizing his accomplishments in creating and commercializing PLA, the new plastic synthesized from corn starch. Accompanying the award was a scholarship given in Dr. Gruber's name to Iowa State University, where Carver was the first minority student and faculty member.

Jim Greenwood (President, Biotechnology Industry Organization)

Mr. Greenwood opened with an expression of thanks for BIO's staff, who work for many months organizing the conference; several vice presidents and their teams pull together to make the World Congress bigger and better each year. He echoed Brent Erickson's enthusiasm at being in Chicago—the location of BIO's 2006 International Convention—with pride at bringing another signature event to America's heartland.

The World Congress on Industrial Biotechnology and Bioprocessing is focused on an exciting emerging sector. The convergence of biotechnology, chemistry and agriculture is opening doors to new inventions and products that will revolutionize industrial manufacturing, the production of chemicals and consumer goods, new food ingredients, environmental protection, and, of course, our ability to meet our energy needs. The World Congress was initiated to help industrial biotechnology forge its own identity within BIO's big tent. They call industrial biotechnology the "third wave" in biotech innovation, which, judging by the 2008 attendance, is becoming a tsunami.

As the World Congress comes into its own, biofuels are coming in for heated, but unjustified, criticism. In the past two years, biofuels have enjoyed the spotlight as the president and Congress boosted their prospects. Now we are learning what is commonplace in the political world: first the media build you up and then they tear you down. Activists and the media are fueling false and negative perceptions about biofuels and sustainability, and about impacts on food production and climate change. These slings and arrows may be familiar to those involved in the introduction of biotech crops. The lesson from past battles is clear: such challenges must be addressed quickly and decisively. Bad information must be countermanded with accurate information, ensuring that our version is part of the story. We cannot allow public perceptions to harden against biofuels, and we cannot allow scientists who work so hard to solve our critical energy needs to be painted as villains.

At BIO they are doing their part, meeting with journalists and challenging and correcting misinformation on biofuel technology. At the same time, critics should be listened to and valid concerns taken to heart. There are legitimate issues to be addressed if biofuel production is to be sustainable. Dialog is needed with NGOs who want to protect the environment, because we do too. BIO is in contact with Congress; Greenwood was pleased to say that their support has not faltered.

To take their message directly to the public, BIO has formed the Advanced Biofuels Climate Change Information Center, which has become an important part of online debate on biofuels (http://biofuelsandclimate.wordpress.com/). But they cannot do it alone. Greenwood encouraged the World Congress participants to describe their work to family, friends, neighbors, legislators and local media, and anyone else who will listen. It is critical that we share our common vision of sustainable production of biofuels, biobased plastics and other renewable consumer products, new food ingredients, new ways to make biopharmaceuticals to create a green, biobased economy to initiate positive change worldwide.

Biotechnology does not have all of the answers. But we do have good solutions to a host of pressing challenges. We need to do a better job in telling our story, to reach and resonate in people's lives. We will weather this current storm if we continue to act with patience, tenacity, and integrity.

The BIO board recently recommended that, in addition to being a world-class advocacy organization there is need for BIO to become a world-class communications organization. They are acting on this recommendation in order to get ahead of the curve and lead the media with their information rather than being in a defensive posture.

Greenwood observed that this conference provides a focal point for key thought-leaders in this space. He expressed hope for a productive meeting with robust exchanges of ideas that will be fuel further innovation, and he expressed gratitude to the participants for their contributions and for their support of the World Congress.■

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Plenary Sessions

Industrial Biotechnology: Outlook for 2008

Jacques Beaudry-Losique (US Department of Energy)
Roger Wyse (Burrill & Company)

Current sky-rocketing prices of fuel, commodities and food globally, with hunger, food riots and instability in developing countries, are driven mainly by two forces, according to **Jacques Beaudry-Losique**: depletion of fossil fuels and the economic emergence of developing economies. China may become the largest market in the world within a decade, coming out of nowhere. Reports indicate that China will have 500 million automobiles by 2050 (the United States now has 250 million). The Tata Nano car was unveiled recently in India, priced at $2,500 and thus accessible to millions; for many, it is likely to be a stepping stone to larger automobiles.

Concerns over climate change have affected the popular view of fossil-energy supplies. Furthermore, new oil finds have not matched consumption for decades, and need for alternative energy sources has been recognized for many years. The need for clean, renewable fuels is now urgent, compelling, and vital for our economic future. Beaudry-Losique told the audience that they are on the front line of the critical effort to safeguard US energy and economic security, as well as improvement of global well-being. Some have constructed an artificial conflict between food and fuel when, clearly, we need both. Imagine what agriculture and the food chain would look like without fuel and fertilizers for tractors and transportation, refrigeration, irrigation and to improve crop growth. Maintenance of stable fuel and fertilizer prices is fundamental to agriculture and to the economy. We need productive agriculture with improved yields with less requirement for chemicals, and not only are we are achieving that, we can succeed in doing it better in the future in large part through biotechnological innovations by people and businesses represented in the audience.

At DOE they are cooperating in the effort to make cellulosic biofuels an industrial and commercial reality. Biofuels from cellulosic materials offer great promise. New biorefineries will sustainably convert biomass to biofuels, biopower and other valuable products. A wide range of feedstock resources will be available, including agricultural residues, dedicated energy crops and a variety of waste materials. The crops will be of various kinds, adapted to local conditions. With appropriate land-use choices, cellulosic biofuels' life-cycle greenhouse-gas emissions will be reduced by as much as 86% compared to gasoline.

Much work remains. We must accelerate progress, enhance sustainability and reduce costs. Commercial success will ultimately depend on meeting cost goals. We have already reduced the projected cost of cellulosic ethanol from ~$6 per gallon in 2001 to ~$2.50 today. Further reduction, although difficult, is possible. Fortunately, we have a mandate: the law. The Energy and Security Act of 2007 states that US-transportation fuel should include 21 billion gallons of advanced biofuels by 2022, and 2 billion gallons as soon as 2012. It states also that, with these advanced biofuels, the reduction of life-cycle greenhouse-gas emissions should be at least 50%.

At DOE, they engage in many partnerships to meet these important goals. They are investing more than a billion dollars in research, development and demonstration of sustainable cellulosic biofuels. They have announced several commitments since early 2007, including a recent program for examination of several conversion technologies with a variety of feedstocks in seven small-scale biorefinery projects. Over the next 4 years, DOE will put $200 million into this effort. A wide range of methods will be examined by the selected partners. Each of seven small biorefineries—Ecofin, Lignol, ICM, Mascoma, NewPage, Pacific Ethanol and RSE, will produce about 2.5 million gallons of cellulosic biofuels per year. More importantly, they will give the nation the platforms required to build commercial-scale facilities. At the same time, DOE is investing up to $285 million to build six pioneer commercial-scale biorefineries, which, when operational, are expected to produce >130 million gallons of biofuels annually: Abengoa, Alico, Blue Fire, Poet, Iogen and Range Fuels. Ground has already been broken for the Range Fuels facility, and contracts have been signed with three others.

DOE is pushing ahead with efforts to develop more-effective enzymes and microorganisms. Improvements are needed in enzymes that convert cellulosic biomass to sugars for fermentation. Selected as partners in the enzyme work are DSM, Genencor, Novozymes and Verenium, to receive up to $34 million over the next 4 years. Another $22 million are being invested in projects led by Cargill, DuPont, GE Global Research, Mascoma and Purdue University to develop organisms for accelerated fermentation.

In the previous week, DOE announced another solicitation for university research to help groom the next generation to

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meet its energy challenges. Up to $4 million will be provided to up to a dozen institutions to perform cost-sharing research on environmentally beneficial, efficient, breakthrough conversion technologies.

The DOE Office of Science has opened three new bioenergy research centers, for work on biomass feedstocks, *inter alia*, including genomics approaches.

The enormity of the challenge and the urgency of need for sustainable solutions demand leveraging resources to create partnerships, including those that go beyond those described above. They work with other federal agencies and programs, with state and regional groups, industry organizations, non-profits, national laboratories, academia and other nations. For example, through the Biomass R&D Board—a cabinet level organization—they work with at least eight cabinet-level agencies and federal offices to ensure that resources are invested widely and efficiently across government.

The DOE works with state and regional entities. They have contributed $6.5 million for work by the Regional Biomass Feedstock Partnership, the USDA and the Sun Grant universities to help them define important regional constraints. Also, they are working with the Governors Economic Coalition to tackle infrastructural challenges for delivery and use of the growing volume of biofuels in the United States.

A good beginning has been made, but more collaborative effort is needed. It's important to note that many companies are independently pursuing innovative approaches to commercialization of biofuels. There is broad need for new ideas that will lead to significant breakthroughs. The DOE recognizes that a wide range of advanced biofuels will be required to fill the nation's transportation needs in the long term, in the same way that a wide range of petroleum-derived products is currently in use.

Sustainability is the driving force for all of the DOE's R&D work, whether it means reducing the water needs in growing cellulosic feedstock, increasing yields, or decreasing the energy inputs for biofuels production. They are engaged in many projects. With respect to land use, they are funding construction of a model—the first of its kind—to predict land-use changes resulting from biofuels production worldwide, at the Argonne National Laboratory and Purdue University. They have committed >$1 million to Conservation International to identify lands on which to produce feedstocks to preserve biodiversity as well as those that should remain untouched. Scientists at the National Renewable Energy Laboratory are comparing life-cycle water requirements for production of cellulosic biofuels to the life-cycle water needs for petroleum fuel production and ethanol from corn or sugar cane. They are developing a national geographic information system atlas to integrate data on feedstocks and ecology, existing infrastructure and other factors to help decision-makers consider a wide range of factors as they tackle questions of where to grow feedstocks and site biorefineries, protection of fragile ecosystems and other difficult concerns.

Achieving sustainability will remain a guiding principle. Beaudry-Losique expressed the belief that this approach will lead to affordable, secure solutions, ensuring national energy and economic security, greater global stability, and environmental health. With proper standards, thoughtful policies and improved technologies, biofuels can and will play an important role in helping the world address its growing transportation energy needs, reduce greenhouse-gas emissions and reduce dependence on oil. Furthermore, many of the necessary standards are already incorporated into the law.

Roger Wyse remarked that some 400 people attended the first World Congress, and four years later delegates numbered ~1,100 and industrial biotechnology is making the headlines almost daily. We have come a long way very fast and as we look forward we need to be circumspect about emerging issues that will determine how quickly this technology will be implemented and its full potential realized.

A tipping point was reached in 2005. Several aspects came together. As a nation, we became concerned about energy security not only because of geopolitical issues but also because hurricane Katrina knocked out a lot of refining capacity. Concerns over global warming began to grow and we suddenly got the political will; policies were put in place in the United States and in Europe, enabling this industry. That momentum continues, driven by interest in biofuels and sustainability.

It has been an exciting time since then. Massive amounts of venture capital have been invested in this industry. Many new companies have been formed and oil-company capital is driving much of the technology development. We have seen a lot of demand for technologies. A lot of venture capitalists and entrepreneurs have been looking for technologies in the universities. It got frothy. We've been paying very high valuations for some of that early-stage technology, the success of which remains to be demonstrated.

One could argue that we came too far too fast. A lot of ethanol plants are in play, and a lot of corn has been diverted into ethanol production and discontinuities in the value chain and the food chain were triggered. We are seeing a temporary disequilibrium, the importance and implications of which should not be underestimated. The implications include significant revitalization of rural America, particularly in Iowa and the other corn-belt states. Wyse, who grew up in the Midwest, knows that when a farmer makes money he buys new equipment and remodels his wife's kitchen. A lot of spending is occurring, revitalizing that important sector.

Implications of high fuel costs and high food prices are also being seen, not only in the Midwest and nationally, but globally.

New companies are being formed around some exciting technologies. A lot of new money has come into this fast-moving space. This could not be a more exciting time in the life sciences and there is no better time to invest.

The stock market has also treated well companies supplying inputs to the agricultural industry that is producing feedstocks for biofuels. Monsanto has done well as it produces corn and soybean seeds. Fertilizer companies have also prospered over the past two years as have farm-machinery companies, such as John Deere. The processors have fared less well, although this has been a unique opportunity for companies like Bunge, whose stock price has almost doubled. Clearly, investors understand the opportunity and where money is being made in the industries ancillary to industrial biotechnology. Metabolix, as the "poster child," got out early in a successful IPO; however, its stock price is now struggling and Wyse hoped that the lower stock price did not portend for the IPO market in the coming year per concerns expressed by some.

The past couple of years have been tough for ethanol producers. Some that went public saw their stock prices depressed, and some of them pulled their IPOs, largely resulting from the issue of blender capacity and the dramatically increased price of corn. At the same time, energy prices have soared, keeping interest in this industry from waning.

Thus, there are many important issues that we have the opportunity to address, but stress factors prevail on the industry.

Important industry-driving policies have been put into place, providing opportunities for development of the cellulosic industry—addressing some of the issues discussed by the previous speaker—*e.g.* by lowering feedstock and sugar costs while maintaining environmental sustainability.

Late in 2007, storm clouds began to form on the horizon and realization set in that it's not just about the technology, it's about the social, economic, environmental and sustainability issues that surround the application of the technology. A lot of negative publicity resulted. The perfect storm of 2005 is merging into a second perfect storm: high fuel prices, high cost of food, and the social, economic, environmental and sustainability issues, which, if we don't address, development of the industry will be delayed. For those already investing and for those who will invest, time is money and a delay could be devastating to the investors and to new sources of capital. The food-versus-fuel debate is not a simple one. Sound bites that are familiar to us fail to do justice to this complex issue, and the press will beat us every time because we will attempt to explain it in complicated terms, describing nuances whereas all they want is a simple headline. The press is having a field day with everything from sustainability to food prices, not only in this country but also in Europe and Asia. It's easy to get an inflammatory headline, whereas supportive articles are usually relegated to secondary positioning with small headlines. We cannot beat them. We are going to have to join them and engage them in discussion of commonalities toward a solution.

There have been many support subsidies for food programs and food-export constraints, and, as a result, when things change as quickly as they have over the past couple of years, discontinuities are exacerbated. Clearly, the high cost of fuel is increasing the cost of production of food. Furthermore, global warming will cause increased extremes of climate, causing, for example prolonged droughts. We must understand how to manage the global food supply and minimize these discontinuities. We *can* produce both food and fuel.

Certainly, the re-allocation of part of the US crop to biofuels has been a factor, but it is only one on a long list. In 2007, farmers increased the acreage of corn and decreased that of soybean. On the global scale, the decrease in soy planting caused an increase in soybean oil futures, which tracked in parallel with palm-oil futures in Asia. In March 2008, when the USDA predicted increased soybean production in the United States, the price of palm oil dropped dramatically. So, there are connections between what happens in the Midwest with what happens globally.

The sustainability issue must be addressed head-on. Wyse was pleased to learn that DOE and others are developing necessary databases.

He predicted that 2008 will be when this industry comes of age. It will be very important how we, collectively, with BIO in the lead, address these important issues. If we falter, not only will the implementation of the technologies be delayed, but the financing needed to drive the industry may be elusive and hard to come by.

These social and economic issues are exacerbated by the fact that the United States is entering a recession—of uncertain depth and longevity—which will affect access to capital. As the companies listed above look for new financing to build pilot and pre-commercialization plants, the perception that this is a growing industry will be critically important, to avoid delays resulting from political winds blowing against it.

The IPO market has been largely closed. However, a number of companies in this space have filed for their IPOs and, since they have revenues, Wyse expressed the hoped that they would set the precedent for future IPO opportunities. Significant advantage accrues from having a great story: biotechnology can contribute in a multitude of ways to solving problems of incrased agricultural productivity, of utilization of some of those sustainable sources of biomass with conversion into myriad products while reducing environmental impact.■

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Industrial Biotechnology: Growing the Global Economy

David Morris (Institute for Self-Reliance)
Wei-Ming Jiang (DSM China)
David Berry (Flagship Ventures

David Morris stated that, in the beginning, industrial economies were carbohydrate-based. In the United States, before the civil war, corn ethanol was the best-selling chemical for use as an illuminant and as a solvent. Its principal competitor was wood-derived methanol. As late as 1920, biochemicals comprised 60% of the chemical market, and, in the 1920s and 1930s, the leading scientists and entrepreneurs, George Washington Carver, Thomas Edison and Henry Ford, led a national effort to expand the carbohydrate economy, in large part as a strategy to solve a two-decades-long depression in rural America. Their vision was to marry industry and agriculture, providing new markets for an oversupply of crops and creating industries in rural areas and perhaps even value-added, on-farm processing. Four regional R&D centers established by the US Department of Agriculture still exist, focusing on regional crops and non-food/feed products and their markets.

In 1941, Henry Ford unveiled his "biological car," the body of which was made of vegetable fiber, seat covers and steering wheel were from soybean and tires were from golden rod grown by Edison on his farm in Florida, after retirement from inventing. The fuel, of course, came from corn.

After World War II, the carbohydrate economy all but disappeared. The price of oil dropped to less than $1 per barrel and the chemical industry emerged as a major force. By the 1960s, plastics were made only from natural gas and petroleum, synthetics had captured almost 70% of the textile market and over 90% of the chemical market, and not a drop of ethanol graced our gas tanks. The nation had moved on.

In the 1970s, the pendulum began to swing back—slowly at first and then with increasing speed—for three primary reasons:

- Technological advancements in the biological sciences
- Rising costs of hazardous-waste disposal
- Increasing oil prices.

Usually, technological advancements are aimed at high-value sectors, such as medical products, which applied to the first biological products. As volume of production increases and further advances occur, the price drops, opening up higher-volume, lower-value non-medical markets. For example, in the early 1960s, polylactic acid was a natural fiber used in disintegrating sutures applied within the body. It cost ~$200 per pound, but since less than an ounce was needed for an operation, it never showed up in the beginning as an itemized expense. Today the price of polylactic acid (PLA) is less than $1 per pound.

Last year, when oil was approaching $85 per barrel, for the first time since World War II, bioplastics became directly competitive with petrochemicals in many markets. While technological developments have lowered the prices of bioproducts, increasingly rigorous environmental regulations have raised the price of fossil fuels and their derivatives. Biofuels made it into the US gas tank initially primarily because of air-quality concerns. The enormously profitable year for ethanol in 2006 resulted from the abrupt phase-out by oil companies of the gasoline additive MTBE, because of fears that it was contaminating groundwater.

Countries, states, counties and even cities are now mandating degradable plastic products, which virtually guarantees a market for bioplastics.

As costs of hazardous-waste disposal rise, biochemicals become competitive because biological manufacturing processes generate few such by-products. And as nations focus on greenhouse-gas reductions and embrace low-carbon standards, carbohydrates and biological processes become even more attractive; living carbon becomes competitive with dead carbon.

The price of oil spiked in the late 1980s, dropped and rose again during the first Gulf war, then dropped and rose again with the second Gulf war. That increased volatility in itself caused some manufacturers to seek alternatives at a more stable price. Since 2004, oil prices have increased steadily, from $35 to $120 per barrel today without a single disruptive event, convincing most that the era of inexpensive oil is over.

Speculation has accelerated these price increases, and speculators may leave the market. But if the price drops, few expect it to be by much and most expect that increasing global demand will lift prices again.

In 1992, Morris wrote a widely disseminated report titled, "The Carbohydrate Economy," which predicted its revival. Sixteen years later, he is surprised by the speed of developments. As of April 2008, the carbohydrate economy had come a long way. Vegetable oils are replacing mineral oils as lubricants and waxes, sugars are replacing oil as the basis for chemicals and fuels. Ethanol consumption—about 3% of the transportation fuel supply—will increase at least six-fold in the next 14 years, with about two thirds of the expansion based on cellulosic feedstocks. More than $1 billion in venture capital flowed into the currently very small cellulosic ethanol industry in the previous two months. Some of world's largest chemical companies, including DuPont and Dow, are moving aggressively into biochemical manufacturing.

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However, in April 2008, the carbohydrate economy is still, to a large degree, a creature of public policy, begging the question: “Are the rules that we’ve developed for the new materials economy well designed?” We make the rules, and the rules make us. The rules that we make—whether regulations, laws, mandates, incentives or zoning codes—channel entrepreneurial energy, scientific genius, and investment capital into the creation of specific technologies and institutions So far, the US government has developed rules to encourage bioproducts and biofuels in virtually the same manner as rules to encourage use of wind and sunlight. But biomass has two crucial distinguishing characteristics:

- Plant matter has many possible end uses—food, feed, chemicals, construction materials, textiles, fuels, fertilizers, *etc*. Care is needed that our policies will not divert plant biomass and acreage from higher to lower uses.
- Sufficient quantities of biomass will be available only if we persuade farmers and foresters to cultivate and harvest it. Therefore, we should design policies to maximize benefits to farmers and rural communities.

The best use for land is production of plant matter for food for people. The current concern over impact of biofuels policy on prices and shortages of food is wildly overstated, in Morris’s opinion. But the concern stems from a fundamental truth: we have a limited area of arable land, and a growing, increasingly wealthy global population that is eating higher on the food chain. There is no question that ethanol policy has raised the price of corn, but the data are overwhelming that this accounts for only a minor part of the increase in retail price of food. The production of ethanol in Brazil from sugar cane has kept pace with the increase in ethanol production in the United States from corn, yet the price of sugar has declined. The evidence is compelling that the increased price of oil, the devaluation of the dollar and speculation have played much more important roles in raising food prices worldwide than have biofuels.

When Morris heard that ethanol was blamed for the tortilla riots in Mexico, he knew that the blame was misplaced, although there is a connection between corn and tortillas. When he heard that the soaring price of wheat was a result of ethanol production, he wondered about the connection since little wheat acreage has been diverted into corn. And when he recently read that rice riots around the world were blamed on biofuels, he began to get angry. Rice and corn are only remotely related, if at all; rice is fed to humans, primarily, whereas corn is fed to animals; 80% of the world’s rice is consumed within the country that grows it, whereas corn is the world’s most traded crop. Nevertheless, the concern over whether the rapid expansion of acreage devoted to energy and industrial materials will lead to hunger is valid, forcing us to confront an important question: Do we need to use plant matter at all for non-food and -feed purposes? Morris’s answer is yes, because if we are to create a renewable-resource-based economy, then it has to be based on renewable resources. 25252832And wind and sunlight lack one fundamental characteristic: they don’t have molecules for conversion into physical products.

We must recognize that land area is limited, therefore we must maximize crop yields and move from a food feedstock to non-food feedstocks. China has capped its ethanol production, based on its needs for food and feed, at 600 million gallons per year. And the renewable fuels standard—little known—has capped the production of corn ethanol at 8 billion gallons in the United States. Morris suggested that, at the top of the non-food hierarchy should be chemicals and other bioproducts for two reasons. First, bioproducts stimulate investments in biological production processes, which, in turn, stimulate investments in the technological and biological sciences. Secondly, we have sufficient plant matter to displace virtually all of our dead-carbon-based chemicals. Next in the hierarchy may come energy, but even within that category there should be another hierarchy. Heat, for example, is often overlooked, and yet when we convert biomass to heat we often get efficiencies exceeding 80%. Next would be transportation fuels, our largest consumer of oil; biofuels may displace up to 25% of our liquid fuels. And at the bottom of the energy hierarchy would be electricity production, because it’s a low-efficiency proposition and there is insufficient biomass to provide more than 5% of our electrical energy needs.

Currently, public policy is inconsistent with this hierarchy. The government awards incentives for the conversion of biomass into electricity and transportation fuels, but there are no incentives for biomass conversion into heat or bioproducts. Ethanol gets an incentive if used as a transportation fuel but not for industrial purposes; corn sugars converted to ethanol get an incentive, whereas corn sugars converted into plastics do not. Vegetable oils converted into lubricants do not get an incentive, whereas vegetable oils used for biodiesel do. We need to redesign these policies.

Another distinguishing characteristic of biomass is that it needs farmers to grow and harvest it, therefore the carbohydrate economy should be viewed as in integral part of farm policy, which Congress has failed to do so far. Its biofuels focus has been on getting more, not on getting better. It assumes that increased biofuels production will increase the prices of crops, which will, in turn, benefit farmers and rural communities. For the first 25 years, the biofuels policy failed in this regard; only in the past 18 months has it been successful, although there is no guarantee of continuance. Students of agricultural history know that the primary problem is that millions of small, vigorously competing farmers sell into an increasingly concentrated buyers’ market. The result is that the prices received for their crops increasingly lag behind the prices they pay at the store. The government created a farm policy that dealt with this by, in effect, allowing farmers to sell their crops below cost of production while making up the difference with subsidies paid for by the taxpayer. This has had pernicious effects. Farmers are seen as welfare cheats, and under-priced US crops not only undermine farmers' self-confidence but undermine agricultural production in other countries. Ironically, this policy leads more to hunger in other countries than does biofuels.

A strategy to address this situation is to have farmers gain a share in the value-added process. Farmer-owned biorefineries were the original basis for the ethanol industry. In 2002, 80% of new biorefineries were majority-farmer-owned, whereas in 2007 90% were absentee-owned with farmers and rural communities no longer directly benefiting. Congress should revisit the incentives for biofuels to take into account scale and ownership.

Morris ended with a quote from Bertrand Russell: "Change is inevitable, whereas progress is problematic. Change is scientific, whereas progress is ethical." We will have change whether we want it or not, but we will have progress only if we establish rules that channel entrepreneurial energy and investment capital and human genius in the directions of creating technologies and—even more important—institutions that are compatible with our long-term vision.

Wei-Ming Jiang described three phases of expansion of industrial biotechnology with special reference to China—initiation, development and commercialization. The first stages require appropriate government policies and financial support and capital investment. In China, government support is strong. Another national advantage is the potential to produce large amounts of biomass. National competence in biotechnology is improving rapidly, and Jiang expressed confidence that China will be a dominant center for biotechnological development in the foreseeable future. Market potential is also strong.

