# Summary Proceedings

## Fourth Annual World Congress on Industrial Biotechnology and Bioprocessing

Linking Biotechnology, Chemistry and Agriculture
to Create New Value Chains

Orlando, Florida, March 21–24, 2007

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## Industrial Biotechnology and Bioprocessing

# Foreword

The *Fourth Annual World Congress on Industrial Biotechnology and Bioprocessing* convened in Lake Buena Vista, near Orlando, FL, March 21–24, 2007, organized by the Biotechnology Industry Organization (BIO), the American Chemical Society (ACS) and the National Agricultural Biotechnology Council (NABC). Some 160 presentations were made in five plenary and five parallel "break-out" sessions, twenty-six presentations were made as posters, and three workshops provided practical lessons to advance the biobased economy. Eleven hundred delegates attended.

The chief organizers—Brent Erickson (BIO), Peter Kelly (ACS) and Ralph Hardy (NABC)—thank event partners BIOTECanada, EuropaBio and the European Federation of Biotechnology and the program committee for their hard work and dedication in screening submissions, locating speakers and in organizing the program. Members of the committee were Roland Andersson (Chemical Institute of Canada), John Argall (BioAtlantech), Aristos Aristidou (NatureWorks), James Barber (Metabolix), William Baum (Diversa), David Bransby (Auburn University), Stephanie Clendennen (Eastman Chemical Company), Bruce Dale (Michigan State University), Larry Drumm (International Biotechnology Corporation), John Finley (A.M. Todd), Brian Foody (Iogen), Rory Francis (Prince Edward Island BioAlliance), David Glassner (NatureWorks), Jack Grushcow (Linnaeus Plant Sciences), Bernhard Hauer (BASF), Jack Huttner (Genencor), Birgit Kamm (Research Institute Biopos), Ron Kehrig (Ag-West Bio), Stephan Kutzer (Lonza Biologics), Colja Laane (DSM), Mike Ladisch (Purdue University), James Lalonde (Codexis), Gerson Santos Leon (Abengoa), Florence Lindhaus (DIREVO Biotech), Mahmoud Mahmoudian (Merck), Blaine Metting (Battelle Pacific Northwest National Laboratory), Thomas Nagy (Novozymes), Erin O'Driscoll (Dow Chemical), Glenn Nedwin (Dyadic), Myka Osinchuk (Alberta Research Council), Adrien Pilon (National Research Council of Canada), John Ranieri (DuPont), Anna Rath (Ceres), Manfred Ringpfeil (BIOPRACT), Sally Rutherford (BioProducts Canada), Chris Ryan (NatureWorks), Garrett Screws (Novozymes), Bill Seaman (University of Florida), James Seiber (USDA/ARS), Alan Shaw (Codexis), Feike Sijbesma (DSM), Leendert Staal (DSM), James Stoppert (Cargill), Mark Stowers (Broin), Gordon Surgeoner (Ontario Agri-Food Technologies), Larry Walker (Cornell University), Todd Werpy (Pacific Northwest National Laboratory), Roger Wyse (Burrill) and Paul Zorner (Dow Chemical).

The unflagging efforts and careful organizational oversight of Matthew Carr, Amy Ehlers, Jocelyn Modine and Kimberly Scherr (BIO) were vital to the success of the conference.

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

This *Summary Proceedings* provides broad coverage of the plenary presentations and highlights of the breakout sessions. It is largely the product of first-rate work by Colleen McGrath, Sarah Munro, Aaron Saathoff and Deborah Sills (Cornell University) as recorders. Thanks are due also to Susanne Lipari (NABC) for her excellent page-layout/design work.

Allan Eaglesham
Executive Director, NABC
Summary Proceedings Editor
July 15, 2007

# Contents

# Welcoming Remarks

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

Mr. Erickson observed with satisfaction that many of the participants had been present at the first *World Congress*. He expressed pleasure in welcoming the delegates—over a thousand of whom had registered—and hoped that they had enjoyed the workshops and breakout sessions that morning and had had a chance to visit the exhibit hall. A robust and interesting program was in store, in terms of both the plenary presentations and the breakout sessions.