The Chinese government fosters industrial biotechnology for three reasons:

- Political—decrease dependence on foreign oil,
- Environmental—reduce greenhouse-gas emissions,
- Social—increase incomes to farmers and along the value chain.

China's reserves of oil are equivalent to only 21 days of current usage. The national goal is to have contributions from renewable energy of at least 7% by 2010 and 15% by 2020.

Over the past 20 years, economic development in China has been rapid, but at great cost. Environmental pollution is a particular problem. Another national goal is a 20% saving in energy by 2012. China is a signatory of the Kyoto Protocol and the Bali Roadmap.

Despite China's recent economic growth, 800 million people still live on less than $1 per day. Land in the western part of the country is infertile. Instead of subsidizing those populations with food, it would be advantageous to subsidize farmers to grow sufficient crops to provide income. One possibility is to produce feedstocks for bioprocessing and production of value-added materials.

Under the current eleven-year plan, *i.e.* until the end of 2012, foci are on:

- Biofuels and biomass development with incentives provided,
- Biorefineries for conversion from petroleum-based chemicals to biochemicals, and
- The biobased environmental industry for reuse of waste materials to produce heat and energy.

Objectives are to produce 2 million tons of fuel ethanol and 0.2 million tons of biodiesel annually by 2010 (replacing 4% of China's fuel consumption), and 300,000 tons of biopolymers, *i.e.* 1% of consumption of plastics. On June 1, 2008, a tax would be levied on plastic bags used in supermarkets. Biobased processing is also being encouraged, using biocatalysts to replace chemical processes.

The northeast of China, traditionally the source of feed, is now a source also of feedstocks for biofuels and bioproducts. Corn yields in this area are poor compared to those in Europe and the United States; recently, scientists at China Agricultural University expressed interest in collaborating with US scientists to achieve yield improvements. Sweet sorghum is grown in the central-west of the country, and sweet potato in the southwest. Sweet potato and non-food cereals are grown on the infertile lands further west. The south, where cassava is the staple crop, is the chief source of biofuels.

China produces 1.5 billion tons of cellulose annually, of which a third may be channeled into biofuel production by 2020, representing 60% of fuel consumption. The government is enthusiastically supporting the development of biomass production.

Not only are foreign companies showing interest in developing industrial biotechnology in China, home-grown companies are forming—producing biofuels, biopolymers, food and feed additives, and biobased fine chemicals

In 2007, Chinese production of biopolymers was small, but has grown rapidly since. Tianjin Green Bio-Science is expected to have a PHA-production capacity of 10,000 tons annually by 2009; if so, it will be the largest single producer globally.

Annual GDP is expected to increase by 10% annually until 2020, with concomitant increases in need for oil or alternative feedstocks. Clearly there is huge potential for the development of a biobased economy, particularly considering that oil is likely to continue to increase in cost.

Jiang's company, DSM, started out, a century ago, as a coal producer and developed into a producer of specialty chemicals, marrying bioscience with material science. Already, more than 20% of their products are created through biotechnology, and their objective is to increase this to 50% within three years.

David Berry began by asking, "What is different about opportunities in the bio-industry in today's market?" Compared to what was happening in the 1970s, for example, much more information is available. We have an ability to understand what is going on at the genome and gene levels, in terms of DNA and proteins and in terms of networks within the cell. This information results partially from investments in technology. Helicos BioSciences, for example, has been pioneering single-molecule DNA sequencing, which helps answer fundamental questions about what is happening in any given cell and how is it different from any other cell. For example, this approach provides information on fundamental molecular heterogeneity in cancer cells—a level of understanding unavailable hitherto, presenting a range of research opportunities. Another area of significant improvement in technology is in rapid DNA synthesis, which impinges on synthetic biology, for example. Technologies like that of Helicos tell you what sort of DNA you want and those like that of Codon Devices allow you to rapidly produce the DNA you want in order to engineer a cell. In 2002, it took about 2 years to make 7.5 kilobases, whereas in 2008 it is possible to make tens to hundreds of kilobases in weeks to months.

The employment of synthetic biology, depending on the size and cost of the product, facilitates targeting a range of industries with the potential of profound effects. Energy and agriculture have been primary areas of focus, and opportunities are opening in the pharmaceutical industry by allowing companies to explore the true opportunity-space for any given protein.

What are the key market drivers for biofuels and bioenergy? We hear much about supply, security and the environment, and many hope that any given venture, investment or technology can solve all three. In fact, such problems usually have to be tackled independently. Reducing dependence on foreign oil is one problem we can tackle aggressively through renewable fuels. Regarding environmental issues, available biofuel approaches have some benefits, but are not necessarily the be-all and end-all. It is important to realize that there is ability to segregate these opportunities.

Clearly, the venture-capital industry has been investing heavily in the energy space, very broadly including cleantech. In 2007, 9% of all venture investment went into the cleantech space, driven largely by increased demands for biofuels. In making investments, we look for fundamental aspects that indicate that a company offers solutions that will last and make a significant difference—production of a fuel, substance or something else that will compete with what is already on the market without requiring a subsidy. Intrinsic to that, operation at scale is essential. Many interesting technologies operate well in the laboratory, but do not translate to a fully operational company. Furthermore, they are looking for people who want to change the way things are. Flagship Ventures has been spending a lot of time in the biofuels arena. In their view, a handful of generations have occurred in this space. In generation one, they characterized the corn-to-ethanol players. Generation two has involved thinking about the diversity of what can be made, including the diversity of feedstocks, in ways to make significant differences in cost and environmental benefit. In early 2006, they invested in Mascoma at which the cellulose-to-ethanol process was being simplified to a one-step fermentation. Mascoma is now commercializing its process at multiple sites throughout the country.

Beyond ethanol, some fundamental questions are begged. If we could make any biofuel, what would it be? With the development of appropriate technology, can it be made and, if so, is it scaleable? With the development of synthetic biology, the next generation of biofuels is beginning to emerge. Combining traits from several sources in a single organism, with a given input desired output becomes possible. This is the basis on which LS9, Inc. was established: why not make petroleum? It's already in use, has high energy-density and other advantages. Examining how nature stores energy and fundamental aspects of the biology of the cell, a pathway may be produced, utilizing a renewable feedstock such as glucose, to produce hydrocarbons at high efficiency. Only with high efficiency can the approach be economical. Using this as a platform to "tailor" molecules, a range of opportunities emerges in the petroleum space, not only for fuels but also for chemicals.

Another advantage of producing petroleum accrues from reduced separation costs. For several biofuels, the separation cost is ~30%, whereas producing something that naturally separates from water reduces this cost and adds to its competitiveness. Not knowing what the price of oil will be in the future, Flagship Ventures uses a baseline of $45 per barrel, to appraise competitiveness of an unsubsidized biofuel. Accordingly, they see the LS9 technology as realistic.

A fundamental limitation of biology is its intrinsic variability. Mutations occur spontaneously and it is difficult to predict how much a gene will be expressed. Therefore, with any new biological process, there is a degree of unpredictability. One opportunity that takes advantage of this is the development of new catalysts that take, for example, carbon monoxide and carbon dioxide under low-cost reaction conditions to produce common everyday polymers. The advantage is in rapidly producing a range of highly desirable, specific products of use in several opportunity spaces. For example, when produced biologically a single polyhydroxyalkanoate is obtained, whereas, when produced chemically a range of discrete PHAs may result, each with unique properties with particular market opportunities such that products can be tailored to consumer needs. This technology, commercialized by Novomer, uses carbon

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monoxide and carbon dioxide as feedstocks, thus benefiting the environment while reducing need for petroleum.

Feedstocks constitute another opportunity. As mentioned, separation costs 30%, and feedstock cost was also 30% (and fermentation 40%) until the price of corn rose recently. The corn pricetag is a key cost driver, and some corn-based ethanol plants are now working on unattractive margins. As we think of future technologies, the feedstock factor is important. Opportunities exist to obtain lower-cost feedstocks from abroad, for instance from Brazil and China. But one of the objectives is to reduce dependence on resources from foreign countries. It's important that we in the United States focus on lowering our own costs.

Realization of the original optimism in this field has proven to be illusive. At the rate at which the current economy is growing, it is unlikely that the biobased economy will be self-sustaining. Better understanding is needed to optimize biology—making the inputs, outputs and processes as efficient as possible—to meet our eventual needs. This raises the question of whether low cost is really the next frontier. Early on in the petroleum industry, much of the development evolved as a result of investigation of what products could be made. Yet, today, the value of the petroleum industry is not controlled by the refineries but by those who own the wells. A similar distribution is happening in the bio-industrial space; it will soon emerge that the controller of the feedstock will control the value chain, which will present opportunities. JOULE Biotechnologies was recently founded, based on some fundamental innovations in synthetic biology and bio-industrial engineering, with the emphasis on breaking through the feedstock bottleneck and making low-cost domestic biofuels a reality.■

A Taste of the Future: Innovation in Food Ingredients and Flavorings

Kent Snyder (Senomyx)
Tjerk de Ruiter (Genencor-Danisco)
Pradip Mukerji (Abbott Laboratories

Kent Snyder opened with the observation that the role of diet in human health, *e.g.* removal of certain soft drinks and fast foods from schools to combat childhood obesity, are subjects commonly covered in the media. There is no recommended daily allowance for sugar intake, but it is generally thought that 40 to 50 grams—about 10 teaspoonful—is sufficient. The average American consumes ~150 pounds of sugar per year (~170 grams per day); a 12-ounce can of soda contains 40 to 50 grams of sugar. There is growing concern not only about obesity, but also its link to diabetes, arthritis, heart disease, stroke, and certain cancers. The US recommended daily allowance for salt is 2,400 mg per day, although it is thought that the average adult needs only ~500 mg per day. A turkey breast sandwich alone is likely to contain ~500 mg of salt. There is a link between sodium intake and hypertension and heart disease.

Food and beverage companies are trying to address these concerns by offering low-fat chips, diet soft drinks and low-sodium soups. However, at Senomyx they feel that this will have only limited benefits. They are borrowing from biotechnological approaches used by pharmaceutical companies, using proprietary screening technologies that are based upon human-taste receptors to discover and develop flavor enhancers and taste modulators. Six programs are being pursued.

In the savor-enhancer program the objective is to reduce or replace added MSG, and also to create new savory flavors by combining compounds with MSG. The sweet-enhancer and salt-enhancer programs aim to reduce the amounts of added sugar and salt in products. They have a bitter-blocking program, to improve the taste of some healthy foods, and a high-potency sweetener program and a cool-agent program to provide ingredients that don't have the limitations of currently available agents.

The savory flavor ingredients, discovered and developed in-house, are marketed by Nestlé.

They are not involved in any genetic engineering.

Humans have a single sweet receptor, and a single savory receptor and about twenty-five bitter receptors. It is thought that only one primary receptor is involved in salt-taste reception, similarly for sour-taste reception.

Taste-buds are located in three places on the tongue detecting sweet, salty, bitter, sour and umami (savory). In the taste-bud, each taste has a specific cell on top of which is a receptor (ion channel) which, on contact with salt or sugar, *etc.*, sends a signal through the taste-bud to the brain. The enhancer binds to a site separate from that of the receptor, activating the receptor, sending a stronger signal to the brain. Thus, the amount of sugar, for example, may be reduced—making a product more healthful—yet the enhancer increases the "sweet" signal to the brain.

Starting with isolated taste-bud cells, they build an assay around the sweet-receptor cells and through robotics and high-throughput screening, they can examine hundreds of thousands of compounds and identify those that interact with the receptor. These are optimized for potency or to improve physical characteristics then moved into taste tests, through product development and the regulatory process to commercialization.

In the sweet-enhancer program, the objective is to decrease the amount of sweetener by at least a third, while incurring no taste effect of the enhancer, which is used at a low level. Key discoveries have emerged from this program. Enhancer S2383 is specific for the high-potency sweetener sucralose, allowing a 75% reduction while maintaining sweetness. GRAS approval is expected within the year, and internal and external evaluations of product prototypes are in progress. A future target is fructose reduction.

The key objective in the salt-enhancer program is to reduce salt in products by 33% yet maintain desirable taste. The key discovery is SNMX-29, believed to be the receptor involved in salt-taste perception.

Receptors and ion channels for the following remain to be discovered: fat taste; mouth feel; metallic taste; and astringency. And further issues to be addressed include: role of genetics in food preference; loss of smell and taste with aging; and loss of taste-perception in cancer patients.

Danisco's acquisition of Genencor in 2005 brought together leaders in industrial biotechnology and food ingredients, making them uniquely placed in the food industry, according to **Tjerk de Ruiter**. Their product portfolio is built around textural ingredients (thickening, gelling and stabilizing agents), emulsifiers, active ingredients (cultures), sweeteners, protective ingredients (antimicrobials, antioxidants), and enzymes. They are unique in the food industry in having a technology platform that brings together food applications and understanding of nutrition and biotechnology with production capability. They can work from molecule to metabolism.

Trends affecting the food industry include safety, health and wellness, socioeconomic aspects (in developing countries—is the root of the food-versus-fuel issue because of corn or because India and China are growing faster than the food supply?) and the market. On the last aspect, the food industry is unusual in that, although there are large corporate players, most of their customers deal with local markets and local taste preferences. This fragmentation makes the food industry difficult. However, Danisco provides Genencor a global network with customer proximity, understanding of local applications in taste and texture and ability to bring products rapidly to market. A single product is insufficient; a range of products adapted to each local market is needed for success.

Genencor's areas of focus are underpinned by the philosophy of "sustainable value creation": health and wellness, and food safety. They define sustainable solutions for value creation not only in terms of cost reduction but in new opportunities that allow the customer to differentiate the product. They continuously look for opportunities to improve the quality of the eating experience. Quality improvement from the industrial point of view often means improving longevity, so that taste is not lost during transportation. An increasingly prevalent objective is to devise "green" solutions.

Their recently launched product, GRINDAMYL POWERFresh, is an enzyme that provides an example of value-creation. It helps the customer extend the shelf-life of bread and has the unexpected effect of improving the flexibility of tortillas. Sometimes, by understanding local needs, new applications are discovered.

An enzyme for degumming edible oil provides another example of value-creation. Not only does this enzyme improve the yield of the oil-extraction process and reduce levels of unwanted compounds, it improves the phytosterol content for added health benefits.

A few years ago, discussion of health and wellness often focused on fats; the Atkins diet is a good example. Many large companies now view health and wellness as an opportunity to differentiate themselves. Nestlé representatives now talk about their nutritional strategies, which represents a significant shift eliciting confidence that health and wellness is an attractive trend rather than a fad.

Where should the health-and-wellness priority be focused? Of the many priorities that may be identified—digestive health, weight management, healthy snacking, *etc.*—enzymes can play multiple roles.

Although food scares regularly make headlines, transient sickness probably results from spoiled food more often than is thought, with the problem under-reported. At Genencor they are putting together a range of products that moves away from "traditional" chemicals to more natural solutions. Their Care4U portfolio ranges from natural extracts, such as GUARDIAN products—extracts from rosemary and tea—to specialized milk fermentates, Natamax and Nysaplin, and their enzyme range. These allow their customers to devise customized solutions for their food-safety issues.

Abbott Laboratories' recent key innovations include Juven, a protein for AIDS, cancer, *etc.* patients to prevent muscle loss, Glucerna, a carbohydrate to treat diabetes, and Oxepa a tube-fed fatty acid for critical-care patients, according to **Pradip Mukerji**.

Innovative research at Abbott is driven both by consumer need and by science. All targets are based on clinical research. Their in-house segments comprise process development, analytical research and services, strategic discovery and clinical research, clinical operations and package development. They keep in mind that their business is about the science of combination not the science of separation, as is more familiar for pharmaceutical and other divisions. They take protein, carbohydrate, lipid, vitamins, flavonoids and other ingredients and put them together, based on discovery science, and test them in clinical trials, being cognizant of the fact that these are not sold in capsules or tablets, so flavor and texture are modified for consumer appeal, and package development is required to ensure appeal in the target country.

Focusing on specialty oils as ingredients, Mukerji dealt with two aspects: therapeutic and economic. Regarding polyunsaturated fatty acids, people are familiar with fish oil and omega-3, but less familiar with arachidonic acid, which we get plenty of, derivatives of which are pro-inflammatory or cause aggregation of platelets, neither of which is beneficial. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which counteract the effects of the arachidonic acid, are obtained from fish oil or from vegetable oils that contain linoleic or gamma-linolenic acid (GLA) by the action of desaturases or elongases in the body.

Patients critically ill through trauma, sepsis, burns, *etc*., have less ability to utilize vegetable oils to produce EPA and DHA. At Abbott they are examining the possibility of using fatty acids to counter this scenario. They theorized that two fatty acids in combination—GLA, a precursor of arachidonic acid, and EPA, a component of fish oil—would act synergistically. To prove this, they examined patients with acute respiratory distress syndrome (ARDS)—which can result from a variety of situations—a condition with a 40% mortality rate, to test whether nutritional treatment could be beneficial: can a combination of GLA and EPA, with antioxidants, modulate pulmonary inflammation? In a randomized, double-blind, multi-center study with a placebo control, this formula shortened ventilator time, reduced pulmonary inflammation, increased oxygenation, reduced new organ failure, and improved clinical outcomes. After 28 days, survival of the experimental group was 67%, compared to 47% in the control group, on top of all the drugs, *etc*, usually administered.

They want to extend this technology for use in products for the general consumer, but maintaining profitability is more difficult in the retail market. Therefore, they chose to use bioreactors as sources, either via fermentation—the bricks and mortar approach—or using crop plants.

With respect to the latter, oil-producing plants (safflower, sunflower, *etc*., which contain a lot of linoleic acid) engineered to synthesize desaturase cloned from a fungus that produces GLA, provide a one-step method for production of GLA. Current sources, borage and evening primrose, provide this product at $20 and $5 per pound, respectively. With the bioreactor, 60% of the seed dry weight is GLA, taking advantage of the high content of the precursor (linoleic acid at 80%) in the safflower.

EPA can be produced in soybean, which has a high content of α-linolenic acid, engineered to produce several new enzymes.

Results from field trials are promising, and retail-market entry is expected in a couple of years.■

Views from the Top: CEO Perspectives on the Evolution of the Industrial-Biotechnology Value Chain

Richard Hamilton (Ceres)
Jeff Broin (POET)
Steen Riisgaard (Novozymes)

Richard Hamilton described the work at Ceres on dedicated energy crops—low-carbon, non-food feedstocks for biofuels. An advantage of dedicated energy crops is that they allow biofuel production at scale. As refining technologies are improved for cellulose conversion and commercial speed is reached, there will be a transfer from corn-kernel to cellulosic feedstocks.

The recent US Department of Energy's "billion ton report" stated that a billion tons of biomass may be obtained annually from forestry and agricultural residues and (about a third) from perennial crops. That report assumed that perennial yields would remain static, whereas Hamilton suggested that there is much room for improvement in energy crops, which is their objective at Ceres. Technologies developed for the Human Genome Project—sequencing, micro-arrays, functional genomics, *etc*.—are being applied to plants. In conjunction with Monsanto they are working with traditional row crops, *e.g.* corn and beans, and are independently taking that technology and applying it to switchgrass, miscanthus and sorghum.

Hamilton opined that genomics applications can have significant impact on these feedstocks, by expanding their environmental adaptation, by increasing biomass yield in tons per acre, by reducing fertilizer and pesticide inputs, and by improving composition and conversion for more gallons of biofuels per ton of biomass. Furthermore, ease of processing may also be improved for lower capital costs. And with funding from the US Department of Energy, in conjunction with Rohm and Hass, they are developing plastic precursors as co-products, also employing genomics.

Biotechnology and breeding, especially marker-assisted breeding, are employed in conjuction, as they are with corn. It takes 7 to 10 years to produce a new variety, so the time to get started is now.

A number of genes have been identified that affect plant biomass. Much of Ceres' gene discovery work has been done in *Arabidopsis*, then examined in rice in high-throughput field trials to examine function in a monocot species. The genes are then moved into switchgrass and miscanthus where there is high likelihood of beneficial effects.

Not all biomass is the same in terms of convertibility. Different feedstocks require different enzyme treatments to maximize sugar release. Differences exist even among cultivars of switchgrass. A priority is to identify "melt in your mouth" types that are easy to process.

Traits beneficial to other crops have been found to "plug and play" in energy crops such as switchgrass. Genes for drought tolerance add to switchgrass's ability to withstand moisture deficiency; thus, high yields will be possible on lands that are marginal for food production.

The 2007 Energy Act set out a higher renewable fuels standard (RFS) of 36 billion gallons of biofuels by 2022, and it's time to put steel in the ground. The United States is ready—not only on the refining technology side but also on the feedstock side—to get on the learning curve and move this technology towards commercialization.

Although some see energy crops as something that will be needed in time, when the industry is up and running, at Ceres they see a role in the early stages. Some ask, "If I'm going to erect a biorefinery, where should it be?" To this, the corollary question is, "How do I figure out the yield potential for any given geography?" The fact is, few data are available for switchgrass or miscanthus or high-biomass sorghum in terms of environments in which they will thrive. This is why Ceres is conducting extensive field trials nationally and internationally. Understanding yield potential for a particular geography is critical for determining where a biorefinery should be located and how large it should be. It's not too early to be thinking about biomass, because long-term contracts will be in effect.

Hamilton expressed pleasure in announcing that Ceres' first commercial product would be launched in the fall, for planting in 2009: Blade Energy Crops. Its premium seeds and traits—a combination of switchgrass and high-biomass sorghum—will produce feedstock for biofuels and biopower.

The production of high-yielding varieties of switchgrass has been in conjunction with the Noble Foundation. In Ceres' 2007 trials, some varieties yielded over 10 tons per acre with low-input requirements. Lowland varieties will be available commercially in 2009.

High-biomass sorghum can provide over 15 tons per acre of dry biomass in central Texas in 90 days. In conjunction with Texas A&M University, even higher production of biomass is expected. This robust annual, grown from seed, gives growers a great deal of flexibility. No long-term field commitment is involved, it can be rotated with other crops, and its input requirements are less than for corn.

Ceres is part of the US Department of Energy's farm-scale "seed to pump" venture with the new ICM, Inc., biorefinery near St. Joseph, MO, providing switchgrass and sorghum germplasm to local growers.

Several business-model options are available for feedstock production, not necessarily the same as that for corn. Some biomass growers will form arms-length relationships with biorefineries, whereas others will have fully integrated long-term contracts. Predictable, sustained delivery of biomass will be essential for biorefineries, particularly if the feedstock has been improved to work well in a particular conversion process. Intermediate business models are possible also.

Whether conversion to sugars is by thermochemical or biochemical means, biomass is a common denominator. With the rise in the price of natural gas, there is increased interest in converting biomass to methane. And biomass may be pelleted and burned directly with coal to provide power. Since December 2007, China's need for coal has outstripped its domestic supply and coal prices have increased significantly. If we put a carbon tax on top of that, the economics of biomass to power become very interesting indeed.

"What will the biorefinery of the future look like?" asked **Jeff Broin**. Current biorefineries use grain as feedstock and most are powered by natural gas and some electricity. The main product is the high-performance transportation fuel ethanol, which is blended into gasoline to increase octane, reduce pollution and help reduce the nation's dependence on foreign sources of energy. The starch in the grains is used for ethanol production, whereas protein, fat and micronutrients remain intact as distillers grains—the chief co-product—used as animal feed. The third product is carbon dioxide, which is captured at some plants for beverage carbonation, food processing, water treatment, *etc*. Thus, biorefineries have worked for several decades, with improvements in technology over the years increasing yields and reducing energy usage. High-gravity fermentation has reduced energy inputs, and POET's BPX raw-starch hydrolysis process, which converts starch to sugar without heat, reduces energy and water consumption while increasing ethanol yields. Such innovations continue to make corn-based ethanol the most viable alternative to fossil-based transportation fuels. A study at the Argonne National Lab that compared ethanol plants, 2006 versus 2001, revealed that ethanol yields per unit of corn increased by 6.4% for dry-mill plants while energy usage decreased by 22% and water consumption decreased by 27%.

Tomorrow's biorefinery will accept new feedstocks. In addition to grain, other renewable materials will be delivered: wood chips, crop residues, and even trash. Initially, these materials may be used to displace some of

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the fossil fuels used for power. POET's plant in Chancellor, SD, provides a good example: a solid-waste-fueled boiler is under construction. Starting in August, 2008, they will take delivery of up to 350 tons of wood waste per day, and the steam created from burning will displace more than half of the plant's natural-gas needs.

POET has signed a contract with the city of Sioux Falls, SD, to install a pipeline from the local landfill to transport methane to the Chancellor plant, which has the potential to eventually displace the rest of the natural-gas usage, making it essentially fossil-fuel independent.

Grain-based ethanol has come in for severe criticism. Yet, consideration of the facts shows that it has been a very good thing, and will continue to improve. Whereas gasoline requires 1.2 units to create 1 BTU at the pump, grain-based ethanol requires 0.67 units of fossil energy to create 1 BTU at the pump (based on 2001 dat); and cellulosic ethanol is expected to require 0.1 unit to create 1 BTU at the pump. The data from the recent Argonne study show that fossil-fuel needs to produce grain-based ethanol have dropped significantly, and with alternative fuel sources for plant operation, the gap between gasoline and grain-based ethanol will be increased further.

We need to understand that when land that could produce energy sits idle, we will never get that energy back—we are literally stealing it from future generations.

The biorefinery of the future will also accept cellulosic feedstocks for ethanol production, anything from wheat straw to orange peals to trash. POET is participating in the US Department of Energy's Project Liberty, converting a grain-based ethanol facility in Emmetsburg, IA, to one that will accept grain and cellulosic feedstocks. Construction was slated to begin in 2009, with the primary source of cellulose being corncobs. Construction of a cellulosic pilot plant was expected to begin in May, 2008, in Scotland, SD.

Broin stated his expectation that cellulosic ethanol will become a commercial reality within a few years, contributing to the US goal of 36 billion gallons of renewable fuels annually by 2022.