At the 2006 conference in Toronto, Erickson had described industrial biotechnology as being in the middle of a perfect storm, with the convergence of concern over energy prices and national security, important federal policies coming into place, and with commercial development of new technologies around the globe. This year—what a difference a year makes—the perfect storm is producing amazing growth in commercial initiatives in the industrial biotechnology space.

(L–R) Peter Kelly (American Chemical Society), Ralph Hardy (National Agricultural Biotechnology Council) and Brent Erickson (Biotechnology Industry Organization) formally open the exhibit hall of the *Fourth World Conference on Industrial Biotechnology and Bioprocessing*.

# Plenary Sessions

## I.I. Beyond the Hype: Global Growth in the Biofuels Industry

Jens Riese (McKinsey & Co.)

Although the focus of his presentation was on biofuels, **Jens Riese** stressed that this was not because the industrial biotechnology bubble as a whole has burst. The initial phase of investing has come to an end and smarter ways are increasingly needed to create value. To identify success factors in the creation of new value, McKinsey initiated a project a year ago that entails collaboration with leading academics and discussions with more than eighty companies, and drawing on the experience that McKinsey has gained from twenty-five projects in conjunction with biofuel "shapers." Riese distilled the information and insight gained into ten key messages.

1. This is not a niche market.

Biofuels will capture a large share of this growing market. Up to 2020, it is expected that biofuels will provide a third of the total growth in the fuels market, and, in that year, will contribute more gallons that Saudi Arabian oil.

2. Large returns drive double-digit growth.

The price of crude oil and the level of government subsidy are determinants of the economics of biofuel production. In the United States (2005 prices), in the absence of subsidies, crude oil has to cost approximately $60 per barrel to make ethanol production economical, whereas in the presence of subsidies, ethanol production would be economical even at $40 per barrel of crude. For biodiesel, in the absence of subsidies, crude oil would have to cost in excess of $90 per barrel; in the presence of subsidies biodiesel production would be economical at $60 per barrel of crude but not at $40 per barrel.

3. Biofuels can contribute substantially to greenhouse-gas reductions.

Corn ethanol utilization results in a $CO_2$ saving of about 20% in comparison with oil-based gasoline. With biodiesel the saving is approximately 80% and about 90% with cellulosic ethanol. Of renewable energy sources in general, biofuels are among the most cost-effective ways of abating greenhouse-gas emissions. However, the cost depends on biofuel type and the feedstock. Assuming a crude-oil price of $40 per barrel, the cost of abating 1 ton of $CO_2$ via rapeseed diesel is ~$400, ~$500 via wheat ethanol, and ~$50 via cellulosic ethanol.

4. There are sufficient feedstocks to replace up to 50% of transportation fuel.

By the year 2020, with moderate increases in agricultural yields, without impinging on food and feed needs and without cutting rainforest, feedstock potential—from wheat / corn, sugar cane, agricultural residues, energy crops and forestry—is estimated at 3,900 tons yearly, sufficient for the production of 360 billion gallons.

5. Oil price and regulation influence supply, demand, and global trade heavily.

Similar to item 2, each US$ per barrel change in crude-oil price (or subsidies) makes a huge difference in biofuel penetration of the market.

6. Super-high margins are likely to disappear in many markets.

In 2005 with crude oil at $60 per barrel, corn costing $1.90 per bushel, capital expenditure of $1.50 per gallon of ethanol and with subsidies, the earnings before interest and taxes (EBIT) was ~50%. In the future, if the price of crude falls, say to $40 per barrel, increasing demand causes corn to increase to $3.00 per bushel, the capital expenditure increases to $2.00 per gallon and subsidies are lowered, the EBIT could drop to less than 15%—still attractive but less so than hitherto.

7. Watch out for new, low-cost geographies.

Low-cost feedstock countries will offer attractive business opportunities. In Germany, ethanol imported from Brazil costs only slightly more than that produced locally, $2 vs. $1.80 per gallon, respectively, largely because of the importation tariff and less so because of transportation cost. Even with transportation included, ethanol from Romania, Bulgaria or Mozambique costs less (~$1.35) than that produced in Germany. Similarly, EU-produced rapeseed biodiesel costs more ($2.90 per gallon) than palm-oil biodiesel from Malaysia ($2.10) and jatropha biodiesel from India ($1.80) including transportation and tariff costs. Ethanol from sugar cane produced in, for example, east African countries may impinge on the global market.