The biorefinery of the future will have many more inputs than today's. Outputs will continue to be ethanol, distillers grains and carbon dioxide, but, most certainly, there will be additional products. Oil can be extracted from corn grain and used for biodiesel production, which is already being done. Value-added proteins can be produced commercially from corn, for use in feed, food supplements and industrial products. Chemicals—household cleaners, paints, solvents and even polymers—will come from tomorrow's biorefineries. Production of many of these is made possible via Bfrac, a POET-developed process that separates the corn kernel into fiber, germ and endosperm, allowing each to be converted into high-value products.

As fossil fuels become scarce and more expensive, the US economy is changing from one based on hydrocarbons to one based on renewables. Many products synthesized from petroleum will, in the near future, come from biorefineries. For many centuries, our world was driven by agriculture. Then came the industrial revolution and then the information age, and now the renewable revolution. In many ways, as a society, we are returning to our agricultural roots. In addition to harvesting crops for food, feed, and fuel, farmers will harvest biomass also. We will see advancements in productivity and yields of these biomass crops, and farmland that has sat unproductive for decades will be used to produce fuel, chemicals, *etc*. In this new age, value will be placed on sustainability.

We, in this industry, are in the right place doing the right things for our country and our environment. These are exciting times and the opportunities are tremendous. As John F. Kennedy said, "The problems of the world cannot possibly be solved by skeptics or cynics whose horizons are limited by obvious realities. We need men and women who can dream of things that never were." The *status quo* is not an option—we need a revolution. A renewable revolution.

Steen Riisgaard suggested that the first part of the Kennedy quotation may be applied to European politicians; still, there are people in Europe who can dream.

After a long debate in Europe, the Commission and the Heads of States agreed that biofuels may be a useful tool, with emphasis on greenhouse-gas reduction rather than on energy security. A directive states that, by 2010, biofuels should contribute 5.75% to total transportation-fuel consumption. However, only Sweden is likely to achieve this goal; average EU-country usage of renewable fuels stands at 1.4%. Achieving the goal of 5.75% has become even more unlikely with increased grain prices—making it difficult for European biofuel producers to be competitive—concern over environmental benefit of biofuels, and the food vs. fuel debate.

Nevertheless, a proposal supported by the Heads of States is on the table: by 2020, there should be a mandatory target of 10% biofuels. Environmental concerns are addressed in terms of biofuels delivering a 35% life-cycle carbon dioxide emissions gain over conventional fuels.

A number of pilot plants are under construction—small in comparison with their US counterparts—for most of which the feedstock will be wheat straw. Funding is poor and scattered at the national and EU levels. Riisgaard predicted that cellulosic ethanol will enter the market in the United States at approximately the same time as in Brazil and China and before Europe.

Nevertheless, several European companies are operational in this space, including Novozymes.

Novozymes has a unique global effort—at several sites—developing enzymes for conversion of cellulose to ethanol, including partnerships with POET and ICM.

The effort is partially funded by the US Department of Energy. They expect that, by 2010, commercially relevant enzymes will be available at commercially relevant prices, for conversion of corncobs and stover and of bagasse for Brazil.

They plan to build on the sugar platform so that products will include building blocks for the chemical industry, as pioneered by DuPont in conjunction with Genencor-Danisco, Cargill and NatureWorks, *etc*. The objective is to compete with products synthesized by petrochemistry. Although this becomes easier as oil prices rise, it remains a challenge since "traditional" chemical synthesis has been improved over a long period of time. In a recently formed partnership with Cargill, they will use renewable resources to produce acrylic acid, which has applications in superabsorbers, fibers, coatings, adhesives, polymers, *etc*.■

Industrial Biotechnology: Sustainable Climate-Change Solutions

Steve Koonin (BP)
Mauro Gregorio (Dow Chemical)
Michael Walsh (Chicago Climate Exchange)

Steve Koonin began by stressing that if you want to store energy in a dense, easily transportable form, the best choice is liquid hydrocarbons. It's hard to see liquid hydrocarbons disappearing from transportation for many decades. With that conclusion, what do we do regarding the problems that liquid hydrocarbons bring with them, notably security of supply and greenhouse gasses associated with their production and use. In Koonin's opinion, as a physicist, the fundamental question is "Where do we get our carbon from?" because the chemists, the chemical engineers and the biologists have wonderful processes for "decorating" carbon with hydrogen or oxygen. A variety of carbon feedstocks is available—natural gas, coal, biomass, petroleum, *etc*.—for production of fuels, chemicals and power. Globally, about 2 gigatons of carbon are consumed as transportation fuels per year, from crude oil. What other carbon sources might be tapped for additional road-fuel use? There's plenty of carbon in coal. Annual global use of coal runs at 5.3 gigatons, and there's plenty more where that came from. China is increasingly using coal to drive transportation; it is converted into methanol, which works well in many internal combustion engines. Much discussed also is Fischer-Tropsch conversion of coal into diesel as practiced in South Africa, for example. Another fossil source of carbon is natural gas, but it's already in relatively short supply.

The trouble with fossil fuels is that getting them out of the ground and using them emits carbon dioxide. Considering corn as a source of carbon, if all of the corn in the world were converted to ethanol with a 100% efficiency, its maximum contribution would be 15% of transportation fuels. Therefore, to access a significant amount of carbon beyond petroleum, either it must be dug out of the ground or obtained from biomass, which is a powerful rationale for biofuels.

The key issues regarding biofuels are cost, compatibility with existing infrastructure and vehicles, land availability after addressing food needs, and patterns of land-use globally. A third of the world's land is non-arable, 11% is used to grow cereals and other crops and 55% is in pasture, prairie, savannah and forest. It appears that there is plenty of land.

Environmental sustainability is also important. Lifecycle carbon dioxide emissions relative to fossil fuels, agricultural practices, water use, nitrogen use, ecosystem diversity, *etc*., are germane. And there is the perennial question of energy balance, particularly for corn ethanol. Most studies suggest that "a bit more" energy is obtained from ethanol than is invested.

To do a good job with biofuel production, we must make what is currently an unnatural fusion between the petroleum and agricultural value chains. The petroleum value chain, which involves exploration, extraction, and moving and distributing it has evolved and been optimized over the past 150 years to a fraction of a percent. The agricultural value chain, which involves crops chosen, cultivation, harvesting and distribution has also been optimized, over a couple of millennia. Needs for the biofuel value chain raise interesting issues: the plants grown, yield, structure and composition of the cellulose, farming practices, harvesting, transportation of harvested material, cellulose processing, and the final product.

As a non-plant person, Koonin expressed amazement at the achievements of food-crop breeders. Comparable genetic improvements in energy crops are possible. With respect to farm practices, pest damage will be much less important than for food crops. The product may be a slurry, *etc*., for transportation; much opportunity exists for innovation in such practical aspects.

And then there is the issue of the final product. Ethanol has been made for millennia. It has wonderful uses, but it does not predominate as a fuel. It has only 70% of the energy of gasoline, it absorbs water and it's corrosive. As the carbon number increases, up to a point, fuel quality improves. Butanol is better than ethanol (four carbons instead of two), and an octane, perhaps 2,2,4-trimethylpentane, would be about optimal as a gasoline substitute. However, the difficulty

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in biological production of these fuels generally increases with their complexity. There is a crossover somewhere, and biotechnology may be employed to optimize the process and the product.

Several years ago, BP, in conjunction with DuPont, initiated a biobutanol project. Butanol has ~90% the energy density of gasoline and it's not corrosive. They are making progress towards commercial production of biobutanol by fermentation. In Koonin's opinion, this is only the beginning of what should be possible.

Biology is the most rapidly developing of the sciences and is likely to remain so for several decades. Novel technologies spring most readily from fields in which the science is moving rapidly. Without doubt, biology *is* generating disruptive technologies.

About 80% of the world's energy is based on carbon—the fossil fuels coal, oil and natural gas—and another 10% is based on traditional uses of biomass. At the same time, all of life is based upon carbon; those fossil fuels were once living organisms and nature has had 3½ billion years of evolution to optimize how she deals with carbon. Some tricks were learned and we need synergies between what we can do and what occurs naturally.

Most of the funding for, and applications of, biotechnology have been biomedical. Despite the size, scale and breadth of interest of the Biotechnology Industry Organization, most biology is driven by biomedicine; investments in agriculture, biomaterials and biochemicals have been much smaller. There's a lot of "white" space to turn biology and biotechnology to energy and other needs. Potential applications include plastics, hydrogen production and enhanced oil recovery. The world captures only 35% of the oil known to be in the ground. The rest is held in pores. Microbial enhancement of oil recovery has been discussed for decades, and Koonin opined that it will become a reality as the price of oil rises and the biology gets better. Carbon sequestration—biological methods for pulling carbon dioxide out of the atmosphere to deal with climate-change issues—is another possibility. Bioremediation, capture of methane from coal beds, and biological processing of crudes are also possible applications.

To pursue some of these, in December 2007 BP established and brought into operation the Energy Biosciences Institute, a dedicated research organization examining application of biology and biotechnology to energy issues. The partnership involves BP, the University of California at Berkeley, the University of Illinois at Urbana-Champaign, and Lawrence-Berkeley National Laboratory. Their hope, to pursue both open basic research and applied proprietary research within the same organization, is novel. The initial focus is on the entire biofuel-production chain, from the germplasm through to the fuel molecule, and significant funding will be applied to the examination of ecological and socioeconomic impacts. It's a half-billion dollar commitment in funding over 10 years, bringing together BP, academia, biotechnology firms and the government to make a coherent push. This kind of arrangement, in Koonin's view, will be increasingly important as we focus on the major problems that the world is facing related to food, water, energy and the environment.

Mauro Gregorio opened his presentation by stating that commitment to sustainability underpins everything they do at Dow Chemical. Since 1990, they have reduced their greenhouse-gas emissions by 20%, significantly better than the Kyoto-Protocol targets. They have reduced their energy use by 22%, representing 900 trillion BTUs, equivalent to California's annual energy use. This required an investment of $1 billion, but the savings represented $4.4 billion. This effort is on-going. They are developing improved Styrofoam-like construction materials, lighter plastics and photovoltaic roofing for use in homes and businesses to generate electricity. They are applying the power of chemistry to create less energy-intensive methods for synthesis of high-volume chemical feedstocks such as ethylene and propylene.

One such project in Brazil is the first fully integrated industrial scale polyethylene complex to use a renewable feedstock, *i.e.* ethanol derived from sugar. Synthesis of polyethylene from ethanol has been possible at small scale for many years, but the challenge has been in scale up to industrial capacity, which is now being launched. Dow is a leading company in technology development, production and commercialization of polyethylene, with a strong presence in all continents. They are the largest producer in Latin America and this project will allow participation in the growth of that region. In conjuction with the sugar producer Crystalsev, they are using sunshine, air and rain to produce plastic.

Polyethylene is a component of diapers, food packaging, trash bags, *etc*. They start with a typical fermentation of sugar to produce ethanol, which is dehydrated to ethylene for conversion to polyethylene as in the "traditional" process. Polyethylene from sugar cane requires significantly fewer fossil resources, because most of the carbon comes from carbon dioxide in the atmosphere.

An important consideration is that the entire complex will be energy self-sufficient, since the bagasse will either be converted into energy or used as fertilizer. Each ton of polyethylene produced removes 2.1 tons of carbon dioxide from the atmosphere; in contrast the traditional method puts 1.8 tons of carbon dioxide into the atmosphere. This will be the first fully integrated, renewable-chemistry plastic plant, bringing benefits that reflect Dow's sustainability goals.

Dow's customers need make no equipment adjustments to accommodate the polyethylene from renewable sources, but it provides them a competitive advantage. The operation is located in the southeast of Brazil, far from the rainforest, where many sugar cane plantations are already in operation and which is being developed with the highest standards of environmental protection. There will be more than 3,200 employees, which will help to stem emigration.

Mechanical harvesting means that there will be no burning of leaves. The power produced from the bagasse will be more than required for plant operation and will be sold to support the local grid, sufficient to power a city of close to half a million people.

Other areas of focus for Dow include fermentation, natural oils, glycerine, syn gas conversion, cellulosic ethanol, bioengineering, new routes to olefins, sugar chemistry and methane activation.

Eight years ago, **Michael Walsh** and colleagues came to the conclusion that market-based cap-and-trade-style mechanisms would be the preferred tool on an international basis for managing emissions of carbon dioxide and of other greenhouse gases. They recognized that governments were having a hard time doing the necessary groundwork, so they sought out those in the private and public sectors who wanted to start to build a voluntary framework from the bottom up to find answers as to how to establish a cap-and-trade system for all sectors, integrating all mitigating options.

They activated a voluntary cap-and-trade system whereby participants who commit to reducing their emissions execute legally binding contracts. The contracts and the rules and standards are subject to external independent verification. From the outset, they felt that integrating solutions for all of the greenhouse gases would be the optimal *modus operandi* for solving the problem at least cost and for achieving near-term solutions. Immediately they were advised by members that a “US only” approach would be short-sighted, therefore they adopted a NAFTA approach and quickly learned that corporations and public enterprises around the world wanted to participate immediately, to be ready and to help drive the legislative and regulatory processes.

The Chicago Climate Exchange has spawned a sequence of exchanges—commodity market mechanisms—around the world. They activated the European Climate Exchange to host trading and regulated futures markets for the European Union emission allowances and the international instruments established under the Kyoto Protocol for mitigation projects in developing countries. They also host futures trading for the conventional pollutant-reductant trading systems—sulphur dioxide and nitrogen oxide reduction-trading programs here in the United States—which have had enormous success in driving down emissions at least cost. That’s what this is about.

A massive global investment—trillions of dollars—in mitigation efforts is required. The question is how the market is to be harnessed to get the maximum bang for the buck, to keep the economy strong and living standards improving yet reducing emissions. These are challenging goals. On May 30, 2008, at the Montreal Climate Exchange, they plan to offer their exchange services—low-cost, rules-based transactions with transparent pricing—wherever there is such a need globally.

In the North American context, they invited involvement and received an encouraging response, initially with fourteen members, including major utility and agrichemical companies. Seventeen power companies have now joined the Exchange, including one in Australia. Diverse entities are now members, including governments and states.

The quantification and verification challenges are, by and large, surmountable. Many of the inexpensive reductions of greenhouse-gas emissions are other than carbon dioxide: methane, fluorocarbons, nitrous oxides, *etc*.

They have opened up the process, so that city governments such as those of Chicago, Portland, and Melbourne, Australia, can lead by action rather than rhetoric. A significant number of forest-product companies are involved, including Brazilian and Chilean firms, taking on the legally binding, independently verified emission-reduction commitment. These are long-term-thinking enterprises that recognize that biofuels open up a range of new opportunities.

Universities—Michigan State, Minnesota, Oklahoma, *etc*.—have campuses that operate like small cities with their own power plants, and they wanted early involvement for the learning opportunities. Walsh suggested to the audience that if they were not already active in this space—membership in the Chicago Climate Exchange is one route—then their companies were at risk, because the rules were being written; a 300-page piece of nuanced legislation is in the pipeline, which they will either understand through direct experience or they will become a “victim” thereof.

More emissions are covered under the Exchange’s legally binding reduction commitment than in any country, including Kyoto signatories such as Germany and the United Kingdom. About half a billion tons equivalent of carbon dioxide are under a cap, primarily in the United States and Canada. Including the caps that are in place in the European Union, the total is about 2 billion tons. The current market value of the European allowances is ~$35 per ton, therefore a $70 billion crop of emission allowances is being distributed and consumed each year. Even in these early days, this is more than the annual value of soybeans, corn and wheat in the United States. In time, the US program is likely to be two to almost three times the size of that of the European Union. Canadian, Australian, Japanese and other systems are developing. These are significant economic propositions, presenting opportunities for those involved and some risk for those unprepared for what is coming. For other mandatory regional programs being discussed or prepared in North America, there is a question as to whether there is sufficient mitigation-cost diversity. Without such diversity, cap and trade becomes a management system, but not necessarily a cost-reducing system. The emission cuts for the years 2003–2006, the first phase of operation of the Chicago Climate Exchange, exceed the total emissions that will be included in the regional programs.

They started with an emission baseline for the years 1998–2001, and agreed to a 1% cut per year, so that by the year 2006 the members had to be, in a verifiable manner, more than 4% below their baseline. Those who find it expensive to make the cuts internally are allowed to trade with someone else in the system who has made an extra cut or who has completed an eligible mitigation project, such as a methane catcher or a reforestation venture, in order to achieve the goal in the macro sense at minimal cost to the consumer.

About halfway through the first phase, the members suggested continuance, with the objective of 6% below baseline by the end of 2010. They have been criticized for aiming for only a 6% cut by 2010, yet in comparison with legislative proposals under consideration in Washington and being activated elsewhere in North America, they are far ahead. This was enough to initiate the process, and to get the systems built to determine a price for carbon.

Reasons for joining the Exchange range from altruism, through concern over climate change, to strategic business advantage, opportunities to shape legislation and risk management. Some said that if they didn't become proactive for specific programs they would be failing in their commitments to manage obvious risks. Many members simply believe in the need to find solutions to mitigate climate change.

They have many "microparticipants" who are providing packages of mitigation projects, such as agriculture and forestry ventures, and traders providing investment liquidity, *e.g.* the Iowa Farm Bureau, the North Dakota Farmers Union and the Environmental Credit Corp. Senator Lugar of Indiana is a supporter and is keen to show that agriculture can be part of the solution.

To maximize mitigation options, one has to go beyond the corporate boundaries to find and bundle small projects. The Intergovernmental Panel on Climate Change has identified three dozen viable mitigation options—no new technology required, of low cost and needing only market penetration—but of which about half need to be activated, *e.g.*:

- Who can "kill off" methane emissions from coal mines, landfills and agricultural facilities?
- Who can use land under forest to absorb carbon?

A 500-page rulebook governs the system, setting the standards to provide the verification goals for the carbon-emissions program. Yet, offsets have gotten a bad name; there is a misperception that neither standards, rules nor procedures apply. In fact, the Chicago Climate Exchange employs the most respected verification companies in the world, following codes and standards via 200 pages of guidance in their rulebook, which is a supplement to the core cap-and-trade system. The total mitigation provided by the project-based credits—farm and forest—are only about 10% of the total cuts realized in the system, but important nonetheless. Walsh told the audience about a dairy farmer in Minnesota who captures methane by means of a low-tech cover over his lagoon. By using that pollutant as an energy source he earns a $10,000 check, which may constitute a 20% boost in yearly income.

They wanted to standardize crediting rates for agriculture and consulted soil scientists with regard to conservation tillage, which is used on a continuous basis on only about 5% of the cropland in the United States and Canada. The scientists estimated that 0.75–1 ton of carbon dioxide would be absorbed per acre per year, which may extrapolate to a net farm-income increase of 10–20% in the next 5–10 years while billions of tons of mitigation services will accrue to the system. Standards are being drawn up for grazing-land-management practices and they are working on inclusion of nitrogen-use efficiency and N_2O emissions.

There is now a commodity price for carbon throughout North America: about $6.50 per ton, less than the $35 per ton at the European exchange, largely because the US system has voluntary participation. On a typical day the Chicago Exchange handles $1–2 million, between buyers and sellers.

The pieces are assembled and the system is working and draft legislation—the Lieberman-Warner and Bingaman-Spector bills—include many of the features that the Chicago Exchange has proven in the field.■

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Synthetic Genomics: Solutions and Challenges

James Newcomb [Bio Economic Research Associates (bio-era)]
Jack Newman (Amyris)
Robert Friedman (J. Craig Venter Institute)

James Newcomb observed that those who have attended more than one World Congress have witnessed the meteoric progress in synthetic genomics and synthetic biology on the industrial biotechnology landscape. Only a few years ago, this field seemed tantalizingly exotic. Jay Keasling and a few others told us about possibilities, and we wondered how long it would be between what was happening in the laboratory and the emergence of commercial reality. We are now acutely aware of the emergence into the commercial landscape of synthetic biology and synthetic genomics. The potential applications of synthetic genomics are seen not only in the fields of industrial biotechnology and biofuels, but across a much wider landscape. The traction of these technologies is seen in companies like Amyris, which recently announced a partnership with Crystalsev in Brazil, to produce, ultimately, a billion gallons of diesel annually from sugar cane. Also, the CFO at Synthetic Genomics, Inc., recently announced likely securement of $100–200 million of new financing for technology development, and similar progress is occurring at companies like LS9 and Gevo.

The broader set of issues around synthetic genomics amounts to what can certainly be considered to be a technology revolution with implications across the full scale of industrial and economic activities. With respect to the wide landscape of potential applications, Newcomb focused his remarks on:

- What's driving this revolution and how robust is it?
- How fast will it happen?

Most importantly, the revolution is being driven by rapid progress in enabling technologies, including sequencing and synthesis of DNA. These changes are happening on a global basis, with a profusion of facilities able to sequence and synthesize genes, with economic implications in terms of the scope of the activity and important implications from the regulatory perspective. It is difficult for US policymakers and other national governments to regulate the activity in this arena. Cheaper and wider access to the technology means that more people will use it, including students.

Another aspect is combinatorial evolution. It has been said that technology creates itself out of itself through combination and recombination over many layers of parts, sub-parts and sub-sub-parts. Analysts have modeled these autopoietic systems and described their dynamics. To get a sense of the power of combination and recombination, Newcomb invited the audience to think of a system of only ten parts and the ability to create combinations without duplication of those parts: about a thousand assemblages are possible without any redundancies. With forty parts, a trillion assemblages are possible. Therefore, even if only one in a million recombinations is useful, the proliferation of useful assemblages is exponential with the growth of the parts. This is the fundamental approach to obtaining useful building blocks on which synthetic biology is based.

But, this is nothing new to nature. Studies of the metabolic systems of *E. coli* at the macro, meso and micro scales indicate that the networks of pathways are, indeed, modular. Most of the changes in the *E. coli* genome over the past 100 million years have come from horizontal gene substitution, not from duplication. Therefore, biology works by much the same process: a process that is deeply conservative in a way, but adds new modules through horizontal gene transfer at the periphery of the network of systems of metabolism.

There is much to learn as we bring the tools of synthetic biology into practice to bring new modules into existing biological systems.

This amounts to an inflection point in the reach and power of biological technology, an inflection point that differentiates from the era of recombinant-DNA technologies pointing toward dramatic acceleration in innovation in industrial biotechnology as well as across a broad range of applications. This acceleration is evidenced by a "bow-wave" of increasing applications at the US patent office.

This brings us to the question of how fast this can happen. While technology-adoption curves are often steep, significant adoption can take a long time. This is less the case for innovations in consumer electronics, but more the case in imbedded industrial infrastructures, which are relevant with respect to biofuels and other bioproducts. It took 20 years from Edison's development of central-power generation and the carbon-filament lamp before 3% of US households had electric power. It took another 20 years before a 50% adoption rate was reached. Three factors relate to such diffusion lags.

- Technology-network effects, *i.e.* the build-up of ancillary technologies. For example, although cellulosic biofuels are a prime mover of processing technologies, the latter lags in comparison with the ancillary technologies of harvesting, managing, storing and delivering cellulose to processing plants.
- Arrangements of use by institutional and social factors that shape the speed of adoption of new technologies. This will certainly be the case in the arena of synthetic biology.

- The Marvin-Frankel cost, as termed by economists, *i.e.* the costs of replacing existing industrial-chemical processes with biological alternatives.

The economist Carlota Perez has described a pattern common across business development—from information technology back to the railroad era—which includes a "crisis in the middle." We saw this with genomics; in 2001 there were great expectations of rapid emergence of cancer therapeutics from genomic information then there was a crash because those expectations were over-inflated. Almost invariably, the "gilded" age of a revolutionary technology is followed by a crash, which immediately precedes the "golden" age, the build-out of the technology when it finds its place in the economy.

At bio-era they used a classical Royal Dutch Shell methodology to appraise the future of synthetic biology, looking at basic scenario elements, the driving forces, major areas of uncertainty, prime movers on the landscape that can change the game, *etc*. From that, they built a set of scenarios to scout the range of possible outcomes. Newcomb offered comments on three key aspects that emerged:

- Government policy in this arena is complex. It is difficult to imagine a scenario in which prohibition of these technologies could be effective without an extraordinary degree of international cooperation. In fact, it is easy to imagine scenarios in which prohibitions could backfire and result in unregulated or even perverse areas of activity.
- The evolution of the technology depends not only on the engineering side, but on other institutional structural elements, such as patent law and the workability of patent law.
- Social attitudes need to be accommodated. Archetypal stories exist across cultures, from Collodi's story of Pinocchio to Frankenstein and the creation of golems in Jewish mysticism, which warn against tampering with nature and capturing life for human purpose. Thus, social reactions to synthetic biology that are cautionary come from deep-seated traditions. This reality demands to be addressed and dealt with carefully and soberly.

Carl Woese wrote an article, "A New Biology for a New Century," in which he stated, "Today we face a choice between a biology that solely does society's bidding and a biology that is society's teacher." Newcomb expressed the hope that synthetic biology will be a bridge between these two, that it will be a biology that *does* society's bidding and, by providing fuller understanding of biological systems, it will provide biology as a teacher.

Jack Newman reminded the audience that we learned to splice pieces of DNA, using restriction enzymes, in 1973, soon after which Waclaw Szybalski coined the phrase "synthetic biology," saying that we were no longer limited to what is available from nature but that we had the ability to creatively combine DNA in our metabolic engineering endeavors.

In synthetic biology they regard the microbial cell as the sum of chemical reactions that may be reprogrammed. The code resides in the cell's DNA, and new DNA can be made synthetically. Although Newman believes that creation of an entire genome will be possible in the near future, at Amyris their objective is more modest.