8. New technologies will change the name of the game.

A number of small-scale commercial plants are already in existence, using various cellulosic feedstocks—agricultural residues, perennial grasses, wood, *etc.*—with various methods of pre-treatment, digestion and fermentation under examination. Grants from the US Department of Energy are providing impetus for improvements in efficiency of the various lignocellulosic technologies with the objective of large-scale commercialization. The production-cost advantages that will accrue from transferring from starch and sugar-cane feedstocks to cellulosic feedstocks will vary with crop and with country. A stronger cost advantage may be expected in China than in the United States, for example. The future of biodiesel remains uncertain. Biomass to liquid syndiesel holds more promise than vegetable oil biodiesel in terms of production cost, but long-term success will depend on the regulatory support—subsidies and import tarrifs—and the efficiency of the best technology.

9. Managing uncertainty is a key factor.

Given the high degree of uncertainty, good investment decisions will result only with thorough understanding of the industry—trends in crude oil prices, regulatory aspects, value chains and technologies. Highly recommended are a portfolio approach and partnerships.

10. The race is on.

In spite of the uncertainties, the outlook is positive. Biofuels represent significant business opportunities and the race for privileged access to intellectual property, prime locations and partnerships is already on.♦

## *I.II. Biofuels: Think Outside the Barrel*

Vinod Khosla (Khosla Ventures)

In 2005 **Vinod Khosla** made five assertions that some thought were implausible:

- We don't need oil for cars and light trucks.
- We definitely don't need hydrogen.
- We don't need new car / engine designs / distribution.
- Rapid changeover of automobiles is possible.
- There will be little cost to consumers, automakers, government.

He wasn't thinking in terms of replacing 10% of gasoline consumption with ethanol, he was thinking in terms of replacing 100%. Different corn-ethanol production methods have different emissions of $CO_2$; typically, corn-ethanol production reduces carbon emissions by 20%. However, cellulosic ethanol can achieve dramatic reductions in greenhouse-gas emissions.

The projected trend in gasoline prices from 2006 is significantly different from that from 2005. In 2005, the price of crude was expected to stay below $40 per barrel into the 2020s, whereas it now is expected to stay above $50 per barrel during that timeframe. When the price of oil is above $40 per barrel, biofuels become economically competitive. Also germane is the trend in yield of ethanol from sugar cane in Brazil; with little technological development, it has increased at 3.8% per year over the past 30 years, without indication of levelling off. Khosla raised the question of whether 30,000 liters per hectare is feasible in the long term (currently it is ~7,000). Many approaches are available—thus, so are many opportunities—for making biofuels economically feasible.

Most promising are crops like *Miscanthus* which, with zero tillage and a little water and fertilizer, produces abundant and relatively inexpensive biomass for cellulosic ethanol. If projected increases in yields of sugar cane (in particular "energy" cane and with utilization of bagasse as a cellulosic feedstock), of biomass crops like *Miscanthus* and of oil crops are realized, Khosla believes that 100% of gasoline can be replaced with ethanol and biodiesel by ~2030, even taking account of increased demand for transportation fuels.

Many pathways exist for the production of biofuels, variously using feedstocks such as natural oils, sugars and starches, algae, biomass of many kinds including wastes for the manufacture of biodiesel, butanol, ethanol, biogasoline, BTL diesel, *etc*. Khosla Ventures has provided funding for a range of companies that are employing various production methods, depending on the feedstock. Trade-offs exist; the larger the supply of a particular feedstock, in general the less it costs and the greater is the technological difficulty in economic production of the biofuel. The current situation led President Bush, in his 2007 State of the Union address, to call for US production of 35 billion gallons of renewable fuels per year by 2017—hardly imaginable even a year ago.

Beyond biofuels, Khosla addressed plastics, suggesting that it would require a relatively small area of land to produce sufficient crop feedstocks to meet the US yearly need for 300 billion lb, replacing oil refineries with biorefineries. The US Department of Energy has listed twelve "platform" chemicals—for the production of a range of compounds with many uses, including polymers for plastics—that can be synthesized from glucose derived from starch and cellulose, including hydroxypropionic, succinic, lactic and levulinic acids, opening new markets requiring agricultural feedstocks.