The microbial cells are fed carbon as sugar and the output is a useful product, ethanol, acetate, lactate, *etc*., which the cell naturally synthesizes. The research he was involved with in Jay Keasling's laboratory—and which started Amyris—used feedstocks that are made within the microbial cell and transplanting a heterologous pathway—of a dozen genes—on top of that to make an antimalarial precursor. In the continuum of metabolic engineering this is just the next level up in abstraction. The protein therapeutics revolution was about inserting one gene to make a protein product; now many genes can be inserted to put together new pathways to make chemical products. The ultimate objective is complete control of the chemistry within the cell.

The biggest challenge is figuring out how best to use synthetic biology to bring products to market. To some extent, this is a matter of scalability.

At Amyris a priority is reducing cycle time, *i.e*. time from formulating an idea to building a microorganism and testing it. If it takes 3 months from having an idea to generating an organism to test, this can be done only four times per year. Four "learnings" per year are much too few. The process of target identification, gene optimization and pathway assembly must be done quickly then function in the cell is appraised. Chemical and biochemical engineering follow and then fermentation scale-up and process development.

Unless there is confidence in writing the correct genetic code for the desired product within ten attempts, then running the debugging program quickly through many iterations becomes important. Standardization of parts is essential, *i.e*. every "nut" must fit every "bolt." Thus rapid creation and testing of systems becomes possible, and, ultimately, cycle time is limited by the ability of the organism to double and it is even possible to decrease doubling time. Thus, thousands of "learnings" per year become feasible, rather than a handful. With this approach, yield of the anti-malarial drug, artemisinin, that they seek to produce, was increased from 0.1 to 25 g/L. The other component of scale-up, is the production of enough material to significantly address needs. The amount needed to treat malaria runs to 400 tons annually, which would translate into 6 million tons if plant material were the source. Microbiological production will require a fifty-fold scale-up, which they hope to achieve in partnership with Sanofi-aventis. This is a non-profit endeavor for Amyris and Sanofi-aventis.

Lack of compatibility of ethanol with gasoline and with vehicles already on the road, led scientists at Amyris to think in terms of producing hydrocarbon-based fuels for aircraft, diesel engines and gasoline engines from carbohydrate feedstocks.

One of the issues mitigating against adoption of biodiesel is cloud point. In the cold, vegetable oils, fatty acids and methyl esters form dense gels. In designing fuels *de novo* that microbiological systems can synthesize, they have been able to circumvent this problem and produce biodiesel that has cold-point and cold-flow properties superior to the specifications of petroleum-produced diesel.

Synthetic biology presents the possibility of producing and distributing biofuels that require no retrofitting of infrastructure without compromising performance, yet produce significantly less carbon dioxide.

Robert Friedman jokingly said that he had drawn the short straw. The speakers ahead of him had had the enjoyable task of discussing the promise of synthetic biology whereas he was left to deal with societal concerns. He suggested that there are four key issues:

- The use of this technology by bioterrorists,
- Potential harm to the environment,
- Concerns over laboratory safety, and
- Religious and ethical questions.

Policy experts from the J. Craig Venter Institute (JCVI), the Center for Strategic & International Studies (CSIS), and the Massachusetts Institute of Technology (MIT) recently authored a report, "Synthetic Genomics: Options for Governance," which outlines areas for interventions and policy options to help mitigate potential risks with this promising area of research. The report, funded by a grant from the Alfred P. Sloan Foundation, resulted from 20 months of in-depth study, review and analysis by the teams above and a core group of fourteen experts. It elucidates policies covering governance approaches—including self-governance of scientists within the field—for enhanced biosecurity, laboratory safety, and environment and community protection.

These concerns over synthetic genomics are not new. They are part of society's unease over biotechnology as a whole. Furthermore, especially since the events of September 11, 2001, the bioterrorism concern has come to the forefront of the national policy agenda.

The environmental concern is not in terms of making synthetic DNA *per se,* it is whether a specific, engineered organism poses a risk to the environment. This is part of the larger societal debate over recombinant DNA since the mid-1970s. Synthetic genomics has become a particular focus of concern as a result of rapid increases in speed and scale: the number of experiments that can be performed per unit of time and the power of those experiments.

Concerns over lab safety, again, are not about synthetic DNA itself, but possible risks to users from engineered microbes.

Some people view all forms of biotechnology as "playing God," and intellectual property issues remain controversial.

Returning to bioterrorism, a chief concern is the possibility of a "superbug" being constructed, a pathogen with increased virulence or resistance to known treatments. Synthetic genomics is a new aspect of this issue, offering new ways to produce pathogens. On the other hand, although physical confinement of dangerous pathogens works well, most pathogens would be easier to obtain by conventional means than by synthetic biology.

The events of September 11, 2001, roughly coincided with the achievement of capability to synthesize a complete genome. The first major synthesis-technology paper published in the post-9/11 world was "Chemical Synthesis of Poliovirus cDNA: Generation of Infectious Virus in the Absence of Natural Template," in *Science*, by Eckard Wimmer and his colleagues at Stony Brook, which caught the attention of the Washington policy establishment. It took Wimmer and his team about a year to synthesize the 7,400-nucleotide genome. A year later, a group at the JCVI synthesized a slightly smaller virus, phage phi X 174. In February 2008, "Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome" was published in *Science* by JCVI scientists. Although this parasitic organism has the smallest known genome of any free-living bacterium, the way is open to synthesizing other bacteria.

Having worked in Washington for more than 20 years, Friedman suggested that it is easy to cook up options for others to try, whereas the hard part lies in evaluating them and the costs, burdens and trade-offs to government, industry and other users of a technology. The report provides as easy-reference guide to seventeen policy options evaluated in terms of ten criteria to help those in government and others assess risk-cost tradeoffs.

Probably the most effective point of intervention lies with commercial providers, "gene foundries," of which there are two dozen in the United States and about fifty worldwide, as well as dozens of oligonucletide manufacturers doing business via the Internet. At least at this stage of development, it's a small community and a reasonably tractable problem. Policies governing this commercial activity might involve screening methods. When an order is received for a nucleotide sequence to be synthesized, identifying software could be used to determine whether it's for a cellulase or a segment of the 1918 influenza virus. Approaches similar to those controlling chemical sales in the United States could be adopted. A powerful means of control would be to identify those with *bona fide* reasons to be working with pathogens. ■

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Breakout Sessions

Biofuels and Bioenergy

The US Department of Energy Bioenergy Research Centers: Accelerating Transformational Breakthroughs for Biofuels Production

Martin Keller (Oak Ridge National Laboratory)
Tim Donahue (University of Wisconsin)
Jay Keasling (Joint BioEnergy Institute)

The single dominant objective of the BioEnergy Science Center (BESC) team is to apply an unprecedented large-scale, comprehensive, integrated interdisciplinary approach to overcome the problem of the recalcitrance of biomass to enzymic degradation. The first challenge is to understand which plant genes are responsible for cell-wall recalcitrance and what impacts modification of those genes would have. Both random and targeted gene identification and modification techniques are being employed for this purpose. The second challenge is to understand and elucidate the microbial aspects of the cellulose "ecosystem" by developing a consortium of high-temperature-tolerant microbes—cellulolytic, xylanolytic and ligninolytic microorganisms—as more effective biocatalysts for consolidated bioprocessing. Laser confocal microscopy, high-throughput isolation using flow cytometry and high-throughput sensor arrays are techniques being employed to screen and study pretreated biomass degraded by microbial consortia.

The primary objective of scientists at the Great Lakes Bioenergy Research Center (GLBRC) is to advance fundamental research in order to remove hurdles in the biomass-to-bioenergy pipeline. Major areas of focus include breeding plants for energy conversion by understanding their biochemical and regulatory pathways in order to facilitate conversion of plant carbon into more-digestible polymers. One of the approaches is to use genomics-enabled, high-throughput techniques to increase plant oil biosynthesis since biomass crops with up to 20% oil content are energy sources that do not require distillation or fermentation. Stochastic and rational strain alteration as well as microbial experimental evolution are employed for microbe screening; samples are analyzed by comprehensive "multi-omics" assays and computational models are developed for reengineering of selected strains. Another goal at the GLBRC is to predict the behavior of bioenergy-production systems in order to help develop a sustainable biobased economy leading to carbon neutrality and reduction in net greenhouse-gas emissions.

Joint BioEnergy Institute (JBEI) researchers are using an interlocking approach consisting of three scientific divisions and a technology division supported by a genomics knowledgebase to tackle the challenges of converting lignocellulosic biomass to fuels. In the feedstocks division, their endeavors include developing bioenergy crops, by studying cell-wall properties in plants with altered enzyme expression affecting lignin composition. The deconstruction division team is studying the changes resulting from various biomass-pretreatment approaches and interactions between lignocellulolytic enzymes; directed evolution is employed to produce more-active and stable enzymes from microorganisms from new environments. In the fuel-synthesis division, they are engineering microorganisms to consume lignocelluloses and monomers, as well as to produce and tolerate high concentrations of biofuels.

Advancing Enzymatic Hydrolysis of Lignocellulosic Biomass

Charles Wyman (University of California)
Shi-You Ding (National Renewable Energy Laboratory)
Bin Yang (University of California)
Colin Mitchinson (Genencor)

At the National Renewable Energy Laboratory, nanoscale imaging, molecular labeling, and single-molecule tracking are being used to understand and elucidate biomass recalcitrance. Molecular interactions need to be understood because plant-cell walls are inherently resistant to degradation. In the Biomass Surface Characterization Lab, atomic-force and other types of microscopy are used to characterize cell-wall assembly at the nanoscale. It has been determined that cellulose is synthesized as macrofibrils whereas hemicellulose forms microfibrils surrounding thirty-six chains of cellulose, *i.e.* six core chains, twelve transition chains and eighteen surface chains. Plants from varied sources show differences in structure. Imaging techniques have been used to compare structural changes induced by various pretreatment methods.

The cost of enzymes used for degradation of lignocellulosic biomass can be reduced by increasing specific activities of cellulases, increasing their thermostability and enhancing the hydrolysis rates. Experimental results suggest that disruption of lignin is essential for achieving high enzymatic hydrolysis rates. Interference by lignin of fungal cellulase activity can be eliminated by the use of lignin blockers.

Treatment of lignocellulosic biomass with bovine serum albumin, a lignin blocker, yielded increases of 5% to 20% in enzymic hydrolysis rates depending on the type of pretreatment used. Comparisons of continual and interrupted hydrolysis of a model cellulosic substrate, Avicel, with β-glucosidase supplementation showed that substrate reactivity remained constant or increased with conversion for interrupted hydrolysis.

Genencor's enzyme cocktails are targeted towards second-generation biorefineries, Accellerase 1000 being the first of a series of such commercial products. Launched in October of 2007, Accellerase 1000 has high β-glucosidase activity to minimize residual cellobiose inhibition, an unclarified product, to make nutrients available for yeast during simultaneous saccharification and fermentation (SSF). It has a minimized formulation, preventing downstream chemical interference. This new product delivers enhanced performance over previous enzyme complexes, on a variety of pretreated biomass samples in terms of faster conversion, and lower cellobiose concentrations. *Trichoderma* fungal cells in the product eliminate the need for yeast nutrients during SSF. Genencor seeks partnerships at the enterprise level and strategic alliances to integrate dedicated enzyme products into biorefineries to enable cost-effective synthesis of ethanol.

Creation and Commercialization of Next-Generation Advanced Biofuels

William Roe (Coskata)
John Melo (Amyris)
Robert Walsh (LS9)
John Ranieri (Dupont)

At Coskata, they aim to be a global leader in the synthesis gas-to-biofuel platform to produce low-cost ethanol. Backed by a team of industrial partners, venture capitalists and research institutions, proprietary anaerobic bacterial strains are used to convert syngas—across a range of carbon monoxide:hydrogen ratios—to ethanol. Coskata's bioreactor design enhances biofilm formation, which maximizes productivity. The process is energy efficient, cost effective (less than US$1 per gallon of ethanol produced) and offers feedstock flexibility when compared to enzymic and chemical-based processes. Successful process operation has been demonstrated at their model plant in Madison, Pennsylvania.

The chief drivers for change from crude-derived carbon to bio-derived carbon are:

- cost of oil,
- need to diversify sources of energy, and
- mitigation of greenhouse-gas emissions.

At Amyris, breakthrough technology in synthetic biology is being used to develop microbial solutions to energy problems, by engineering microorganisms to convert sugars to fuels. The global transportation-fuels sector involves a trillion-dollar market and requires a "no compromise" product that has to be competitive in order to meet or exceed the standards set by gasoline. Scale is what matters most; feedstock has to be hugely scalable and the infrastructure for production has to be scaled accordingly. Amyris fuels include hydrocarbons, renewable diesel, jet fuel and gasoline that can be produced in existing ethanol plants, can be blended up to 50% with petroleum fuels to provide the greatest economic value, have been tested to reduce emissions by 80% and are compatible with current distribution infrastructure and engines.

LS9 is a 3-year-old Renewable Petroleum company that produces biocrudes and biofuels to provide sustainable replacements for diverse petroleum products. For an alternative fuel to succeed in the market, it has to be renewable, scalable, compatible with the current infrastructure and distribution systems, domestically produced, and cost competitive. With proprietary enabling catalysts and processes, LS9 is producing DesignerBiofuels with composition and performance precisely controlled for specific end uses by genetic modifications. LS9 biofuels have greenhouse-gas benefits equivalent to those from cellulosic ethanol, utilize 70% less energy for production as they require no distillation and allow pipelines to be used rather than trains and trucks. LS9 has a small number of strategic partners for product development and commercialization and a larger number of partners for feedstock sourcing and pre-treatment.

The strategy employed at DuPont Applied BioSciences is to focus on large, market-driven opportunities, transform the targeted industries with their integrated knowledge base, create new partnerships to expand market opportunities and accelerate speed to market. An integrated approach requires sensing and adapting. DuPont's partnership with BP to resolve ethanol constraints involves biobutanol production. Butanol delivers significantly improved logistics and fuel performance across the value chain as it has higher energy density and is synergistic with ethanol. Also, it has the potential for higher blend levels without vehicle modification and no increase in emissions of carbon monoxide, hydrocarbons or nitrogen oxides. DuPont's technology allows selective production of biobutanol without the usual byproducts.

Comparison of Key Conversion and Feedstock Technologies

Jim Schumacher (Mascoma)
Gregory Pal (LS9)
Neal Gutterson (Mendel)
Randy Cortright (Virent Energy Systems)

Mascoma scientists aim to achieve a low-cost configuration for cellulose hydrolysis and fermentation through the integration of pretreatment and their proprietary technology of consolidated bioprocessing. Technology validation has been underway at a pilot plant at Griffis Technology Park, NY, where multiple feedstocks are being evaluated by an integrated process. A demonstration plant has been set up in Tennessee through a strategic partnership with the University of Tennessee with switchgrass as feedstock. Mascoma has several technology partners, capital partners and value-chain partners who will be critical in achieving technology scale-up and product commercialization.

At LS9, the business model is to produce and market wholesale fuels and chemicals either alone or through strategic partnerships. To be successful, a biofuel must be compatible with the existing pipeline network, fueling stations and modes of transportation. LS9 uses proprietary enabling catalysts in combination with proprietary processes to produce DesignerBiofuels products. Applying synthetic biology allows determination of specific fuel composition and control of performance of end-products.

The focus at Mendel Biotechnology is on breeding perennial C4 grasses such as miscanthus as dedicated non-invasive energy crops. The Council on Sustainable Biomass Production (CSBP) has a goal of generating a broad multi-stakeholder consensus on the performance metrics for second-generation biofuels in the United States, and guidelines for sustainability principles with support from farmers, germplasm providers, social and economic interests and biorefinery owners. Strict standards are necessary for deployment of appropriate technologies to ensure long-term sustainability of the biofuels industry.

Virent Energy Systems is commercializing a proprietary low-temperature catalytic process, BioForming, for conversion of biomass into hydrocarbon fuels, chemicals or hydrogen. The composition of BioForming's "green gasoline" is the same as that of unleaded gasoline. Currently, the technology has achieved high yields from five- and six-carbon sugars, corn syrup and sucrose feedstocks and demonstrated the exothermic nature of the integrated process. The completion of the first year of a multi-year development program with Shell was recently announced; commercialization of the BioForming process is underway.

Biofuels in Aviation

Wayne Seames (University of North Dakota)
Robert Dunn (US Department of Agriculture)

Sustainable Energy Research, Infrastructure and Support Education (SUNRISE) is a coalition of four universities in North Dakota, dedicated to the development of sustainable energy options to improve the state's economic development. Biodiesel has poor cold-flow properties, low energy density and problems with oxidative stability due to unsaturated esters. Proposed solutions for the design of a renewable aviation fuel include the use of a more complex alcohol during esterification to lower its freezing point and of shorter-chain triacylglyceride oils like coconut oil. The SUNRISE Renewables, Inc., biojet fuel meets JP-8 specifications and bench-scale development of the process has been completed. A pilot plant will be commissioned in late 2008.

Key concerns surrounding the use of biodiesel as an aviation jet fuel are its cold-flow properties, its stability during storage, the effect of contact with moisture and its nitrogen-oxide emissions. Suspensions of monoacylglycerols that settle in storage tanks are resistant to melting back into solution. Hydrolytic degradation of biojet fuel has been observed on contact with moisture leading to reduced oxidative stability of the fuel. Flight trials conducted at Baylor University with 20% biodiesel in jet-A1 showed significant differences in the exhaust stains left by the two fuels with the blend being lighter and easier to clean.

International Developments in Cellulosic Biomass Technologies

Hideaki Yukawa (RITE)
Trevor Stuthridge (Scion)
David Turner (Lignol)
Sonti Ramakrishna (PRAJ)

The Research Institute of Innovative Technology for the Earth (RITE) has developed an ethanol-producing strain of *Corynebacterium glutamicum* that has cellobiose-uptake capacity. Simultaneous utilization of mixed sugars by the RITE bioprocess is further advantaged by tolerance to fermentation inhibitors. Cellulosic-ethanol production at the pilot scale is work in progress. Establishment of technology for the production of biobutanol as a renewable fuel is also a top priority at RITE.

With New Zealand striving to be 100% carbon neutral by 2040, the development of a self-sufficient biorefinery is the focus of the New Zealand Lignocellulosic Bioethanol Initiative. Plantation-softwood-based lignocellulosics are the only significant resource for biofuel production and no significant technological barriers exist to such use of softwoods. Although lignin removal by pretreatment decreases glucose content, it improves saccharification efficiency, hence a trade-off between process impacts has to be considered. Process optimization and demonstration-plant construction are the immediate steps toward commercialization.

Lignol Energy Corporation's biorefinery produces a clean pulp that is rapidly converted into sugars at low enzyme costs and value-added products generate revenues that mitigate production and commodity risks. Lignol acquired and modified a solvent-based pretreatment technology originally developed by General Electric then sold to Repap and used commercially in pulp production. Other common pretreatments result in much lower amounts of fermentable components. High-purity lignin with potential industrial applications is also produced. The roadmap for commercial demonstration includes pretreatment and ethanol production in an industrial-scale pilot plant and further development of the range of applications for lignin.

PRAJ Industries has an R&D center focused on biofuels to help address India's energy needs. Feedstock for biomass is derived from sugar-cane mills as bagasse, and from farms as agricultural residues. The primary limitation appears to be the collection of biomass due to decentralized farming infrastructure. To address feedstock diversity, PRAJ researchers are making extensive comparisons of pretreatment technologies, hydrolysis and mixed fermentations *vis-à-vis* gasification followed by catalytic fermentations to determine the best combination for cost-effective ethanol production. Integration of large-scale lignocellulosic plants with existing sugar-mill and paper-mill models is under examination.

Joint Process Developments in Biomass

Scott Kohl (ICM)
Gerson Santos-Leon (Abengao)
Steen Jorgensen (Novozymes)
Mark Stowers (POET)

The need of the hour is to get the existing technology to the market. The entire value chain for cellulosic ethanol production has to be integrated via partnerships. Feedstock genetics and production need to be coupled with high fermentability and enhanced processing properties with benefit accruing also to farmers. Pretreatments need to be effective, enzyme hydrolysis must be cost competitive, and fermentation must achieve high yields. Partnerships among companies allow them to complement activities to complete the entire value chain for ethanol production, since no single company has competence in all technologies. At ICM, the aim is to orchestrate the technologies and incorporate them in two stages. A "1.5"-generation plant will use captive fibers, produce food and fuel while refining aerobic processes for higher-value products. A second-generation plant will be co-located with current starch-based facilities and eventually will become an independent cellulosic ethanol plant.

The goal at Abengoa Bioenergy is to develop and implement cost-effective biomass-conversion technologies by improving existing dry-mill-process yields of ethanol. A multi-stage effort is combining biological and thermochemical pretreatments to enzymically hydrolyze wheat and barley with separation into xylose, cellulose and lignin streams. A biorefinery pilot plant has been set up at York, Nebraska, and construction of a biomass-demonstration plant is underway in Salamanca, Spain. Catalysts are also being developed for thermochemical conversion of biomass. Abengoa's hybrid-plant concept has its project site at Hugoton, Kansas.

Scientists at Novozymes want to improve the catalytic efficiency of enzymic hydrolysis while reducing enzyme dosage to achieve cost-competitiveness. Dose reduction can be achieved by increasing enzyme-mix complexity and requires genetic modification of some components. The enzyme dose is highly dependent on the type of pretreatment. Washed, acid-pretreated corn stover has been found to be optimum from an enzyme digestibility point of view. There is urgent need to keep the momentum behind the current political support for biomass-to-fuel in order to achieve economic feasibility by 2010. Collaboration among leaders in their respective fields is essential. The decision to pick a particular technology needs to be made soon.

Project LIBERTY recently launched by POET involves ethanol production from a cellulosic feedstock, involving integration of unit operations including corn fractionation, a solid fuel boiler and anaerobic digestion; projected annual capacity of this facility is 100 million gallons. The main avenues for collaboration are in feedstock collection, scale-up and construction. Corn cobs—a good cellulosic feedstock, normally left on the field after harvest—have higher carbohydrate content and twice the density of stover. This density makes collection and separation a logistically feasible operation. The main challenge is to engage farmers in collection, storage and grinding of cobs, requiring equipment modifications. Finding and managing multiple collaborators are key for successful scale-up.

Biofuels Technology to Address Climate Change

Maxes Ringpfeil (BIOPRACT)
Robert Kramer (Purdue University)
Tom Kalnes (UOP)
May Wu (Argonne National Laboratory)

The combustion of fossil carbon leads to climate change while the combustion of regenerative carbon is climate-neutral. Scientists at BIOPRACT aim to amplify, intensify and economize regenerative carbon production and make it available for use. Almost any organic compound is microbiologically convertible into methane via anaerobic bacterial metabolism. Methane-formation rate can be maximized by identifying and eliminating limitations in processes intermediate to biogas formation. Statistical experimental design methods have been used to achieve the most efficient results. Future plans include use of neural networks to control the biogas-production system for continuous improvements.

A research team at Purdue University is using microorganisms to produce hydrogen from waste-biomass materials by anaerobic digestion. Solar energy is used to preprocess the feed material. Fuel cells are used to convert the hydrogen produced to electricity, which can be supplied to remote locations. The system has the potential to sequester carbon dioxide by the use of organometallic nanocatalysts as well as to produce fertilizers.

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A joint development of UOP and Eni SPA uses their Ecofining hydroprocessing route to convert vegetable oils to high-quality diesel. The feedstock-flexible Ecofining process produces “green diesel,” which is compatible with conventional diesel engines and which has superior product properties to other options, *i.e.* higher cetane levels, lower cloud point and lower emissions. Life-cycle comparisons with ultra-low-sulphur diesel (ULSD), biodiesel, and syndiesel have shown that greater fossil fuel savings can be achieved with green diesel. Green diesel provides reductions of 42% to 85% in green-house gas (GHG) emissions compared to ULSD when calculated per ton of biofeedstock.

The greenhouse gases, regulated emissions, and energy use in transportation (GREET) model is a biofuel life-cycle-analysis model that includes activities from fertilizer manufacturing to vehicle operation. Coskata uses a hybrid approach for syngas-to-ethanol production by gasification and syngas fermentation. Analysis of the production-process features showed that substantial fossil-energy savings could be achieved, while electricity co-generation and steam export reduced GHG emissions even further. The syngas-to-ethanol-process design and production options showed a positive net energy balance. Stand-alone plants with co-generation of electricity (through flue-gas heat recovery) and co-located plants with utilization of excess steam (by the accompanying plant) were found to achieve substantial fossil-fuel savings from well to wheel.

Biofuels from Microalgeae

Paul Roessler (Synthetic Genomics)
Lisa Morgenthaler-Jones (LiveFuels)
John Beneman (International Network on Biof xation of CO_2 and Greenhouse Gas Abatement with MicroAlgae)
Jonathan Wolfson (Solazyme)

High crude-oil prices, high prices of starch and oil crops and global-warming concerns provide the perfect storm for the development of algal biofuels. Expanding the toolbox for strain manipulation is key to making them commercially viable. Numerous closed photobioreactor designs have been tried out, with flexible-film tubular bioreactors being the most popular. Heterotrophic growth of algae on sugars in fermentors is another avenue for development. Product recovery is the crucial bottleneck to cost reduction. The application of synthetic biology and synthetic genomics will allow improved growth-module designs and lower capital and operating expenses.

The technology used in most commercial algae systems relies on open ponds producing products such as *Spirulina* and beta-carotene which have relatively high-value but low-volume markets. More than a hundred companies are using photobioreactors to grow algae; they are ideal for genetically engineered organisms but are about ten times more expensive than other options. A key challenge is that algal blooms require a lot of water, with yields of only around 0.2 to 0.4 grams per liter. At LiveFuels, scientists are developing an algal biocrude with 150 million gallons of algal water, using agricultural run-off and neutral lipid-extraction techniques. At $116 per barrel oil, algae-to-biofuels facilities are scarcely commercially viable. The diversity in the range of algal companies will be key to tackling the challenges currently faced by this industry.

Commercial photobioreactors (PBRs) and covered greenhouse-pond systems have been unsuccessful. With capital costs of millions of dollars per hectare, even high-value-product ventures have failed, indicating that PBRs are impractical for biofuel production. A feasible alternative to high-cost PBRs appears to be “high rate” ponds, which are shallow raceway mixed ponds developed at UC-Berkeley in the early1950s, which are used by 98% of commercial microalgae systems today. However, the total world production of all microalgae is only about 10,000 tons. Techno-economic analyses of open-pond microalgae biodiesel production conclude that we are not yet in a position to make algal biodiesel economically. Long-term R&D is necessary.

Solazyme, Inc., is a synthetic-biology company at which marine microbes are used to produce biofuels and oils. A proprietary microbial fermentation process allows algae to produce oils in the absence of sunlight in large bioreactors. The key feature of the process is feedstock flexibility; various non-food feedstocks—waste glycerol, woodchips, corn stover, switchgrass, *etc.*—have been utilized to produce a 100% blend SoladieselRD. The chemical composition of this new product is similar to that of petroleum-based diesel; it is compatible with existing transportation fuel infrastructure.

Linking the Pulp and Paper Industry with Lignocellulose Bioconversion

Thomas Browne (Paprican)
Adriaan Heiningen (University of Maine)
Thomas Amidon (SUNY ESF)
Junyong Zhu (US Forest Service)

The forest sector manages a sustainable, non-food, non-agricultural crop and understands the supply chain of the pulp and paper industry. However, that industry is in need of novel products to manufacture. It could form links with the chemical industry by directly substituting existing chemical raw materials with bio-olefins, *etc*., to provide biobased products. For economic feasibility, initial transformation of feedstock could be done at a pulp mill and intermediates shipped to chemical plants. Identifying potential industrial chemicals that can be derived from hemicellulose and lignin will help build strategic partnerships and create new markets.

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The pulp mill is ideal as a biorefinery for processing biomass other than wood since it already has the basic infrastructure and operating permits. The ideal forest biorefinery process would involve fractionation of feedstock into cellulose, lignin and monosugars so that each could be separately processed into suitable products. The near-neutral green liquor biorefinery process involves value prior to pulping, uses kraft pulping conditions, yields about 47% pulp and produces ethanol at $1.8 per gallon while the American value-added pulping (AVAP) biorefinery process is more feedstock-flexible.

Hot-water extraction of woody biomass prior to pulping allows preservation of product value and ease of separation. Fractionation of the products of wood extraction allows recovery of acetic acid, oligomers, furfurals, *etc.* Fermentation by unmanipulated and recombinant strains produces fuels such as hydrogen, butanol and propanol, as well as ethanol. Pentose fermentations need to be commercialized and co-feeding of biomass crops and agricultural residues needs to be developed. Water-based technology and the availability of willow hardwood are near-term advantages for the biorefinery at the College of Environmental Science.

A new pretreatment process for woodchips uses moderate temperature and pH with low dosages of chemicals followed by size reduction, thus requiring low-energy inputs leading to economical saccharification by biological or chemical conversion. The amount of substrate digested and the yield of enzymically hydrolyzed glucose are comparable to those obtained by organosolv pulping. The production cost for ethanol by this process is lower than that by steam-explosion pretreatment. Future developments include scale-up, process optimization and commercialization through industrial partnerships.■

Renewable Feedstocks

The Business of Bioenergy from Purpose-Grown Trees and Other Woody Biomass

Ron Barmore (Range Fuels)
Tim Eggeman (ZeaChem)
James Mann (ArborGen)
Denny Hunter (Catchlight Energy)

Range Fuels was formed in 2006 by Khosla Ventures to commercialize cellulosic ethanol. Ground was broken in November 2007, in Soperton, GA, for construction of a cellulosic ethanol plant using local woody biomass resources. Second-generation biomass-conversion technology will be used with a novel two-step thermochemical process to convert biomass solids to synthesis gases and then to liquid alcohols using a proprietary catalyst.

Short-rotation trees incorporate many desirable features for a bioenergy feedstock, including perennial growth, low inputs, high yields and low costs. Greenwood Resources, a producer of hybrid poplar trees, is partnering with ZeaChem to supply feedstock for their hybrid biochemical/thermochemical process. Estimated yields for the hybrid process are higher than those estimated for biochemical or thermochemical alone. Improvements in feedstock management and harvesting, conversion technologies and automobile efficiencies will further increase yields in the future.

ArborGen is an integrated tree-improvement company and the world’s largest producer of trees for planting. Biotechnology is being used to add value-enhanced traits, such as increased growth rates, shorter rotations and increased yield to their products for fiber and energy applications. *Eucalyptus* genotypes developed by ArborGen show increased growth rates in the southeastern United States, comparable to growth rates in Brazil, with frost tolerance. Biofuel-specific resource management could improve yields of short-rotation trees. Logging infrastructure already exists to bring this feedstock to conversion plants.

Catchlight Energy is a joint venture of Weyerhauser and Chevron to commercialize large-scale production of liquid transportation fuels from sustainable forest resources, taking advantage of the parent companies’ existing infrastructure. Weyerhauser will provide the feedstock to a conversion plant managed by Catchlight Energy. The resulting product will take advantage of Chevron’s ability to distribute liquid transportation fuels. Catchlight will initially be focused on R&D to commercialize a conversion process, likely incorporating third-party technology partners.

Cellulase Expression in Plants

Simon Warner (Syngenta)
Bruce Ferguson (Edenspace Systems)
Elizabeth Hood (Inf nite Enzymes)

Syngenta Biotechnology has adopted a three-horizon technology-development timeline for efficient and sustainable biofuels. Presently, the goal is to reduce biomass-feedstock costs through maximizing yield; in the short term, innovations within the industry such as tailored crops and processing changes to increase efficiency will be important. The long-term goal is to induce plants to express new enzymes to produce self-processing feedstocks, making sustainable, cost-effective cellulosic ethanol a reality. The company has already successfully expressed multiple cellulases in plants.

Biotechnology will play an important role in reducing the costs associated with cellulosic-ethanol productions, providing the modern genetic techniques necessary to develop new feedstock crops, such as domestic grasses, and to improve utilization of existing feedstocks such as corn. Stover is a potential cellulosic resource that benefits from years of agricultural study and acceptance, and is grown simultaneously with a cash crop, the corn grain. Edenspace Systems is using biotechnology to improve these cellulosic feedstocks, including the development of endoplant enzymes, therefore reducing costs associated with enzymic processing.

Significant cost factors associated with producing enzymes in a plant include expression of enzymes, economy of scale, regulations regarding transgenic products, and optimized production and purification. Using corn as a model system allows researchers to boost expression levels in a well characterized system, and tailor where in the plant enzymes are expressed. In this scenario, the entire corn plant can be used for biofuels production. Infinite Enzymes has demonstrated the ability to express new enzymes with measurable activities in corn.

Feedstock: From the Farm to the Bioref nery Gate

Spencer Swayze (Ceres)
Dick Carmical (Price BIOstock)
Bud Cary (AgFuture Energy)
Rob Meyer (ICM)

Technology-enabling mandates require that 44% of alternative fuels be made from cellulosic sources by 2022, requiring a five-fold increase over current production levels. Advanced biofuels feedstocks will be critical to the growth of the industry and biotechnology will allow optimization of biofuels crops to maximize yield and density per acre. Improvements in biomass composition and conversion technologies will reduce capital costs and provide sustainable economic returns for producers. Once a suitable crop is identified, the seed should go in the ground at the same time as the steel for building the conversion plant.

America's renewable-energy resources are patchwork at best, with wood dominating the East, corn in the Midwest, wind along the coasts and a combination of wood, wind and solar in the West. One of the key challenges for any renewable-energy conversion plant is bringing resources to the plant itself. Raw-material procurement presents several challenges to biomass conversion facilities: supplier trust, competition for resources, moisture management and the volatility of cost of raw materials. Solutions to these challenges include long-term contracts, fair prices for suppliers and changes to technology to minimize processing costs.

The USDOE "billion ton" report projects 800 million tons of biofuels feedstock coming from agriculture, which works out to 110,000 trailer trucks loads of biomass per day. An integrated solution is needed to bring together the producer and the converter and to guarantee appropriate returns for both, before significant capital is invested in hard assets. BioYield will combine the knowledge and technologies from AgFuture Energy and the Texas A&M University system to deliver dedicated biomass feedstocks to conversion facilities at fair and economic prices.

Current commodity prices are high, making traditional crops more profitable and increasing prices of inputs and land. At the same time, however, farming marginal land is more profitable. Growers will be interested in producing energy crops if they can achieve the same profits as with traditional crops with similar input costs. On the other hand, they may have to purchase new equipment to adapt to biomass harvest schemes. Biorefineries need feedstock of consistent type, quality and shape, to maximize conversion efficiencies.

Algae For Energy

Thomas Byrne (Byrne)
Ben Cloud (XL Renewables)
Quinn Goretzky (Alberta Research Council)

Algae are a promising technology for sequestration of carbon dioxide emitted from power plants and for creating value-added products such as biofuels. Innovations Canada (I-CAN) is researching microalgal photosynthesis for direct utilization of flue gases, fixation of carbon and production of a biomass feedstock. Over the next 5 years, I-CAN will work on proof-of-concept, strain selection and scaling up from the laboratory to the pilot plant to an ultimately integrated system working year round in Canada, generating a positive return.

For algae to be feasible as a biomass feedstock, production systems need to be large-scale and economical. XL Renewables has developed a trough system for growing and processing algae that is scalable to 40 acres, is low cost using proven agricultural concepts, with potential production of triglycerides, fatty acids, and meal for animal feed as well as biomass for biofuel synthesis. A demonstration facility will be open to the public in November 2008.

Location and operating requirements are important considerations in constructing an algae plant. Algae require carbon dioxide, light, heat, water and other nutrients to grow and while these may be found in a variety of locations, it is important to match the location to the desired end-product. Location options include large animal operations, which would have good sources of waste heat and wastewater; ethanol plants, which would have clean carbon dioxide streams for producing food-grade algae; and coal-fired power plants, which would have waste heat, but possibly with carbon-dioxide streams contaminated with sulfur and/or heavy metals.

Swedish Developments in Industrial Biotechnology

Cherryleen Garcia-Lindgren (Processum)
Bo Mattiasson (Lund University)
Rajni Hatti-Kaul (Lund University)

Processum is an integrated lignocellulosic biorefinery in the north of Sweden. Traditional pulp and paper industries need to adapt to new technologies and products to thrive in the current environment, by producing: high-value chemicals, ethanol, lignosulfonates for the concrete and oil industries, *etc*. Waste products are delivered to combined heat and power plants. Future research will focus on scaling up production, operating continuously, raw-material flexibility and process integration.

Biogas, a combination of methane and carbon dioxide produced by anaerobic digestion of biomass or waste, is an efficient energy carrier and could be integrated with natural gas in Sweden to supplement energy supplies. Challenges to widespread biogas use include process intensification, improved hydrolysis of lignocellulosic materials, improved process control, and distribution. In some municipalities, biogas is already being used to manage wastes and run mass transportation.

Specialty chemicals and materials may provide more economic value than energy, which is an important consideration in the switch to a biobased economy. Greenchem is a national program for the enzymatic production of industrial chemicals, based at Lund University, that unites producers of raw materials, producers of chemicals and users of chemicals. Greenchem will identify novel enzymes from extreme environments and transfer the genes to appropriate hosts for biocatalyst production.

Capitalizing on Biomass: A Comparison of Energy-Crop Economics for the Bio-Revolution

Roger Samson (REAP-Canada)
John Baker (Eastern Lake Ontario Regional Innovation Network)
Frank Dohleman (University of Illinois)

Various options are available for bioenergy production. Warm-season perennial grasses can efficiently capture solar energy on marginal farmland in many areas of the United States. Pelletizing is the most efficient way to transport and distribute the biomass; pellets are convenient to handle, have greater energy density than bulk biomass, and provide greater control over burning. Without government support, barriers to grass pelletization are economic, not technical.

Hemp is another biomass option for energy and materials production. Not yet optimized, hemp could be improved by breeding. It produces significant amounts of biomass in diverse environments, including many areas of Europe and Canada, and can be harvested with minimal investment in specialized equipment. Products using hemp include automobile components, insulation, paper pulp and building materials, as well as biofuels feedstock. Bio-prospecting for new varieties could lead to improvements in yield and composition.

The selection of biomass feedstock will vary by geographic region. In temperate Midwestern climates, some grasses may not be viable. In states like Illinois, it is necessary to produce a feedstock that is as profitable as corn when grown on marginal lands. Miscanthus possesses many of the qualities that make a good biofuels feedstock, including high yields, recycling of nutrients to roots, compatibility with conventional farming equipment and low inputs. Improvements in miscanthus germplasm may increase yields and allow the crop to be tailored to fit more areas of the country.

Fractionation, Pretreatment and Hydrolysis

Rajeev Kumar (Dartmouth College)
J.Y. Zhu (US Forest Service)
Ed Lehrburger (PureVision Technology)
Steve Hutcheson (Zymetis)

Accessibility to cellulases is an important factor affecting cellulose hydrolysis. A study by researchers at Dartmouth College investigated the accessibility of cellulose, xylan, and lignin in various cellulose and biomass substrates and how that accessibility changed after applying several pretreatment technologies. Accessibility was influenced by pretreatment type and substrate composition.

Physical biomass-size reduction by mechanical means—the necessary first step to increasing substrate accessibility for lignocellulosic conversion to biofuels—is energy intensive, particularly for woody material. A methodology developed by the US Forest Service characterizes the shape and size of mechanically reduced biomass to determine accessibility and effectiveness of the reduction method. The study showed that disk milling is more effective than hammer milling for cellulose accessibility and hydrolysis.

At PureVision Technology, a two-stage fractionation and pretreatment process has been developed for biomass conversion, separating the stream into cellulose solids, a five-carbon-sugar stream and a lignin-derivatives stream. PureVision is working with the Canadian Triticale Biorefining Initiative to develop the wheat-rye hybrid into an industrial crop for producing energy, fuel, chemicals and advanced materials in a dedicated biorefinery.

Enzyme hydrolysis is a critical factor in the effectiveness and cost of a pretreatment scheme, as enzymes applied require a high degree of specificity, synergism, substrate utilization and low cost. Zymetis is taking advantage of a new source of hydrolytic enzymes from the gamma-proteobacterium *Saccharophagus degradans*, isolated from the Chesapeake Bay, with a known genome sequence and extremely robust degradative abilities. Enzyme mixtures produced by Zymetis are applicable to a wide range of biofuel feedstocks, have high activity, and contain multiple specific enzymes, such as cellulases, xylanases, pectinases and ligninases, making the mixtures a "one-stop-shop" for biomass hydrolysis.

Seed Oils as Industrial Feedstocks

Steve Fabijianski (Agrisoma Biosciences)
David McElroy (Targeted Growth)
Jack Grushcow (Linnaeus Plant Sciences)
Jitao Zou (National Research Council Canada)

Agrisoma Biosciences is using engineered trait loci (ETL) to improve the composition, reduce the processing requirements and improve the final product from oilseed feedstock crops for biofuels. ETL technology can be used to introduce, express, combine and manage multiple new traits in any crop, with consistent expression and good control over random delivery of multiple genes. This technology will allow producers to design their oils, with monosaturate levels and chain lengths specific to applications, for high-value products.

Targeted Growth, Inc., is proposing using Camelina sativa—like canola an oil-seed brassica—as a low-cost dedicated biofuel feedstock. It can grow on marginal land with low input costs and presents opportunities for improvement through classical and molecular breeding, mutations and transgenics. Improvements in yield and fatty-acid composition could lead to improved feedstock for production of biodiesel and other chemicals.

Oilseed crops may provide alternative feedstocks for other currently petroleum-based processes, such as plastics or lubricants. Castor oil could be particularly valuable as a trait genetically engineered into other species, as it has many patented industrial applications, established industrial chemistry, and recognized oxidative stability and lubricity. This requires identifying several varieties of non-food oil seeds for agronomic testing and selection for regional breeding programs. Testing of transgenic meal will be needed with a view to possible food and feed uses, with development of life-cycle data to quantify greenhouse-gas reductions. Biodiesel production would be the first refining step, with oil fractionation and purification and testing for suitability for polymer production and other applications.

Researchers at the Plant Biotechnology Institute of the National Research Council Canada will apply molecular biology techniques complementary to traditional breeding methods to modify the fatty acid composition and yields of oilseed crops to produce oils with novel characteristics and compositions. Key genes for oil production will be identified through natural variation, serving as molecular markers for more traditional breeding methods. The challenges to this include adapting to a novel crop platform and overcoming metabolic bottlenecks to oil production.

New Fuel-Crop Alternatives

Thomas Todaro (Targeted Growth)
Nhuan Nghiem (USDA ARS)
Ashok Dhawan (Haryana Agricultural University)

Jatropha is an oilseed tree that could be grown on wastelands in India to improve green cover and air quality while producing oil that could be made into biodiesel. In order to make jatropha commercially profitable, new propagation techniques and plant varieties tolerant of high salinity must be established. A national network of fourteen research facilities in India is working towards making jatropha a biofuel crop with some success already gained in micropropagation. Development of salt-tolerant lines is in the planning stage.

Barley as a biorefinery crop is being developed by the USDA ARS to produce food, feed, and fuel in areas outside the traditional corn belt. Barley fuel ethanol could be produced from both the grain and the straw once cellulosic ethanol is cost effective, bringing new sources of income to farmers and rural economies. Barley could be used as a cover cropp in winter after more profitable crops such as corn or soy, thus reducing soil erosion. Research is underway to address technical challenges to barley, including hull-less barley to reduce abrasion in harvesting and processing equipment, dry-fractionation processes to separate fermentable and non-fermentable fractions, and enzyme research to reduce mash viscosity.

Targeted Growth, Inc., has increased the yield of canola and soybean using a cell-cycle-regulating gene to create oilseed feedstocks with lower input requirements. Camelina is being developed as a biofuel crop through breeding; researchers hope to increase productivity further with yield genes. Additionally, a lower-cost corn crop, specifically for integration with existing ethanol infrastructure, is being developed, and they are looking into developing algae as a second- or third-generation biofuel feedstock through molecular biology and engineering.

Harvest, Storage, and Transportation of Biomass

Stuart Birrell (Iowa State University)
Robert Anex (Iowa State University)
Jeffery Frost (AgRefresh)

In order to make biofuels profitable, every aspect of the value chain must be considered. Harvesting systems and technologies must be adapted for a biofuel-feedstock regime, harvest storage and transportation have to be optimized for low-density crops and all new or adapted technologies must be acceptable and profitable to producers. Harvesting-system development at Iowa State is based on modular attachments to a standard combine to lower capital conversion costs and maintain flexibility.

Iowa State is also developing the New Century Farm as an integrated research and demonstration entity for biomass production, processing and utilization. Research areas will include biomass-germplasm development; crop production; harvesting, storage and transport; biochemical and thermochemical biomass processing; byproduct utilization; and ecological, social, and economic systems impact. The farm will be located west of Ames, IA, on an existing ISU research farm, with construction beginning in the spring of 2008 and a completion goal of spring 2009.

Complete life-cycle analyses of biofuels production and processing are necessary to hold the industry accountable. Problems with biofuels must be addressed and LCAs used to assess energy, greenhouse gases, and sustainability. Analyses should include harmonized accounting of the benefits, ownership of carbon-market benefits, and improved greenhouse-gas science and monitoring.■

Renewable Chemicals and Biomaterials

Polyhydroxyalkanoates: Plastics of the Future

Eve Wurtele (Iowa State University)
Christopher Nomura (SUNY-ESF)
Stevens Brumbley (Australian Institute for Bioengineering & Nanotechnology)
Kristi Snell (Metabolix)

Polyhydroxyalkanoates (PHAs) are a family of naturally occurring polyesters. Many PHA polymers have been described, with a wide range of characteristics that are significant for use in the plastics industry. Economically viable production of PHAs in plants is limited by detrimental effects on growth. Following up previous work to increase production of the PHA polyhydroxybutyrate (PHB) in *Arabidopsis*, researchers at Iowa State University have found that PHB can be selectively accumulated in plastids without affecting organelle function. This accumulation is dependent on the light regime, constituting up to 15% of dry weight. Ongoing work seeks to document the metabolic changes that cause increased PHB production in conditions of long-duration light exposure. Additional research efforts are being conducted on PHB accumulation in transgenic switchgrass, the metabolic profile of which is significantly different from PHB-producing *Arabidopsis.*

At the State University of New York College of Environmental Science and Forestry, researchers are investigating the production of PHAs in bacterial fermentations to overcome the challenge of controlling polymer formulation in transgenic plants. They have found that low-value glycerol, a byproduct of biodiesel production, can be used as a carbon source by the bacterium *Pseudomonas putida* KT2440 for PHA production, and that the PHA polymer composition can be adjusted by manipulating the quality of the glycerol. The use of low-value glycerol as a carbon feedstock could significantly lower the price of PHA production, as well as create a market for anticipated excess by-product glycerol from the biodiesel industry.

Scientists at AIBN are working on PHA production in field sugar cane. They have a three-gene pathway that allows one-step transformation of sugar cane, and have demonstrated successful subcellular compartment targeting. A greenhouse study demonstrated the constitutive production of PHB in non-photosynthetic tissue of the transgenic sugar cane. A high-throughput method for screening transgenic plants for high productivity allowed examination of 10,000 transgenic lines prior to greenhouse-scale evaluation. Additionally, they have constructed a genome-scale metabolic flux model of sugarcane to help in the choice of future targets for improving PHA production.

Metabolix, a Cambridge, MA, bioscience company, has formed a joint venture with Archer Daniels Midland called Telles to begin commercialization of Mirel, a family of bioplastics produced through large-scale fermentation of sugar by highly engineered microbes. The production facility is currently in the construction phase, with start-up expected in early 2009. Independently, research continues at Metabolix on biobased plastics in non-food crops. A multi-gene expression system has been developed with inducible promoters for *Arabidopsis*, thus avoiding the reduced growth caused by some constitutive PHB systems. Research on gene expression/transformation systems for switchgrass is ongoing.

Activities Along the Industrial Biotech Value Chain for Renewable Chemicals in Germany

Steffen Rupp (Fraunhofer Institute for Interfacial Engineering and Biotechnology)
Markus Wolperdinger (InfraLeuna)
Karin Bronnenmeier (Linde-KCA-Dresden)

Germany is making a concerted effort to link the work of university research, industrial production, and engineering to advance industrial biotechnology. An example of the interdisciplinary collaboration between science and industry is the Fraunhofer-Gesellschaft, a consortium of fifty-six institutes in Germany and around the world which constitutes one of the largest research institutions. It consists of engineers and life scientists, with “white” or industrial biotechnology as a key field of research. The institute

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works with market-research groups, government, and other stakeholders to forecast trends in biotechnology, and to direct research efforts most effectively across the value chain of industrial biotechnology, from resources to final products, with the goal of developing efficient, profitable biorefineries. Through their interactions, the Fraunhofer-Gesellschaft is actively working to be a "transmission belt" between science and industry.

Further efforts to realize the ideal of an integrated biorefinery are underway at the Leuna industrial site, where InfraLeuna GmbH is working to integrate renewable chemical platforms, value chains, and infrastructural requirements for the chemical industry. A scale-up center has been established to close the gap between laboratory- and industrial-scale production. This shared pilot-plant facility offers a neutral ground for collaboration between small and large icompanies, with cost benefits achieved by avoiding the construction of many separate pilot plants at each user's location. In the planning stage, the facility will have a modular construction that allows feedstock storage, and reactor capacity of from 25 to 10,000 L. The facility will foster close integration of financial, engineering, utility, and other industrial users. Stakeholder use of the facility is expected to be for limited duration, with access awarded after a competitive screening process.

The Linde group has experience with industrial gas production, engineering and contracting for chemical, gas, biotech, and pharmaceutical plants, and logistics. Currently leveraging their experience for biorefinery projects, they view the biorefinery as a sustainable source of value-added products (including energy) from biomass inputs. Such a facility requires integrative processing, which is characterized by a high degree of complexity. Engineering constraints include the efficient use of biomass, flexibility of inputs and outputs, high energy recovery, and low emissions. Reference projects displaying the Linde group's experience include a biopharmaceutical venture for Roche in Switzerland for the production of monoclonal antibodies, a starch-based biorefinery in Germany, and a fossil-based refinery and polyethylene plant in Saudi Arabia.

Advances in Crop Biofactories in Australia

Cameron Begley (CSIRO)
Mike O'Shea (CSIRO)
Allan Green (CSIRO)
Victoria Haritos (CSIRO)

Motivated by the global drivers of population growth, climate change, and resource development, and influenced by national commitments to crude-oil security, Kyoto obligations, economic growth, and a positive balance of trade, Australia is working to build its bioeconomy by leveraging its efficient export-oriented agricultural capabilities with increased biorefinery capacity. This effort is being conducted by a variety of academic, governmental, and industrial organizations, including the Commonwealth Scientific & Industrial Research Organization. CSIRO is currently in the first of a three-stage crop-biofuels initiative, working to develop proprietary platform technologies and proof-of-concept production in the non-food crop safflower. Ongoing projects include vernolic oil, fatty acid monomers, and novel protein biopolymers (silks).

Scientists at CSIRO Advanced Materials have broad experience with advanced materials, ranging from polymer banknotes to spinoff companies working with medical polyurethanes. The production of advanced materials from novel plant compounds, specifically the crop-based production of unusual fatty acids, represents a current avenue of active research. Fatty acids are an attractive target because of their functional diversity. Some, such as 2-hydroxypolyacetylenic fatty acids, represent an attractive new platform because of their ability to self-assemble through hydrogen linkages. Intensity of photopolymerization can alter the characteristics of polymers derived from this fatty acid platform, allowing construction of sensors based on shape change. Other fatty acids have demonstrated biocidal activity. Saturated and unsaturated hydroxy-fatty acids are being tested as modifiers for condensation polymers, thermoplastics, and adhesives, leading to a wide variety of material characteristics.