Many consumer biases exist, *e.g.* hybrid cars are good, corn ethanol is not so good, biodiesel is good and nuclear energy is bad. It is not generally understood, however, that the reduction in carbon emissions resulting from driving a hybrid car is about the same as using ethanol rather than gasoline, whereas the hybrid cost is about a hundred times greater than for ethanol use. Furthermore, the reduction in oil use with the hybrid is ~20–30% and with ethanol is ~90%. Comparisons of ethanol with biodiesel reveal that the former is advantageous in several respects, particularly in terms of cost.

The paradigm shift that is needed to embrace the biobased economy and reduce reliance on petrochemicals will result in many benefits from achievement of sustainability to improved national security. The poorest regions of the world have great potential for biomass growth and thus production of new commodities desirable to the developed world. Instead of sending $300 billion per year to the Middle East, we can distribute these funds more broadly and thus help the world while we help ourselves. ♦

## *II. Industrial Biotechnology—It's Not Just About Biofuels: Applications in Foods, Flavorings, and Fragrances*

Colja Laane (DSM), Andrew Morgan (Danisco), Carolyn Fritz (Allylix),
John Finley (Louisiana State University)

**Colja Laane** described the white—or industrial—biotechnology value chain as the conversion of agricultural products and byproducts into sugars by chemical / physical treatment with or without enzymes, and conversion of the sugars via biocatalysis or microbial fermentation to biofuels, base chemicals, biomaterials, and biospecialties including fine chemicals, pharmaceutical intermediates and food ingredients. Annual global sales of food ingredients amount to $35 billion, of which $9 billion (24%) result from white biotechnologies, which compares well with bioethanol, for example, at ~$15 billion.

Growth in the food-ingredients market is projected at ~$8 billion over the next 4–5 years, of which ~$3 billion (35%) will occur for products from white biotechnologies. The food-flavor market—flavors, flavor enhancers and sweeteners—amounts to ~$12 billion. DSM has been active for some time in sweeteners and is increasingly involved in the development of flavor enhancers, using yeast as the source. With old techniques utilizing proteases and peptidases for protein degradation, glutamate is obtained as a flavor enhancer; similarly, RNA can be degraded selectively into its components, *e.g.* 5'-guanosine and -inosine monophosphates. DSM has developed new technologies—Maxarome Select and Maxarome Pure—that produce higher concentrations of nucleotides at lower concentrations of salt, producing sweetness and flavor enhancement with a clean taste free of "yeastiness," boosting "mouth-feel" in low-fat beverages.

A new ingredient for texture control will soon be marketed by DSM. The components of potato starch, amylose and amylopectin, are "reshuffled" to make Meltagel with zero content of amylose. It melts like gelatin and can replace gelatin and fat, with uses including improvement of "mouth-feel" of beverages.

Laane described two processing aids marketed by DSM. Brewers Clarex is a novel protease for cost-effective prevention of haze-formation in beer without negative effects on foaming and flavor. Its addition before fermentation significantly reduces the number of batch operations prior to bottling. Another processing aid—CakeZyme, a phospholipase—has utility in commercial baking, improving crumb structure and softness in sponge and pound cake. Although egg-requirement is reduced by 20%, shelf-life is significantly extended.

In the nutritional ingredient field, biobased vitamin $B_2$ provides a good example of advantages that accrue from adoption of white biotechnology. In comparison with the chemical-synthesis approach:

- twelve raw materials are replaced by sugar,
- seven solvents are replaced by water,
- waste is reduced by >70%, and
- cost is reduced by >20%.

PeptoPro is a protein hydrolysate of di- and tri-peptides. It stimulates insulin to transport glucose to muscles, where it is converted into glycogen, thus aiding muscle recovery after heavy exercise. In addition to muscle strengthening, peptides have many uses, *e.g.* control of blood pressure and satiety, and hypoallergenic infant nutrition.

Ethanol is also a food ingredient. Laane suggested it be thought of in terms of “drink the best and drive the rest.”