To develop a plant-expression platform for these unusual fatty acids, CSIRO researchers are seeking to combine the high yield and high oil content of traditional oilseed crops with the wide natural diversity of fatty-acid structures in wild plants through metabolic engineering. Progress has been made with: enzyme and gene discovery by documenting a family of gene products that modify the Δ12 position of unsaturated fatty acids; substrate enhancement by applying proprietary RNAi technology to remove competing pathways; and working to increase product accumulation through gene discovery for appropriate acyltransferases from plants with high accumulation of unusual fatty acids. Through these efforts, significant increases in the plant-based accumulation of unusual fatty acids have been realized, and through continuing research, commercially viable levels are within reach.

Experience at CSIRO is not limited to fatty acids, oils, and carbohydrates; research is ongoing into other biopolymers, some of which are protein products, such as silks. These are stable, strong, biodegradable, water-insoluble, and natural, but suffer from performance limitations in water, staining in sunlight, and challenges of producing new materials and domesticating spiders, insects, *etc*., to produce large quantities. These silks are also difficult to spin. Their protein structure (extended beta sheet) presents a serious challenge because there has been no full-length expression of the large (>250 kD to 1 MD), highly repetitive gene sequence. Honeybee and lacewing silk have a smaller secondary structure and are easier to purify than spider silk; the gene can be expressed in microbes, fungi, and plant hosts. This recombinant silk, recovery of which is accomplished with mild thermal treatment that does not negatively affect downstream processing, has demonstrated similarity to native silk in terms of protein structure, elasticity, and stiffness, suggesting opportunities for novel silk-protein biomaterials.

Nanobiomaterials

Manjusri Misra (Michigan State University)
John Dorgan (Colorado School of Mines)
Jonny Blaker (Imperial College)

Nanotechnology provides tools that enable the design of materials at the nanoscale. Multifunctional nanomaterials, such as nano-biocomposites, which combine nano-reinforcements with polymer matrices obtained from renewable feedstocks, are an emerging technology with significant promise with various industrial and engineering applications. These multifunctional materials have multiple uses, such as improved mechanical strength, improved thermal and environmental stability, improved appearance, improved barrier properties, reduced flammability, and self-sensing capability. Potential applications of these nano-biocomposites include use in the automotive, packaging, and construction industries.

The integration of biotechnology and industrial ecology has created a new market for bioplastics, potentially greenhouse-gas-neutral polymers. However, these polymers suffer from performance drawbacks (particularly their low heat-distortion point). One method for addressing these drawbacks is the creation of bioplastic microcomposites, which integrate materials such as glass or natural fibers in the bioplastic. Nanoscale compositing materials can realize even greater property improvements. However, the life cycle impact of traditional nanoscale fillers must be evaluated through the application of industrial ecology. The integration of nanotechnology, biotechnology, and industrial ecology may give rise to “ecobionanocomposites,” materials that contribute to global environmental quality while economically meeting industrial-performance requirements. Unlike the inorganic clays commonly used for making nanocomposite materials, ecobionanocomposite feedstocks would be renewable (not mined) and degrade after use into innocuous substances. Researchers at the Colorado School of Mines have been investigating the use of cellulose as a composite filler material for use with bioplastics. Cellulose composites have been constructed previously by grafting cellulose acetate to polylactic acid. However, cellulose acetate is expensive, so the current work targets the supramolecular structure of cellulose microfibrils instead. These supramolecular structures bridge the length scale between the traditional material classes of copolymers and microcomposites, and behave similarly to chopped glass fiber, but are lighter, and should be less expensive. Researchers have demonstrated improved heat-distortion properties, transparency (demonstrating good dispersion at filler concentrations of up to 15% cellulose), and are working on commercialization.

Researchers at Imperial College London are also working to combine cellulosic materials to obtain all-cellulose hierarchical composites. These composites would represent a new class of materials with superior mechanical, environmental, and chemical performance, as well as significantly reduced life-cycle costs. A crucial issue with these green composites is the incompatibility of natural fibers with many polymers used as the matrix material. To overcome this incompatibility, researchers have developed the technique of modifying the natural fiber surfaces by attaching nano-scale bacterial cellulose to the fiber surfaces. The fiber surface was successfully modified by culturing a strain of cellulose-producing bacteria in the presence of natural fibers that had been treated with sodium peroxide. The presence of bacterial cellulose increases the effective fiber surface area, leading to greater interaction with the cellulose matrix. The composite performance can be adjusted by tuning the degree of crystallinity of the cellulose matrix, but the presence of the nano-cellulose attached to the fiber surfaces leads to composites with improved mechanical performance. This research suggests that all-cellulose hierarchical composites may be a substitute for traditional glass-fiber-based reinforced polymers in the near future.

Biomaterials in the Automotive Sector

Doug Pickett (General Motors)
Hamdy Khalil (Woodbridge Foam)
Mohini Sain (University of Toronto)

As with other industries, the automotive sector is subject to commodity pricing, and so it is open to biobased products provided they reduce costs and meet performance requirements. General Motors, now 100 years old, is a global company, with standardized engineering, business processes, and materials across the planet. Therefore, the business requires interchangeable materials. The materials used have evolved from the cotton-canvas and wood bioproducts of the horse-drawn-carriage era to current materials because of the emergence of materials with better technical performance. Biobased materials are still used in automotive production, from balsa wood in Corvette flooring, allowing mass reduction, to natural fibers used in headliners for acoustic benefits, to wood trim in some

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models for satisfying customer preferences. Incorporation in cars of soy-based foam products, bioplastics, and biofibers is expected soon, as well as novel materials for structural applications. Currently, in evaluation of new materials, biobased products are given preference, assuming parity of performance and cost. Priorities with new materials are car-mass reduction and reduced environmental impact while meeting performance and perceptual-quality goals, and managing costs. New materials are constantly under examination, and suppliers are invited to submit biobased products that meet specifications.

Woodbridge Foam Corporation, a supplier to the automotive sector, is currently the market leader for plant-based polyol use in polyurethane foams. The Woodbridge group's sustainable supply strategy involves managing the erratic price of petroleum-based products with renewable, abundant, regionally appropriate biobased feedstocks. Research is commissioned at selected universities and other institutions, and vertically integrated partners supply renewable resources to develop proprietary materials and technology to deliver new materials for their customers. A key challenge of using regionally appropriate biobased feedstocks is the need for global consistency in product supply for their customers. Therefore, at the Woodbridge group they have worked to develop a process to homogenize plant oils to produce a consistent product by chemically transforming the oil to urethane grade polyol. They are targeting automotive interior applications for these biomaterials, and have developed targets for the amount of biomaterial incorporated into each component. As the technology advances, the biomaterial fraction will increase.

The key avenues to introducing new biobased products to the automotive industry are: satisfying the market demands of automotive companies; meeting mandated (legislative) requirements; and reducing costs for automotive companies. To supply automotive components, a vendor must be able to supply the material globally with just-in-time delivery, which means warehousing seasonal crops. Additionally, the biobased products must meet established quality metrics with high-volume production capacity. In other words, the production process must be scalable from laboratory to commercial production, which means that funding must be secured to support a multi-year scale-up process.

Conversion of Renewable Feedstocks to Chemicals Beyond Fuels in a High Crude-Oil-Price World: Perspectives for the Future Biobased Economy

Jim Millis (Draths)
Paul Bloom (Archer Daniels Midland)
Stephen del Cardayre (LS9)
Andy Shafer (Elevance Renewable Science)

Liquid fuel is not the only commodity that tracks the price of crude oil. Other chemical derivatives, such as benzene and other aromatic compounds, are also derived from crude, providing a market opportunity for the production of such compounds from non-fossil sources. At the Draths Corporation, synthetic biology, synthetic chemistry, process engineering, and materials science are being used to produce them from renewable feedstocks. This is an attractive market space because recent programs to develop routes to biobased chemicals have largely focused on shorter-chain aliphatic intermediates (lactate, propanediol, ethanol, *etc*.). However, biobased alternatives to aromatics and benzene-derived chemicals have lagged. Work is underway at Draths to produce the nylon monomer, caprolactam, from renewable feedstocks and to develop a route to the novel intermediate, phloroglucinol. These target compounds are of particular interest because caprolactam is currently synthesized from benzene via a complicated process that produces ammonia and has high energy, water, and fossil-feedstock needs, and phloroglucinol is not widely used because of the current dangerous synthetic pathway, although it is a useful component of adhesives and resins and has the potential for many derivatives.

Most R&D and commercialization efforts for biobased-chemicals and biofuels can be divided up into direct chemical replacements and bio-advantaged molecules. Of these two options, bio-advantaged molecules present the biggest challenge, but have the potential to introduce game-changing platforms for the future. Most large-scale biobased chemical announcements focus on producing direct replacements for petroleum-based chemicals; if economical conversion technology is developed, markets already exist for these products. Both approaches have different risk/reward potential and resource requirements. A balanced portfolio based on resource utilization, including planning for co-products, will be required in any initiative.

At LS9 they are taking an engineering approach to the problem of producing drop-in fuel and chemical replacements from renewable feedstocks. They begin with an economic analysis to define biocatalyst requirements, and then apply synthetic biology to optimize and scale a proprietary microbe to produce the desired product, rather than beginning with a catalyst and designing a process around it. They are manipulating fatty acid biosynthesis for the

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production of biphasic products, allowing product recovery without distillation. Such pathways can be controlled via metabolic engineering to improve the flux from substrate to product; additionally, the structure of the fatty acid products can be manipulated to tailor fuel properties. LS9 focuses on producing biocrudes that can be cracked through a process similar to that used on crude oil to produce products ranging from biofuels to commodity chemicals. Fatty acid esters are being produced for small-scale production of biodiesel; a demonstration plant based on this technology is envisioned within the next year.

The evolution of the petrochemical industry has been characterized by decreasing processing costs and increasing source costs. Biobased chemicals still have high processing costs, suggesting market opportunities if these costs can be reduced. Elevance grew from collaboration between Cargill, Materia and the Department of Energy to take advantage of this opportunity and to develop new products. When the company was formed, emphasis at other companies was on feedstocks or derivative products, but not on commodity chemicals useful as building blocks and materials for further industrial use. At Elevance, products have been developed in the functional oil and personal-care spaces; work continues on products for the anti-microbial agent, lubricant and additive markets.

"White" Biotechnology: From Building Blocks to Bioterials

Steffen Schaffer (Evonik)
Marcel Wubbolts (DSM)
Oliver Peoples (Metabolix)
Joseph Kurian (DuPont)

Those with experience in biotech-based production of goods high in the value chain (such as consumer products) have recognized an opportunity to produce intermediates from renewable sources. At Evonik they are implementing this reverse integration, having identified biobased compounds with superior performance for target applications, such as coatings and polymers, that have limited potential from classical synthesis. Using fermentation for processing instead of classical synthesis opens the door for new compounds. Fermentation processes, traditionally using starch feedstocks, are currently being expanded to exploit a wide variety of other feedstocks, including methanol. Methanol fermentation produces a wide variety of intermediates that have use as building blocks for industrial chemical production. At Evonik they are working to build capacity for metabolic engineering of methanol-fermenting microbes.

As the second generation of biorefineries emerges, life sciences and material sciences are increasingly integrated. This integration introduces great potential for innovation of new and existing biobased building blocks for sustainable polymers. However, the transition to second-generation biorefineries is not without challenge. Although first generation (starch-based) biorefineries use their feedstocks efficiently and have markets for their byproducts, second-generation (cellulosic) biorefineries still pose unanswered questions about logistics, technology, byproduct markets, and feedstock efficiency. At DSM, a life sciences and materials company, they are leveraging their experience with industrial chemical production, enzyme production, and fermentation to address these issues. A partnership has been forged between DSM and Roquette for the production of biosuccinic acid—which can form the basis for biodegradable high-performance materials—from cereal feedstocks rather than crude oil. They are building upon their experience with industrial enzyme production in *Aspergillus niger* by evaluating their existing enzyme products for hydrolase activity that may be applied to lignocellulosic processing. Complete cellulose conversion has been demonstrated in simultaneous saccharification and fermentation (SSF) processes. They are also leveraging their experience with industrial baker's yeast production to use yeast for other fermentation processes. Through classical methods and metabolic engineering, yeasts have been developed that can ferment the common cellulosic sugars xylose and arabinose, as well as glucose and galactose. This broader utilization of sugars suggests better feedstock utilization, and more efficient biorefinery operations. Leveraging experience of industrial enzyme and fermentation processes will enable the development of mature second-generation biorefineries.

At Metabolix, the focus is on the production of biopolymers through fermentation and plant platforms. Their current commercial target is the production of polyhydroxyalkanoates. When these polymers are produced in, and recovered from, plants, the remaining biomass can be utilized for bio-energy or other co-products. In a joint venture with ADM—Telles—Mirel will be commercized, *i.e.* a family of bioplastics produced through large-scale fermentation of sugar by highly engineered microbes. The production facility is under construction, with start-up scheduled for early 2009.

In the past 15 years, the DuPont Corporation has reduced its greenhouse-gas emissions by 72%, partially due to their R&D efforts on next-generation materials and fuels from biomass. Their current goal is to use at least 20% biobased material, as verified by ASTM methods. Biobased monomers are produced at DuPont, such as 1-3 propanediol, for incorporation into polymers to produce cloth, thermoplastic, and a wide range of other products. The production of high-quality, high-performance materials from agricultural feedstocks is no longer a dream; it's an industrial reality.

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Advances in Biobased Chemicals Production

Huimin Zhao (University of Illinois)
Carol Lin (University of Manchester)
Robert DiCosimo (DuPont)
Philippe Soucaille (METabolic EXplorer)

Applications of directed evolution and rational design for protein, pathway, and genome engineering have been successfully demonstrated as engineering tools for industrial biotechnology. Specific examples include new bioprocesses developed at the University of Illinois for the synthesis of phloroglucinol and xylitol. Currently, about 200 metric tons of phloroglucinol are made each year from TNT, a dangerous process with serious environmental consequences. A novel method of synthesis combines biological synthesis for the precursor triacetic acid lactone, which is then chemically transformed to phloroglucinol. However, a phloroglucinol synthase enzyme was recently characterized and expressed in *E. coli*, allowing complete biological synthesis. This enzyme has been improved through direct evolution, and the bacterial host has been manipulated to over-express the enzyme. Since discovery, the concentration of biologically produced phloroglucinol has been increased sixteen-fold. Xylitol, one of the US Department of Energy's top-twelve platform chemicals, is currently produced from D-xylose, the purification of which is expensive. Therefore, it would be industrially beneficial to use hemicelluloses directly, or its hydrolysate, for the production of xylitol. The technical challenge of this approach is that xylose reductase enzymes are not specific for D-xylose and bind also to L-arabinose. By applying several rounds of directed evolution, an *E. coli* strain was produced with a sixteen-fold increase in selectivity for D-xylose, again demonstrating the efficacy of these engineering tools for industrial biotechnology.

Industrial production of chemicals from sustainable feedstocks requires cost-competitive strategies for processing biobased raw material. Research has progressed on the production of a generic wheat-based fermentation feedstock that could be converted into platform chemicals, biodegradable polymers, and biofuels via microbial bioconversion. In developing this generic feedstock, the aim is to use all wheat components for the production of both value-added end-products and precursors for chemical synthesis. Carbon and energy sources are provided through the enzymatic hydrolysis of wheat polysaccharides, and protein is hydrolyzed into amino acids to produce a nitrogen source. The wheat also provides growth factors such as trace elements and vitamins to produce a nutrient-complete microbial feedstock. Researchers at the University of Manchester have evaluated feedstock-formulation strategies based on the production of wheat hydrolysates and fungal autolysate for the microbial production of succinic acid with the facultative anaerobe *Actinobacillus succinogenes*.[4] Comparing media demonstrated that succinic acid was produced more effectively on a hydrolysate than on a semi-defined medium. Use of the fungal autolysate as a nutritional supplement showed that the wheat-derived feedstock contained all the essential nutrients for *A. succinogenes* growth and succinic acid production.

The use of enzyme-catalyzed reactions shows commercial promise in the production of disinfectants. Peracetic acid is widely viewed as the "gold standard" of biocides, but widescale use has been limited by problems related to shelf life and odor. DuPont scientists have been researching the use of enzymes to reproducibly generate an efficacious concentration of peracetic acid *in situ* and on demand using food-grade triglyceride and hydrogen peroxide as substrates. They have screened commercially available lipases and identified several with such catalytic action, but with low perhydrolytic specificity, thus requiring high enzyme concentrations. Subsequently, it was discovered that enzymes belonging to the structural family of CE-7 esterases exhibit significant perhydrolytic activity. These perhydrolases share a conserved structural motif that imparts superior perhydrolysis activity, making them a unique family of enzymes particularly suitable for the generation of peracids *in situ* at room temperature at concentrations sufficient for use in disinfectants. To commercialize this process, fermentations for protein production are being optimized.

METabolic EXplorer is a French biotech company that uses proprietary bacteria to reduce the costs of production of selected compounds by at least 30%. The company's strategy is to choose a target compound, then to design a metabolic pathway for its production, followed by optimization at the laboratory scale before pilot-plant and full-scale production. Their current portfolio of compounds includes L-methionine and glycolic acid, currently produced by *E. coli*, and MPG, propanediol, and n-butanol, which they are working to produce with *Clostridium acetobutylicum*. *C. acetobutylicum* can utilize a wide variety of substrates and has previously been used for the industrial production of solvents; however, there are no publicly available tools for gene deletion or insertion. METabolic EXplorer has implemented tools for this genetic manipulation, and has been able to use *C. acetobutylicum* for the production of n-butanol.

From Polyols to Propylene Glycol

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Hari Sunkara (DuPont)
Yusuf Wazirzada (Cargill)
Sharry Lynch (UOP)

Oxoreductases require cofactors such as NAD(H) and NADP(H) for their catalytic activity. These cofactors

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must be regenerated because they are prohibitively expensive to supply on an industrial scale. A variety of cofactor-regeneration strategies has been proposed, including enzymatic and electrochemical methods. However, these methods suffer from the requirement of a second mediator enzyme, and from detrimental interactions between electrodes and enzymes. Researchers at Seoul National University have constructed a novel biocatalytic electrode that allows electrochemical regeneration with no mediator. This method arose from experiments with a combined electrode and biocatalyst constructed by immobilizing cells and conductive powder on the electrode. They discovered that the close contact between the electrode and immobilized cells resulted in cofactor regeneration without a mediator. They have since refined the technique by immobilizing enzymes instead of cells and using porous metal oxides as the electrode to increase surface area, and have demonstrated the technology for cofactor regeneration in continuous nitrate removal, conversion of 2-propanol to acetone by alcohol dehydrogenase, and deamination of L-glutamate to alpha-ketoglutarate. This technology has a wide variety of potential applications including: synthesis of chiral compounds, alcohols, aldehydes, and ketones; steroid functionalization; enzymatic fuel cells; and enzyme biosensors.

At the DuPont Corporation, scientists have developed a new family of polymers made from 1,3-propanediol, which is produced from fermentation of dextrose in a non-conventional polycondensation process. These high-performance "Cerenol" polymers are long-chain ether-linked diols with properties useful as the soft segments in elastomeric polymers, as well as in personal care products, lubricants, coatings, and inks. Cerenol polymers contain varying non-petroleum sourced fractions, ranging from 50% to 100% bio content. Compared to petroleum-based polymers, the manufacturing of Cerenol is environmentally friendly and safe, with less lifetime greenhouse-gas emission, low toxicity, and high biodegradability. Cerenol is comparable to the commercial polyols polytetramethylene ether glycol (PTMEG) and polypropylene glycol (PPG), but avoids the drawbacks of PPG, and has advantages over PTMEG that include lower viscosity and melting point, suggesting that it is a better elastomer. It has a thermo-oxidative stability comparable to that of PTMEG, and is a good ingredient for thermoset and thermoplastic polymers. Cerenol is commercially available.

At Cargill, a vertically integrated value chain supplies agriculturally derived polyols to manufacturers for production of biobased polyurethanes. They moved from the concept of biobased polyols to commercialization of a first-generation product in less than 3 years, and are working to develop a 100% polyether polyol replacement. These biobased polyols bring new product-supply options, supply stability, and the opportunity for differentiation with novel chemistry. Previously, the successful use of natural oils in flexible slabstock applications was limited due to issues of quality consistency, odor, impact on physical properties, and processing limitations. However, after a performance-focused R&D effort, BiOH polyols are now on the market, produced with chemistry detached from propylene or ethylene oxide, with resolution of the odor and quality-consistency issues previously encountered with other natural oil-based polyols.

The combination of high crude-oil prices and the emergence of biodiesel as an alternative renewable fuel creates new opportunities for the production of propylene glycol (PG) from low cost glycerin (a biodiesel byproduct). The supply of glycerol is expected to far outpace demand, and so the price has already eroded as producers look for alternative markets. Fortunately, glycerol is versatile, and has a low price compared to propylene oxide, the crude petroleum-based feedstock currently used. At UOP LLC, a Honeywell Company, researchers have been working with colleagues at the Pacific Northwest National Laboratory to commercialize a highly selective catalytic conversion process for this biobased feedstock. The catalytic conversion process is more energy efficient and consumes less water than the production of PG from propylene oxide. As this technology comes to market, it has the potential to replace the ethylene glycol (EG) used in antifreeze. In 2008, the EG demand from the antifreeze market was larger than the total PG market, thus representing a significant opportunity. The selective catalyst developed by UOP and PNNL has been implemented at pilot-plant scale with good results, achieving 99.5% PG purity at $0.30 per pound less expensive than synthesis from crude petroleum.

Converting Lignocellulose into Biochemicals

Johann Gorgens (Stellenbosch University)
Johan Van Groenestijn (TNO)
Henk Noorman (DSM)

Current fermentative bulk-chemical production methods depend upon microorganisms selected and optimized on glucose-based media. The development of second-generation biorefineries utilizing lignocellulosic biomass will require the integrated development of biomass feedstocks, processes, and microbes. In this integrated context, the chemical composition of lignocellulosic feedstocks has substantial impact on the requirements for pretreatment and fermentation.

Sugar cane bagasse is one potential source as biomass. Sugar cane can be engineered through classical breeding and genetic methods to develop energy crops with enhanced yields and characteristics amenable to lignocellulosic processing, such as reduced lignin content. Similarly, cellulolytic yeast can be engineered to optimize fermentation of pretreated lignocelluloses. Integration of feedstock engineering and

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pretreatment with advanced cellulolytic yeasts has the potential to substantially improve the efficiency of lignocellulose fermentation.

Optimization of fermentation can take two approaches: a product-oriented approach in which a natural producer of a target compound is optimized for use with available feedstock, or a feedstock-oriented approach in which an organism that utilizes a variety of feedstocks is optimized to produce the target compound. The second approach allows new evaluation of microbial hosts for lignocellulosic feedstocks. Researchers at the Netherlands Organization for Applied Scientific Research have evaluated wild-type strains of six industrially relevant microbial production hosts (*Escherichia coli, Corynebacterium glutamicum, Saccaromyces cerevisiae, Pichia stipitis, Trichoderma reesei*, *Aspergillus niger*) for utilization of lignocelluosic feedstocks, including corn stover, wheat straw, bagasse, willow, and waste glycerol. The feedstocks had thermal pretreatment under mild acid conditions followed by enzymatic hydrolysis or concentrated acid pretreatment and hydrolysis. The microorganisms were evaluated for inhibitor resistance, carbon-source utilization versatility, and general performance. Although all six production hosts efficiently utilized pretreated real-life feedstocks, carbon-source versatility and inhibitor resistance were the major discriminating factors. Overall, *A. niger* was the most efficient host, followed by *P. stipitis*.

Boosting nature's selectivity with the latest achievements in functional genomics and advanced bioprocess design, including smart multifunctional bioreactors and *in situ* product recovery, help the chemicals industry to create new and sustainable markets. At DSM there is a long history of industrial biotechnology, and they continue to leverage existing platforms for enzymes and microbes to develop new tools spanning the range of industrial biotechnology, from feedstock development to process and organism improvement to novel products. DSM scientists participate in collaborations for open innovation, publishing results on sequencing the genome of *A. niger*, improving the production of fumaric acid via fermentation, and the production of 4-hydroxybutyrate and its lactone. Research is conducted also on incorporating green monomers into polyesters, drawing on experience balancing product requirements for stiffness and flexibility. These efforts build upon existing production platforms such as the company's history with yeast technology and hydrolase use in the food industry.■

Fine Chemicals, Food Ingredients and Pharmaceuticals

Cell Factories for Fine Chemicals and Vaccines

Peter Punt (TNO)
Israel Goldberg (Hebrew University of Jerusalem)
Oscara Sánchez (University of the Andes)
John Frenz (GlobeImmune)

At Dyadic, filamentous species are being used in "fungal cell factories" as versatile sources of proteins. Low-protease mutants of *Chrysosporium lucknowense* are producing human antibodies and strains of *Aspergillus* have been genetically engineered to produce bovin chymosin, human lactoferrin, human antibodies and human interleukin 6. System improvements continue, including increased gene-copy number in host strains, improved low-protease strains, new efficient gene-disruption approaches, low cellulase-background strains, improved fermentation conditions and more-efficient recovery approaches.

"Platform" organisms are those that are efficient cell factories but are unable to synthesize and excrete desired metabolites. For example, the glutamic acid producer *Corynebacterium glutamicum* can be manipulated to produce a range of amino acids as well as inosinic and a succinic acid. Citrate-producing *Aspergillus niger* and fumarate-producing *Rhizopus oryzae* are being genetically modified for over-production of lactate (superior in several respects to lactic-acid bacteria) used, *inter alia*, for production of the new biodegradable plastic, polylactic acid. The recently discovered bacterium *Paracoccus marcusii*, which is pigmented due to presence of carotenoids, is being mutated and grown in modified media for over-production of astaxanthin, a powerful anti-oxidant.

Fructooligosaccharides (FOS), produced by plants and some microorganisms, are increasingly popular as natural food ingredients particularly as sweeteners in formulations for diabetics. The key enzyme is fructosyltransferase (FTase). Effects of temperature, pH and initial sucrose concentration on FTase activity were examined in free-living versus alginate-immobilized cells of *Aspergillus*, with particular reference to the transfructosylating:hydrolytic activity ratio. The best conditions for FOS production were pH 5.50, 60°C and sucrose concentrations higher than 50% with free mycelium, with a conversion selectivity higher than 90%, remnant sucrose of about 8% and a FOS yield in the range of 51 to 53% w/w. Fructosyltransferase produced by *Aspergillus* can be considered for FOS production scale-up.

Tarmogens—*tar*geted *mo*lecular immuno*gens*—are whole, heat-killed yeast cells genetically modified to express (a) protein antigen(s) that stimulate the immune system after hypodermal injection. They are taken up by cells that activate the immune system, stimulating a T-cell response against the desired target(s). This platform has a number of advantages over current approaches; Tarmogens generate potent T-cell immune responses, are not neutralized by the immune system, can be rapidly engineered and are simple and inexpensive to manufacture. Tarmogens as therapies against hepatitis-C infection and pancreatic cancer are in phase-2 trials; several others are in the pre-clinical stage.

New Sources of Biopharmaceuticals

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Karen McDonald (University of California-Davis)
Ivonne Naumann (University of Erlangen-Nuremberg)

Statins are cholesterol-lowering drugs that decrease the risks of heart disease, stroke and heart attack. Simvastatin is a semisynthetic derivative of lovastatin, a natural product isolated from the filamentous fungus *Aspergillus terreus*. Simvastatin—significantly more potent than lovastatin in lowering cholesterol biosynthesis—had US sales of $4.3 billion (Merck) in 2005. Simvastatin is synthesized from lovastatin in multiple steps with an overall yield of <60%. By cloning cDNA of LovD (acyltransferase) from *A. terreus* into *E. coli* and overexpressing it, a >99% conversion of monacolin J to simvastatin was obtained. Whole-cell biocatalysis is an attractive alternative to the multistep semisynthetic method.

Threats of global pandemics and bioterrorism dictate the development of cost-effective scalable systems for rapid production of human therapeutics, vaccines, antitoxins, antibodies, *etc*. Transgenic plant-based systems require long timeframes. A novel plant-based expression system is under development for high-yield production of such therapeutics via transient expression in non-transgenic (wild-type) plants and plant tissues. The broad host-range plant pathogen cucumber mosaic virus (CMV) is the vector of gene transfer. The coat-protein RNA is replaced by the RNA of choice, thus virions cannot be reproduced and virus cannot move from cell to cell. Some T-DNA of *Agrobacterium tumefaciens* is replaced by the CMV expression cassette, then "agroinfection" transfers T-DNA to the plant cell and its nucleus; the T-DNA genes are transcribed, and mRNA is exported to the cytoplasm where translation takes place. Tested methods of introduction of genes of interest to whole plants and removed leaves include pressure injection, topical application and vacuum infiltration. Expression of human alpha-1-antitrypsin at 4 days after induction via vacuum infiltration was higher in detached leaves than in intact leaves of *Nicotiana benthamiana*, indicating a scalable methodology.

Herpesviruses—widespread in humans and vertebrates—is the most common agent of fatal disease in immunocompromised patients. The few available antiviral therapies have negative side-effects and lack specificity, and resistance is common. There is pressing need for new antiviral drugs. It has been known for some time that sulfoquinovosyldiacylglycerides (SQDGs) are active against HIV by inhibiting the reverse transcriptase. Nine algal species (Cyanophyta, Heterokontophyta, Chlorophyta and Rhodophyta) were grown in photobioreactors and production of SQDGs tentatively confirmed by thin-layer chromatography then identified by mass spectrometry. None of the three Chlorophyta species showed SQDG activity. A virus-specific peroxidase staining method confirmed anti-human cytomegalovirus activity in MRC-5 host cells of SQDG from *Porphyra purpurea* (Rhodophyta).

Advances in Biocatalysis

Francesco Molinari (University of Milan)
Eng-Kiat Lim (University of York)
David Rozzell (Codexis)

Captopril is an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of hypertension and some types of congestive heart failure. A new chemo-enzymatic method of synthesizing Captopril was developed, using 2-methy-1,3-propanediol—an inexpensive, water-soluble, *meso*-molecule—as substrate. A strain of the bacterium *Acetobacter pasteurianus* converts 2-methy-1,3-propanediol into the key intermediate *R*-HIBA, with 100% efficiency at ~95% enantiomer excess. Ramoplanin is a lipoglycodepsipeptide produced by the bacterium *Actinoplanes*, highly active against Gram-positive bacteria, including methicillin-resistant staphylococci and vancomycin-resistant enterococci. Local tolerability when injected intravenously can be improved by replacement of the original fatty acid chain with different acyl-residues. Screening of 620 strains of *Streptomyces* spp. for amidase/acylase activities revealed a hundred positives, of which one had high membrane-bound activity with ramoplanin. Optimization and repeated-batch operations allowed biotransformations on a multi-gram scale. An extracellular enzyme from a *Streptomyces* sp. allows demannosylation of N-boc-ramoplanins; its purification gave a biocatalyst with high selective/specific activity.

Glycosylation is a key regulatory process that affects the subcellular location of compounds and their transport, alters metabolic flux and yield of compounds and affects bioactivity. Glucosyl transferase (GT) sequences in *Arabidopsis thaliana*—107 in all—were cloned in *E. coli*. The flavonoid, quercetin, was found to be recognized by twenty-nine of the *Arabidopsis* GTs. Small molecules added to the *E. coli* system can be glycosylated and then excreted into the culture medium; thus, glycosides of benzoates, flavonoids, phenylpropanoids, coumarins, terpenoids and phytohormones, can be synthesized for pharmaceutical, *etc*., use. A synthesis system is being developed also in plants, for large-scale field-production of medicinal glycosides.

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Interest is increasing in using biocatalysts (enzymes) in chemical engineering, as chemists come to understand the advantages, particularly for the synthesis of chiral intermediates and from the environmental perspective. Twelve "green" chemistry principles have been enumerated, including waste minimization, involvement of safer chemicals and use of renewable feedstocks. Furthermore, the biocatalysis-use paradigm is evolving. Within the old paradigm, the process was designed around the best available enzyme, whereas the current approach is to design the desired conceptual process using "green" methods and to improve the fitness of the enzyme for the desired process by gene-shuffling. TBIN—the key chiral intermediate for the cholesterol-lowering drug Lipitor—is now synthesized using biocatalysis, as is montelukast, the active ingredient in Singulair, for treatment of asthma and allergies.

Industrial Biotechnology Boosting the Food & Drinks Chain: From Science to Product

Johan Van Hylckama Vlieg (NIZO)
Anthony Borneman (Australian Wine Research Institute)
Carl-Erik Hansen (Nestlé)

Lactic-acid bacteria convert sugar to lactic acid and other functional molecules including B-vitamins. Five species are important to the food industry, within which thousands of food-grade strains exist with diverse metabolic pathways producing many flavors for possible use in cheese-making, for example. A high-throughput-screening system uses 96-well arrays providing variation in medium ph, salt content, moisture content, *etc*., allowing assessment of 600 cheeses/person/day. A miniaturized, disposable microbial culture chip has been fabricated by microengineering a highly porous ceramic sheet with up to one million discrete wells as small as 7×7 µm. This versatile culture format, allows examination of micro-colonies (200–300 cells). The chip has been used for high-throughput screening of >200,000 isolates from Rhine water for ability to metabolise a fluorogenic organophosphate compound, resulting in the recovery of twenty-two micro-colonies of desired phenotype. Intraspecies diversity in terms of the genomics of the lactic-acid bacteria (LAB) has been contrasted and compared with phenotypic diversity as a means of identifying desirable probiotic characteristics in LAB of non-dairy origins. Mannose-specific adhesion to the intestinal epithelium was identified as a probiotic factor; it might be important for competing with pathogen-binding sites in the gut.

Wine is produced in thirty-four countries; grapes are grown on 8 million ha. Of the ~30 billion litres of product, 15% to 20% is surplus. There is need to make more than one type of wine from a single supply of grapes, which is possible by manipulation of the yeast. The primary role of the yeast is the synthesis of ethanol, and the secondary role is the synthesis of metabolites that affect flavor—acids, alcohols, carbonyls, esters and sulphur compounds—and conversion of grape-derived precursors to aromatic compounds such as terpenols and thiols. Employment of GMOs in winemaking is banned in most countries. However, wine yeast is genetically engineered for research purposes to provide insights into how improvements may be achieved by other means. An intra-species self-cloning strategy is used to achieve recombination. It is possible to sequence the yeast genome within a week at a cost of $5,000; five commercial strains have been sequenced and winemaking traits have been mapped to polymorphisms. Metabolomics is being used to custom-design yeast strains for specific consumer preferences.

Open collaboration between industry and academia is increasingly important to Nestlé. In 2007, more than 300 subcontracts were in operation, with emphasis on "disruptive innovations" for consumer benefits in terms of food safety and promotion of good health throughout life. An important priority is flavor—making healthy food taste good.

Biotech in Big Pharma—"Red" Biotech

Steven Projan (Wyeth)
Robert Ryall (Sanof Pasteur)
Steve Farrand (Schering Plough)

Biotechnological approaches to drug discovery were instituted at Wyeth in 2003 in parallel with "traditional" chemical synthesis of small molecules for treatment of cardiovascular and metabolic diseases, inflammation, neurological lesions, cancer, women's health issues and musculoskeletal problems. Overall, biological drug discovery takes two thirds the time taken for small molecules. Biopharmaceutical drugs provide means of attacking unique medical targets. This approach is more logical in that drug discovery in general requires thorough understanding of underlying biology. Biotech drug discovery is beginning to resemble small-molecule drug discovery in terms of development of libraries, use of laboratory automation methods, development of optimization technologies and of structure-activity relationships. After removal of marrow, fat and mineral content from bovine bone and implantation of the extract in rats, eighteen related bone morphogenic proteins (BMPs) were identified, with various roles and biological activities, for possible use in improving bone growth after major physical trauma. Calicheamicin gamma 1 is a potent cytotoxic agent—from the bacterium *Micromonospora echinospora* ssp. *calichenis*—that cleaves DNA, causing apoptosis. Tumor-specific antibody-targeted calicheamicin therapy—Mylotarg—is in phase-III trials as a treatment for leukemia.

In the United States, 5% to 20% of the population contracts influenza annually, >200,000 people are hospitalized from complications and ~36,000 die as a result. Globally there are at least 500,000 deaths from influenza. Vaccination is the

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primary and single most cost-effective measure of prevention; Sanofi Pasteur is a major provider of influenza vaccine at 900 million doses annually, which would be insufficient to tackle a true pandemic. Improvements are being made including use of biotechnology to: increase vaccine supply (new cell-culture methods and recombinant technology); improve vaccine efficacy (with higher dosages, new adjuvants, delivery approaches that target antigen-presenting cells, live vaccines, and recombinant vaccines); improve vaccine administration (patch and intradermal delivery); increase pandemic preparedness (all of the above plus dose-sparing, anti-viral agents, monoclonal antibodies, *etc*.).

Biologics, already an important part of the therapeutic “arsenal,” are expected to be the primary growth driver in the pharmaceutical industry, accounting for approximately 60% of annual sales increases for 2004–2010. Growth in their use as therapeutics for oncology is predicted to be 79% for this period. Biologics provide diversification of risk, complementing small-molecule products, and have approximately double the success rate of small molecules in preclinical/clinical development: 15–25% vs. 7%. Schering-Plough is developing an increasing presence in the biologics marketplace as well as a staff and infrastructure that can serve as a foundation for major biologics franchises across multiple therapy areas. In 1982 and 1996, DNAX and Canji, respectively, were acquired to broaden capabilities in immunology, oncology, gene therapy and biologics, and in 2005 Shering-Plough Biopharma was founded in Palo Alto, to further broaden capabilities in biologics discovery research and early development, with particular emphasis on monoclonal antibodies, therapeutic proteins and gene therapy. In partnership with Organon BioSciences, a system-wide biotechnology network is under development, with laboratories and production facilities on the east and west coasts of the United States, Ireland, Europe and Singapore, producing therapeutics for cardiovascular disease, oncology, disorders of the central nervous system, respiratory disease, women’s health issues, and immunological and infectious diseases.

Production of Nutraceutical Ingredients Using “White” Biotechnology Processes

Hans-Peter Meyer (Lonza)
Michael Katz (Fluxome Sciences)
Volkert Claassen (DSM)
Herbert Woolf (HDW Consulting)

Nicotinates, including nicotinic acid and niacinamide, are vitamin-B3 supplements used in the food, animal feed, and pharmaceutical industries. Lonza’s commitment to green chemistry is illustrated by its process for producing nicotinates, which includes hydrolysis of cyanopyridine to niacinamide by an immobilized enzyme. Inclusion of the biological process means significantly fewer by-products. L-carnitine is a conditionally essential nutrient for fat metabolism, obtained from meat and to a lesser extent from vegetables. Complex chemistry is circumvented by converting butyrobitaine to L-carnitine by a mutant strain of *Agrobacterium*; Carnipure is marketed as a supplement that improves vascular health; it is used by athletes to shorten recovery time. Docosahexaenoic acid (DHA) is an omega-3 polyunsaturated fatty acid, a supplement thought to promote cardiovascular health. The chief source of DHA is coldwater fish, as a result of their consumption of zooplankton, but this is increasingly problematical due to over-fishing and pollutant contamination of fish oils. Lonza uses a non-parasitic/pathogenic, non-toxigenic and non-genetically engineered strain of the marine protist *Ulkenia* in a batch fermentation process to synthesize DHA.

Resveratrol, a polyphenol compound synthesized in the skins of red grapes and in certain plant roots is thought to have anti-aging properties, cancer-prevention activity and to be cardiovascular protective. Its presence in red wine is thought to be an instigator of the “French paradox” *i.e.* low incidence of coronary heart disease in southern France despite high dietary intake of saturated fats and sedentary lifestyle. At Fluxome Sciences, resveratrol is manufactured by a proprietary fermentation process as a pure, white, crystalline powder suitable for tablet and capsule formulations. Fluxome expects to open it for wider use as a functional-food component, in cosmetics, and as a pharmaceutical ingredient. The scale-up phase has been achieved and production partners are being identified for US market entry by the end of 2008.

Although health and wellness are increasingly important factors influencing what food products are brought to market, they are usually positioned as added benefits, secondary to other trends that provide product differentiation. Taste, but also texture, was, is and will remain the most important factor influencing consumer choice. DSM’s Maxarome products are yeast extracts that are natural taste enhancers with many applications. They can be used for salt reduction and, although of low fat content, they promote full flavor and can replace MSG. Meltagel, produced by “reshuffling” amylose and amylopectin from potato starch, is a healthy replacement for gelatin and/or fat that improves mouthfeel. CakeZyme is a microbial phospholipase that enables manufacturers to use up to 20% fewer eggs in baked goods. Addition directly to the cake mixture enhances emulsification, resulting in lower batter density, improved batter viscosity and delayed crumb setting. Consequently, the finished product benefits from increased volume, improved crumb structure and softness, and longer shelf-life. PreventASe is an asparaginase from the fungus *Aspergillus niger* that converts asparagines into aspartate. As a result, asparagine is unavailable for the chemical reaction that forms acrylamide (a toxic and carcinogenic compound) when starchy foods—bread, cake, cookies, potato chips, cereals, *etc*.—are heated.

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Even in the United States, where food is plentiful, there is inadequate intake of micronutrients and vitamins among at-risk groups—due to physiological, psychological, economic and social factors—compromising nutritional status. At-risk groups include those who consume less than the recommended daily amounts of fruits and vegetables, get little exercise, are overweight, are smokers, the elderly and infirm, and those who are immuno-compromised. Production of vitamin B_2 (riboflavin, which aids red blood-cell and antibody production, and cell growth) by the fungus *Ashbya gossypii* was improved. The endogenous isocitrate lyase (ICL) gene was isolated and subcloned into an ICL-defective mutant with improvement in promoter effectiveness. Unlike the hazardous chemical method of synthesis, the single-step fermentation process—with a 60% cost reduction—uses renewable resources and replaces twelve chemical compounds with sugar or soybean oil and replaces five solvents with water. The "natural" image has market appeal and the product is purer and easier to hand and to formulate.

Genomics for Food, Nutrition and Personalized Medicine

Mary Ann Lila (University of Illinois)
Rickey Yada (University of Guelph)

There is strong evidence that flavonoids synthesized by plants—grapes, blueberries, raspberries, *etc.*—confer many health benefits, but specific bioactive compounds and individualized roles have been difficult to identify and assign. A cell-culture production approach was developed to provide abundant, uniform yields of mixtures of flavonoids that correspond to those produced in nature by the same plant genotypes; isolation from cell cultures is more rapid and streamlined than isolation from plant tissues. Introduction of ^{14}C sucrose to the cell-culture medium enabled recovery and the identification of biolabeled flavonoid compounds, which were tracked in the serum of rats after gavage by jugular catheter insertion. Label uptake by gavage was followed as was label loss via feces and urine, and time-courses were constructed for label in blood and interstitial fluids. Label was detected in brain tissues and in bone catechins. The next challenge is to identify the metabolites that are responsible for *in situ* bioactivity.

Canada has twenty-one Networks of Centres of Excellence—linking universities, public and private sectors—in four broad areas: Health and Biotechnology, Information and Communication Technology, Engineering and Manufacturing, and Natural Resources and Infrastructure. The Advanced Food and Materials Network is in the Health and Biotechnology area and has $5.5 million in funding for a maximum of two 7-year cycles. Twenty projects are financed under three themes involving seventy-five professors at twenty-four universities (with 100–150 students, postdocs and research associates) and approximately twenty companies. The three themes are: Science and Engineering of Foods and Biomaterials, Food Bioactives and Health Outcomes, Regulation, Policy and Consumer Health. Six projects are financed under Food Bioactives and Health Outcomes with the mission of identifying safe and effective bioactive food components for optimal human health. Five nutrigenomics projects have had funding since 2006 (see below) at $2.95 million (3 years), renewable for up to 7 years, with $750,000 in industrial and government contributions. Twenty-four researchers are involved at seven Canadian universities (with ~25 students, *etc.*), with eleven industrial partners. Project titles are:

- Nutrigenomics and Biomarkers of Chronic Disease
- Nutrigenomic and Proteomic Approaches for the Study of Functional Peptides to Improve Gut Health
- Use of Conjugated Linoleic Acid as a Nutraceutical for Health Promotion in Humans
- Fish Nutrients and the Metabolic Syndrome: An Integrative Genomic and Metabolic Phenotyping Project
- Social Issues in Nutritional Genomics: The Design of Appropriate Regulatory Systems

The Strategic Transition and Application of Research (STAR) program operates within the Advanced Food and Materials Network to assist in the exploitation of research results and developments that promise to advance knowledge and technology-transfer in emerging areas related to foods and biomaterials research. It supports proof-of-principle and knowledge-mobilization projects that are of benefit and relevance to the social and economic health of Canada, with funding available up to $150,000 per project (1 year, non-renewable).

Biotech in the Fine Chemical Sector: "White" Biotechnology

Hans-Peter Meyer (Lonza)
Robert Holt (Piramal)
Norbert Windhab (Evonik Degussa)

Globally, chemicals constitute a $1,800 billion market of which "white" biotechnology has a $50-billion niche. It is predicted that 20% of global chemicals will be produced via biotechnology by 2020, representing huge business potential spread over diverse markets and products and involving many biotechnologies. Fine chemicals will be the main driver; it is predicted that 60% of the fine chemicals market will be met by biotechnological processes. On the other hand, many situations exist where chemists have need for "green" (biotech) solutions, but none are available. Furthermore, limitations are sometimes met when scaling up from pilot to large-scale production. Conclusions drawn by Swiss companies active in industrial biotechnology are:

- Too much attention is being focused on biofuels and feedstocks; concentration should be on high(er) value-added products.
- Fine chemistry has considerable bottlenecks, but seems to have the best outlook.
- For the time being, focus should be on contained fermentor systems; biotechnological process engineering and process technologies need strengthening.
- Investment models should be developed to efficiently support industrial biotechnology start-up companies.
- Stakeholders and their requirements should be defined.

The importance of chirality in pharmaceutical synthesis became an issue in the late 1980s, and with it came realization of absence of tools for solving the problem. Awareness of the potential of biocatalysis in chemical engineering grew with the acceptance that arose from clear need. Chemists who had avoided enzyme use increasingly turned to biocatalysis to solve problems. For example, in 1990, complex chemical synthesis of an anti-fungal compound produced an entantiomer with undesirable side-effects; this was solved by introducing a commercially available lipase. However, lack of availability of enzymes for specific tasks induced chemical engineers to turn to whole-cell approaches which presented potential for wide diversity of biocatalytic processes, possibilities of simple, rapid screening programmes and simple scale-up procedures. For example, using the yeast *Pichia angusta*, Piramal developed a whole-cell bioreduction method to produce the cholesterol-lowering drugs Lipitor and Crestor. Currently, with huge numbers of nucleotide sequences available in databases (*e.g.* for 15,000 esterases, 23,000 lipases, 18,000 alcohol dehydrogenases, 150,000 proteases), individual gene synthesis possible at $1,000–2,000 with a 2-week turnaround, and simplified cloning methods, there is transitioning back to exploitation of isolated enzymes.

Biocatalysis lends chirality and enantiomorphic selectivity to chemical synthesis. It is used at Evonic for production of D and L alpha-amino acids, (*S*)-beta-phenylalanine, and (*R*)- and (*S*) alcohols. Chiral technologies are key to success for a supplier to the pharmaceutical and fine-chemicals industries. Evonic's toolbox of enzymes gives access to a broad range of chiral building blocks, providing choices for the best route to produce the desired compound. An important development was the use of whole cells at high density. Sometimes, complex multi-step chemical reactions can be replaced by a single fermentation. Furthermore, with creative partners, Evonic seeks to extend the substrate-spectra of their cell-catalysts even beyond microbiology. In conjunction with Phytowelt, they are linking plant science and industrial biotechnology. Through "phytomining" they are identifying, isolating and expressing genes encoding plant enzymes to be introduced into microorganisms to provide new enzyme activities for cell-catalyst and fermentation processes. Evonik's Coatings & Additives unit has been supplying methacrylate polymers to the pharmaceutical and fine chemicals industry for over 50 years. These EUDRAGIT polymer coatings are used for solid enteric formulations administered orally for sustained as well as for immediate release.

Advances in Food Processing and Ingredients

Gaby Tiemi Suzuki (University of Campinas)

Riboflavin (vitamin B_2) is an essential vitamin, widely used in the pharmaceutical, food and feed industries. Produced by microorganisms and micro-algae, its key role is in the metabolism of carbohydrates and amino acids. It is effective in the treatment of migraine, malaria and Parkinson's disease and is protective against cardiovascular illnesses. Global demands for vitamins in nutraceutical products are expected to increase 4.6% annually to >$4.0 billion by 2010. Industrial production of riboflavin is currently achieved with the bacterium *Bacillus subtilis*, the filamentous fungus *Ashbya gossypii* and the yeast *Candida flaveri*. *Candida* sp. strain LEB 130—isolated from a sugar-plantation soil—was examined for effects of carbon and nitrogen sources on riboflavin accumulation in a shaken culture medium (absorption at 444 nm). Thirty-one carbon sources (sugars, sugar alcohols, fatty acids, carboxylic acids and oils) and twenty nitrogen sources (urea, inorganic compounds, organic salts, and amino acids) were examined separately as replacements for sucrose and ammonium tartrate, respectively. D-glucose and xylose increased riboflavin production four-fold. Several nitrogen sources gave significantly more riboflavin than did glycine, despite the fact that the latter is a precursor. Combinations of the best carbon and nitrogen sources have yet to be examined as has the potential for significant improvement in riboflavin production from strain development.

Biotech Production of Cosmetics and Fragrance

Richard Burlingame (Allylix)
Barbara Klein (Institute of Bioprocess Engineering)

Sesquiterpenes are plant-produced aromas that are insect-repellent and attractant. Variously with antimicrobial, antiherbivorial and antioxidant properties, they are multicyclic C-15 compounds with multiple chiral centers that are difficult to synthesize. Aroma chemicals represent a $1.8 billion market, with terpenes representing $0.68 billion. Monoterpenes are C-10 compounds, of which most that are of industrial relevance are synthetic; they are high-volume, low-cost compounds (*e.g.* 5,000 mt of menthol are sold at $10.00/kg). Most industrial sesquiterpenes are extracted from natural sources, representing a low-volume high-cost market. Nootkatone ($4,000–10,000/kg) is Allylix's first

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product; traditionally extracted from citrus peel, its high cost limits use to fruit juices, citrus-flavored beverages, and fine perfumes—lower volatility compared to monoterpenes means that the fragrance is "substantive," *i.e.* it lasts long on skin and clothing. Allylix's new technology will result in step changes in cost of production with broader penetration of the personal-care and household-cleaning markets. Yeast makes the sesquiterpene precursor farnesyl pyrophosphate (FPP), whereas sesquiterpene synthases (cyclases), which convert FPP to various sesquiterpenes, occur mainly in plants. By introducing synthase genes into a yeast strain engineered to produce large amounts of FPP, economic production by fermentation of a variety of commercially attractive terpenes is now possible. Furthermore, protein engineering of terpene synthase genes is improving specificity/activity. Allylix is developing strains and processes for three sesquiterpene products. At multiple grams per liter, yields are at, or are approaching, initial scale-up targets and commercialization is expected in 2008.

Coenzyme Q_{10} is used in cosmetics and medications as an anti-oxidant. Photobioreactor-screening modules were used to grow microalgae for coenzyme production—species of Chlorophyta, Rhodophyta, Haptophyta and Heterokontophyta—assaying with high-pressure liquid chromatography mass spectrometry and electrospray ionization mass spectrometry. *Porphyridium purpureum* (Rhodophyta) was a good source for the antioxidant and scale-up cultivation to 100 L was achieved. Accelerated solvent extraction improved product recovery by fourteen-fold.■

Business Development, Infrastructure and Public Policy

Ensuring the Sustainable Development of Biobased Industries

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Maria Wellisch (Natural Resources Canada)
John Heissenbuttel (Council for Sustainable Biomass Production)

The production and use of biofuels have the potential to address issues underpinning rural economic development, climate change and energy security. Which of these "drivers" is chosen as a focus determines the appropriate policies and programs to incentivize biofuel production. As an energy superpower, Canada can be a major player in the biofuels industry; 22% of its primary energy demand could be satisfied via its current forestry and agricultural production. The optimal use of biological resources depends on the priorities that are established, as different fuel sources have different advantages. Identifying a region with low costs, creating large-scale biomass production, coupling transportation systems to coal replacement and enabling large-scale biorefineries are key. The Great Lakes/St. Lawrence region represents a good opportunity.

Canada's definition of sustainable development is that which "meets the needs of the present without compromising the ability of future generations to meet their own needs." Policy infrastructure is in place at the federal and provincial levels to pursue such development. A piecemeal approach is being used to utilize agriculture beyond food, feed and fiber; research-support programs will promote the conversion of oilseed flax straw into new, higher-value products, including industrial products. The vision is to utilize flax industries as the basis for a sustainable economy, as part of a national strategy. Environmental, economic and social issues associated with sustainable development will be evaluated. In the future, benefits from genetically modified flax may be examined.

The Council on Sustainable Biomass Production has been established to ensure that feedstocks for cellulosic bioenergy and biofuel facilities in the United States are produced sustainably, balancing economic, environmental and social imperatives. Its goal is to generate broad multi-stakeholder consensus on the industry's development. The Council comprises producers, refiners, environmental organizations, private companies, government agencies and academics. It will develop draft principles, objectives and performance metrics through September 2008, and will then seek broad input, adjusting and testing standards. The government can best encourage sustainable development through carbon taxes and facilitating infrastructure development.

Partnerships for Successful Development of the Forest Bioref nery

Virginie Chambost (École Polytechnique)
Doug Freeman (NewPage Corporation)
Edward Pye (Lignol Innovations)

The term "forest biorefinery" implies full utilization of wood biomass for production of fibers, chemicals and/or energy. Volume/margin trade-offs exist for forest biorefinery products; for example, cellulose-based fibers have the greatest volume, but the lowest market price. Product-portfolio composition must be established, addressing issues such as whether co-products will be treated as resources or waste. The forest biorefinery could be a boon to traditional forest-producing nations that face an economic stalemate and to the pulp and paper industry. Forestry companies have key competitive advantages, but lack capital and innovative product-development cultures. To implement the biorefinery, companies will first need technology partnerships, then commercial partnerships and finally value-chain partnerships.

The pulp and paper industry and biorefinery-technology providers bring essential skills to a partnership. A partner must contribute a competitive edge and be a philosophical fit. Pulp and paper producers must first protect their core business, and then move towards reduction of fossil-fuel usage and investigation of biorefinery opportunities. Risk-sharing arrangements should demonstrate a strong commitment, and reimbursement should be provided if biorefinery technology cannot reasonably deliver. Finally, growth potential is a key factor in evaluating possible partnerships.

The need for alternative liquid transportation fuels drives interest in biorefining of lignocellulosic biomass. In addition to transportation fuels, biomass chemical byproducts could be utilized by the chemical industry; 40 million tons of lignin co-product and 23 million tons of other chemicals could be produced per year. Thermochemical, biological and biorefining technologies exist for production of fuels and chemicals from biomass. Organosolv technology represents a superior biorefining process in that it does not destroy fine chemical structures in the feedstock and creates a variety of products and byproducts that hold value. A current project in Colorado under a US Department of Energy program includes a demonstration plant. As production is scaled up, economies of scale in chemical production may be achieved.

Aligning Technologies to Address Market Drivers for Renewable Biobased Products

John Kelly (Clemson University)
Christophe Schilling (Genomatica)
Damien Perriman (Dow Chemical)
Alif Saleh (BioEnergy International)

Disruptive agricultural technologies provide opportunities to address energy and chemical needs. Recombinant chloroplast technology, gene sequencing and identification technologies, increasing volume of published plant genomes and high-yield transgenic plants all have the potential to contribute to a new agricultural era. As prices for petroleum derivatives and renewable energy demands rise, the US dollar falls and crop prices increase, agricultural products become more attractive. Various natural fibers can be used to produce biochemicals. Hybrid energy field crops, such as switchgrass, sweet sorghum and coastal loblolly pine, could be efficient sources of ethanol. Molecular genetic approaches may further enhance these crops. Using a public-private research-center model would optimize resources for commercialization and development. The Clemson Genomics Institute is rapidly expanding such capacity.

Biotechnology can be harnessed to deliver raw material-advantaged conversion technologies for the chemical industry. Genomatica's integrated metabolic engineering platform delivers metabolic modeling and simulation technologies combined with synergistic evolutionary and experimental technologies. A large range of metabolic designs can now be explored, while novel proprietary bio-factories reduce the time needed to make a product. Key criteria for product selection must be evaluated. Producers should begin with a number of pathways for a given chemical and then evaluate which, if any, is feasible.

Sustainable business practices create value. The current market outlook for biobased products, according to McKinsey & Company, indicates fast growth. Markets exist for a variety of products, including industrial goods such as adhesives and coatings. Momentum currently exists in this area, as polyethylene and propylene glycol can now be derived from renewable resources. Accessing feedstocks that are cost-advantaged, renewable and do not compete with food is important. Collaboration accelerates success when asset owners partner in strategic areas, such as for market focus, to mitigate risk, to advance proof of concept, for platform development and to generate a pipeline of market-focused products. Chemical biotech allows access to biobased products without affecting food-crop markets.

A platform for profitable production of biochemicals aligns technology with the market. The right technologies and feedstocks allow production of high-value chemicals. Products take about nine years from discovery to development; there is a dearth of commercially available products, but several could be available within a decade. Market volumes will vary by product. The development process is improving, as better results are being achieved in shorter timeframes. For example, today's technology for 1,4 butanediol is capital-intensive and complex, but scientific advances could facilitate a next-generation version within seven years. Producers should not expect to earn a premium in the market. It is critical to start by producing replicas of oil-based petrochemicals and then move forward, taking a long-term view of development and setting reasonable milestones.

Increasing the Public's Understanding of Industrial Biotechnology: What's the Story?

Michael Harris (BioWorld Today)
Brady Huggett (Nature Biotechnology)
Darlene Snow (Ogilvy Public Relations)

BioWorld's publication portfolio includes its *Biofuels Report 2007*, the *2008 Biofuels Report: Economics of a*

Driven Market and the *Industrial BioWorld Today Newsletter.* In launching an industrial biotechnology trade publication, one must brainstorm the market to find a theme and identify audiences and their needs. News can affect markets, and the media must realize that they bear a responsibility. Companies need to hear both good and bad news, but bad news must be reported without sensationalizing it. External factors must be taken into account: trade publications may need to accelerate publishing schedules to match mass-media coverage and provide balanced perspectives. Ethanol production in the United States is expected to increase significantly through 2012, demanding more coverage.

Nature Biotechnology has a readership of 106,000, 66% of which are located in the Americas. Coverage of industrial biotechnology in *Nature* includes the oil giants' support for biofuels research, analysis of the cellulosic ethanol boom, and commentary on biotechnology's contributions to biofuels. It also provides news of, and views on, academic papers. General media outlets do not cover the real industrial biotechnology story; publications such as *Nature* must give industry members information they need and promote significant industry news and accomplishments.

Several issues exist in the media landscape for industrial biotechnology. For example, the United Nations is still opposed to ethanol, and media cycles fuel both enthusiasm and enmity. The media drove initial enthusiasm over cellulosic ethanol, but now tend to dwell on the downsides. Biofuels are currently in a phase of confusion, as the public does not understand the mix of positive and negative information reaching them. The industry must educate detractors and develop a pipeline of positive stories. It also must generate a response strategy, correcting misstatements and utilizing independent sources to correct its positions. The conversation should always focus on positive developments. When addressing a crisis with the media, it is important to elevate the issue and respond and fix the problem; often the response to a crisis is judged, not the crisis itself. An Ogilvy survey indicated that understanding of industrial biotechnology is shallow and that industrial biotechnology is generally not covered as its own sector. This emphasizes the need to take advantage of opportunities for coverage and to provide accurate information, given the crowded media market.

Economic and Environmental Indicators of the Biobased Economy

John Ferrell (US Department of Energy)
Ron Cascone (Nexant)
John Miranowski (Iowa State University)
William Caesar (McKinsey)

Cost-competitive cellulosic ethanol could be available by 2012 and could displace 30% of US gasoline consumption by 2030. A combination of balanced analyses, constructive dialogue and smart policies is needed to push the biobased industry forward. These factors are being implemented by the US DOE, State Department and Congress. The DOE's biomass program is developing resources, technologies and systems for the production and use of biofuels to grow sustainably. It also addresses indirect land use, climate change, biodiversity and water-use issues. Additionally, the program is engaged in standards-development and principles-setting for bioenergy feedstocks. Cross-cutting efforts are planned to develop national-scale economic and environmental analysis tools and address sustainability challenges. This is supplemented by a regional biomass energy feedstock partnership that engages in field trials and related activities. The Energy Independence and Security Act of 2007 promises to increase demands for biofuels through its mandate and incentives.

Biofuels face critical challenges. Current feedstocks are food-based and limited compared to crude oil, while blended ethanol is inconvenient for use within the hydrocarbon fuel infrastructure. Despite concerns over the price of crude, the drivers are complex: economics, geopolitics, armed conflict and climate are working together to produce price increases. Peak-oil concerns, indicated by declining reserve replacement, also drive crude price and interest in biofuels. Although significant new refining capacity is planned, biofuels are—and are expected to remain—dwarfed by conventional fuels. Refiners are not of a single mind on how we should proceed with biofuels. Many fossil-based energy companies are getting involved, with varying degrees of interest. Logistically, butanol represents a better biofuel solution than ethanol.

Starch-based ethanol is neither a short-term nor a permanent fuel solution. It is useful as a transition fuel while other alternatives are developed. Corn prices are driven not only by ethanol, but also the price of crude oil/gas, sensitivity to the current tax credit, and other issues. The food-versus-feed-versus-fuel debate is driven by these issues, as well as by the weak dollar. Ethanol plants will likely remain concentrated in the Midwest in order to reduce transportation and other costs. For cellulosic ethanol to be feasible, the amount of biomass that suppliers would be willing to sell needs to equal the amount of biomass demanded; currently a large gap exists between the price processors could pay for biomass and the price farmers demand. Biomass ethanol tax credits and tariffs are less important now due to the renewable fuel standard. The United States still needs to address biomass production and infrastructure systems, and must also better utilize bioindicators to understand the biobased economy.

The investment climate for biofuels evolved from irrational exuberance to despair, as ethanol stocks plummeted 40% to 60% after January 2008. Value shifts during the past three years have continually altered the market. Feedstock prices, infrastructure and regulatory support and environmental challenges have all emerged as key drivers for investors. The investment climate around the world varies, from good in Brazil, to mediocre in the United States, to poor in Europe. Investors must decide whom to partner with, what technology to choose and where in the value chain to operate. For next-generation (cellulosic) biofuels, the investment climate is more attractive; R&D in this area is mostly a US play. With next-generation investments, options will expand and additional technologies may continue to emerge.

US Energy and Agriculture Policy Update: Federal and State

Matthew Carr (Biotechnology Industry Organization)
Lloyd Ritter (Green Capital)
Keith Cole (General Motors)
Pete Pellerito (Biotechnology Industry Organization)

The Energy Independence and Security Act of 2007 is a game-changer for biofuels. Its major provisions include corporate average fuel economy (CAFE) standards, a renewable fuel standard (RFS) and appliance and lighting efficiency standards. Starting in 2009, an increasing fraction of RFS products must come from cellulosic or other advanced biofuels. There is no mandate for corn ethanol specifically. Any renewable fuel from a facility constructed post-enactment of this bill must achieve a 20% reduction in lifecycle greenhouse gases over baseline amounts; this includes direct emissions and significant indirect emissions such as from land-use changes. The current biomass R&D program funding has been increased by 50% and extended through 2010, including Department of Energy biorefinery demonstration grants. The DOE bioenergy research centers have increased in number from three to seven.

The 2008 Farm Bill features a tax package, including a cellulosic tax credit. The ethanol blenders credit was expected to be reduced to $0.45 per gallon, the ethanol tariff extended for 2 years and a biodiesel tax credit left out of the bill. A biofuel loan guarantee program provides grants and loan guarantees for biorefineries and biofuels production plants to support advanced biofuels. Support for cellulosic ethanol and biodiesel feedstock purchases were expected to be included as well. The Resource Enhancement and Protection (REAP) Program provides support, including loan-guarantee programs, for all farm-based renewables. Federal agencies will now have to purchase biobased products, and an accelerated timetable for biopreferred label implementation will be established. The Bioenergy Crop-Production Program will encourage the development of the next generation of feedstocks by paying farmers for planting appropriate crops. Funding for the Biomass R&D Act is authorized at $200 million per year, which is not enough to tackle feedstock and related issues in building the bioeconomy. A variety of land conservation and transportation studies and programs will continue to measure the impact of bioenergy and needs for further growth.

Biofuels provide the biggest opportunity to impact fuel usage today. EPA standards, the Energy Independence and Security Act of 2007, state initiatives and proposed carbon-emissions cap-and-trade legislation all provide an environment in which they can contribute and make an impact. A cap-and-trade program would create a market for a finite level of carbon emissions. The Lieberman-Warner cap-and-trade bill carries a low carbon threshold, effectively ignores upstream greenhouse-gas emissions and includes a low carbon-fuel standard. Under it, refiners and importers of fossil fuels remit allowances equal to emissions when their product is burned, while utilities and large manufacturers remit allowances equal to the tons of carbon dioxide they emit. In this framework, capped entities would pay for allowances and for offsets. It is unclear how a cap-and-trade system would account for upstream greenhouse gases, which carries implications for biofuels.

States lead the way on biofuels legislation, with forty-one having passed legislation to encourage company formation. Reality checks for legislatures, however, have come in the form of the public's lack of basic understanding of biofuels, other alternative energy sources and unrealistic commodity expectations. Success will require partnerships between industry, technology creators (universities, non-profits, *etc.*) and policy generators. States are providing incentives for biorefinery construction and expansion, including property-tax relief, exemptions from environmental review and tax abatements. Some states are promoting market creation or expansion; Ohio has designated ethanol plants eligible for Air Quality Development Authority funding and Missouri's Renewable Fuel Standard Act requires most gasoline to have 10% ethanol. Several states are seeking to build capital for start-ups, broaden potential sources of biomass and create multi-state arrangements.

Building Incentives for Biotech Industrial and Environmental Companies

Michael Parr (Dupont)
Joe Keeley (Arent Fox)
Paul Naumoff (Ernst & Young)
Mike Bernier (Ernst & Young)

Dupont's biomaterials strategy features products made with renewable resources that integrate science and sustainable solutions, have equal or better performance than incumbents and have a smaller environmental footprint. Materials traditionally made from oil are now relatively more expensive; a new manufacturing process has led to the development of Cerenol polyols and the commercialization of the new polymer Sorona. Biomaterials have applications in many areas, including lubricants, coatings and cosmetics. Government incentives, such as tax credits and a renewable fuels standard, have helped develop a biofuels industry with economic, security and environmental benefits. Biomaterials provide similar benefits with greater potential for high-margin business creation and broad business ventures, yet they have not received significant federal support. States have provided some assistance, but limited and targeted federal policies would be helpful. Support for R&D would be most valuable.

The future of the biofuels debate will be much like those for the solar and wind-energy industries. The primary issues that were addressed included basic economics and integration into existing networks. Now, the debate is over ancillary issues, such as wildlife impacts and obstruction of scenic views. To encourage the industry throughout the course of the debate cycle, the government can provide a variety of tax incentives and grant programs from federal agencies, and Congressional funding for specific projects can benefit or hinder projects depending on their structuring. In the past, the federal government has implemented tax-incentive programs, mandates, grant programs for test projects and earmarks. The government will likely avoid projects that compete with food sources, such as corn ethanol. Businesses should build alliances with local and state constituencies, and talk to Congressional delegations and staff on Capitol Hill, use one person to coordinate efforts, communicate benefits and needs of projects and realize that government assistance is not an overnight process and that there are downsides to government grants and support.

State and local government and agencies are providing multiple incentives for industrial biotechnology development. Although tax incentives/credits are widely available, they should not make a business, but simply be supportive. Tax incentives are provided in a variety of forms. Financial incentives, such as grants, are popular as well; like tax incentives, a variety of structures is employed. Each of these options has benefits and drawbacks. Intangibles are often touted as inherent benefits that certain states and municipalities offer. These can include access to public transportation, universities and potential partners. Companies can benefit from understanding each step of the process required to obtain the incentives they seek. They must be prepared to identify benefit opportunities, pre-negotiate with governments to establish the value the company brings to the table, negotiate the specific agreement, capture commitments in writing and preserve the agreement by taking steps to meet requirements.

Federal incentives take different forms in the United States and Europe. Europe favors cash incentives, favorable loans and other such devices, whereas the United States prefers tax incentives, such as credits, deductions and waivers. Federal tax credits represent a huge capital subsidy. Tax credits should either be claimed against current tax or monetized. Monetization should be planned nine months in advance of plant construction. It can then be accomplished by soliciting bids from investors who would like to buy tax credits, selecting the most promising bidders and negotiating a final arrangement. Accountants, a legal team and broker constitute an appropriate monetization team to carry out the process so that it is not a distraction to business operations. Levers are generally available to create the optimal result for project developers, so they do not necessarily have to accept what the tax credit equity market is offering.

Development of New Biobased Supply Chains for the Automotive and Chemical Industries

Randy Powell (BioDimensions)
Mike Karst (Entira)
Geof Kime (Stemergy)

Many companies have developed portfolios of products made from oil and natural gas. Biobased materials have the potential to replace all of these fundamental chemicals. Challenges must be addressed, however. New products must compete with the inertia of previous investments in petrochemicals. A small number of petrochemicals feeds the petrochemical complex; these higher-value materials will get the last oil available, even after it is no longer used for transportation fuel. Renewable and abundant biomass supplies, stable and predictable costs, reduced carbon impact, novel materials with superior attributes, positive impact on the balance of trade and rural economies and the potential for higher margins than with biofuels all act as drivers for biomass-derived materials. Biobased materials can be used directly without modification, as modified/functionalized materials or as building-block chemicals. Lignocellulosic feedstock will produce the greatest diversity of biobased materials at many decentralized locations near biomass-production locations. Thermochemical processes are best suited for fuels whereas biochemical processes are best suited for chemicals. Many industries and competencies will be involved in biobased-materials production.

Relatively few companies control what feedstocks enter the market; the grain merchant is at a point of influence. In the traditional commodity value chain, the crop merchant and first-level processor are characterized by large-volume crops dependent on government subsidies and supply/demand cycles resulting in phases of hyper-production and low prices. In a transitional commodity value chain, power lessens at primary grain processors. Finally, in a future commodity value chain, concentration of power will be in the production and processing blocks, with every acre contracted to a specific user every year; acres contracted years ahead will reduce risk. Most lignocellulosic feedstocks are missing from major grain markets. Alternative crops need processing systems to add extra value, a visible revenue model and risk-mitigation tools for growers and

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logistical tools for getting crops from the field to the market. Improvements to risk-management tools and open markets need the most work. Additionally, farmers need a visible revenue model to manage competition for land among crops; one option is multiyear contracts, especially where capital investment is necessary.

Stemergy, a Canadian company, is developing crops and supporting co-products for the automotive industry, and is expanding the capacity of its proprietary BioFibeRefinery technology. Rising energy prices have changed industry economics, driving demand for renewable materials. Biomass converts solar energy and greenhouse gases into useful materials. The latter is resulting in proposed carbon-credit-trading systems. Advantages of biofibers are their large quantities as raw materials, good properties for a variety of applications, and government support and market pull as renewable materials. Flax, hemp, kenaf and other raw-material sources provide useful materials such as cellulose, hemicellulose, and lignin and versatile fiber types. Improved production processes, processing infrastructure and market-access mechanisms afford opportunities for optimization of inputs. Automotive applications of biofibers include interior trim, with the industry demanding primarily a favorable quality/pricing ratio, just-in-time delivery and lot traceability. Innovation trends include new process development, fiber modifications and enhancements, and new product applications.

Building a Successful Bio-Economy Cluster in Canada

George Mallay (Sarnia-Lambton)
Joel Adams (University of Western Ontario)
Archie Kerr (LANXESS)

The Sarnia-Lambton Economic Partnership occupies a central location in the North American market, but the small community has experienced an economic decline in recent years. Oil shocks stunted growth in the 1970s, and 50% of industrial jobs were lost during the 1990s. The chemical industry, meanwhile, has provided private-sector leadership, funding and a council for economic renewal. Sarnia-Lambton is a public-private partnership established to seek further economic growth for the region. Economic clusters rely on interdependence, involving trading sectors, related sectors and supporting institutions. Opportunities for development currently exist in agriculture, chemicals and automobile manufacturing, and biotechnology is relevant to all. With good leadership, the entry barriers and other challenges to developing a robust biobased cluster in Canada can be overcome, benefiting other entities in the region as well.

The University of Western Ontario's Bioindustrial Innovation Centre provides planned property that serves as a focal point for bio-economy cluster development. This is one of Canada's leading research parks, with over $1 million in venture capital available, Ontario's largest medical biotechnology incubator and a significant industrial customer base. The research park's three-phase plan will establish self-sufficient real-estate operations, develop the capacity for regional innovation and build a fully functional park on regional strengths, such as industry, agriculture and chemical end-users. Barriers to commercialization exist, but can be overcome if bioindustry developments provide good value. This unique facility for commercialization will aid that process through its 60,000 square feet of space, a $25 million expansion and $15 million in support from the federal government. The ultimate goal is to attract over $1 billion by 2014 and support over 150 commercialization projects with one to two spinoffs and/or product lines per year.

The chemical industry needs to increase innovation to be competitive, and is currently hampered by lack of scale at plants and eroding sales to Asia. The Sarnia-Lambton region possesses a number of advantages for the industry, such as a good location for natural gas and oil pipelines and close proximity to the Great Lakes A good agricultural base also exists. Currently, the region needs to build a solid portfolio of companies to reduce risk, provide room for alternative business models, promote integration with the existing chemical industry and promote innovation. The Sarnia Chemical Park is a new, large industrial cluster space with cogeneration power available. The Bluewater Sustainability Initiative was recently launched to make the community green and coordinate participants' ideas and sustainability activities. R&D tax credits are available, a $1.5 billion jobs-creation program exists and the end goal is to move companies out of the research park and generate jobs in the broader community to strengthen the region's economy.

Building the Bioref nery Business of the Future

Douglas Hawkins (Rohm & Haas)
Margriet Drouillon (Ghent University)
Alvin Young (Omni Tech International)
Subbu Kumarappan (Michigan State University)

A variety of specialty materials can be produced via biorefineries. Such materials include electronic components, coatings and products for other niche businesses. Growth in this area is driven by supplier pressures, customers changing their preferences, and changes in regulation and other outside influences, such as from NGOs. To respond, companies should reemphasize technological leadership and increase their capabilities in the fastest-growing markets. Industrial biotechnology products represent an avenue for this growth. Specialty-material manufacturers can leverage their expertise to apply biotechnology to their processes, but must still be prepared for large investments, long time-horizons and the need to enter or develop markets. Different technology options present different relative costs and advantages for products.

The Ghent Bioenergy Valley is a public-private partnership that focuses on bioenergy development. It seeks to develop biogas, biodiesel, ethanol and means for green electricity generation. In doing so, it promotes technological innovation, enhances cluster formation and educates the public. Technological innovation is promoted by simultaneous work on different paths to end-products, allowing companies opportunities to cooperate and find the best available processes. Cluster formation is supported through an efficient path to the production of a final product; raw materials arrive and are processed by several companies located in the area, minimizing transportation and other production costs. The valley continues to move towards the integration of its power plant and biorefinery, with the plant moving toward 100% operation on biomass. Outreach events, shows and demonstrations are undertaken to expand public knowledge about products. Ghent was a biotechnology pioneer in the 1970s and now possesses the largest bioport in Europe.

Products made from petroleum can also be made from soybean oil; the question is whether the switch would be profitable. The US Soybean Board manages the soybean supply to increase domestic utilization and exports and to promote the production of a higher quality soybean. Market development, new uses and production-increase work are carried out by the Board. Significant increases in use of soybeans for various materials are currently in progress, driven by the desire for renewable, cost-effective inputs. New uses target plastics, lubricants, coatings and other emerging opportunities. Programs are in place to fund new development, such as $4.5 million per year available through the US Department of Agriculture; seventy projects are currently being monitored in conjunction with this program. There is a large estimated market size for new products, as well as for industrial cleanup applications.

Value drivers for cellulosic ethanol plants include rising oil prices, multiple technologies, feedstock costs and regional differences. Real options quantify future investment values, indicating how much one is willing to pay to keep options available for future use. Future uncertainty erodes extra value from an asset. Real options appear in choosing supply chain investments, technologies to utilize and outputs. This concept is particularly useful in policy analysis: investing in smaller pilot plants creates newer opportunities and "newer options," keeping more options available. It is important to remember that waiting may not always be optimal, as a first-mover advantage may outweigh benefit from waiting to act.■