Genencor, well known in US biotech circles, is a division of Danisco, which is headquartered in Copenhagen **Andrew Morgan** described Danisco as a leading supplier of food-ingredients—emulsifiers, stabilizers, enzymes, cultures, protectants, flavors, sweeteners and sugars—serving the food and feed, grain-processing, cleaning and textiles, pharmaceutical and plastics industries. Danisco has ~11,000 employees in forty-six countries, with annual sales of ~$3.5 billion.

Biotechnology’s connections with food go back thousands of years, with the development of microorganisms and enzymes for making bread, cheese, wine and beer. Fermentation and other microbial technologies are now used for the production of amino acids, vitamins and flavors. And there is increasing use of biotechnological tools in the discovery of bioactive ingredients and taste modulators.

Xylanases are used extensively in the animal-feed industry and in baking. They solubilize xylans, improving crumb structure and volume of high-fiber, whole-grain bread. Danisco has engineered xylanase for resistance to inhibitors in flour.

Hexose oxidase, isolated from the marine alga *Chondrus crispus* and engineered into a microbial system, is used in baking as it oxidizes sugars in addition to glucose and in pizza cheese there is less browning as a result of the Maillard reaction.

Phyzyme hydrolyzes phytic acid in animal-feed grains, releasing phosphorus for the benefit of the animal and negating the need for addition of inorganic phosphate, thus reducing phosphate excretion to the environment.

Probiotics are live cultures of microorganisms used chiefly for immune modulation and improvement of intestinal health. Clinical data generated in the past 5 years support the efficacy of at least some of these products for relief of irritable bowel syndrome, for example. Many yoghurt-type products are popular in Europe and are gaining acceptance also in North America. With a colon simulator, it is possible to examine probiotic effects on microbial populations, specifically effects on expression of genes, such as of *cox* as an indicator of anti-inflammatory effects. With North Carolina State University, Danisco has published the gene map of NCFM, a probiotic strain of *Lactobacillus acidophilus* to aid understanding of the biochemical basis of benefits from probiotics.

Lastly, Morgan reported that Danisco is using biotechnology / drug-discovery tools to understand the molecular biology of taste in the development of bitter blockers and sweetness enhancers.

Allelyx develops proprietary natural products for the flavor and fragrance, and insect-repellent markets. Accordingly, **Carolyn Fritz** stated, her company has a particular interest in terpenes and their derivatives. Hundreds of thousands of terpenes are produced in nature—cyclic molecules with multiple chiral centers. Menthol, for example, is a ten-carbon (C10) monoterpene. The best known diterpene (C20) is a precursor of taxol, and some triterpenes (C30) are steroid precursors. Carotenoids are tetraterpenes (C40). Terpenes have many properties—flavors, fragrances, insect repellents, insect attractants, anti-viral and anti-fungal—therefore, they have broad market potential as flavors and fragrances, pharmaceuticals, nutraceuticals, urban pesticides, and as crop-protection and industrial specialty chemicals.

The flavor / fragrance market, estimated in 2003 at $15 billion, has four segments: flavor blends, fragrance blends, essential oils and aroma chemicals. Flavor and fragrance blends are similar in value (~$5 billion), and of fragrances the most important sub-segments are for personal care and household cleaning. Terpenes are of major importance in this sub-segment, with a value of $700 million, albeit representing only a few of the flavors and fragrances that are possible.

Historically, these compounds have been under-exploited because they are expensive to produce, either by extraction of the small quantities that exist in plants or by complex chemical synthesis.

Allelyx has developed a biosynthetic platform for terpene production. The gene is taken from nature and re-engineered to increase specificity for production of the terpene of interest, then it is inserted into a strain of yeast that is up-regulated for terpene production. The terpene hydrocarbon produced through fermentation is altered either chemically or biosynthetically to the precise compound required. This pioneering technology is protected by broad intellectual property.

Six compounds have been chosen as having significant market potential and may be made commercially available quickly. One of these, Nootkatone—a flavor for citrus beverages and fruit juices—has been publicly announced and is expected to be commercially available in early 2008. With the significant reduction in the cost of production using the biosynthetic platform, Nootkatone will have uses also in personal-care and household-cleaning products. All six products will be commercialized by 2010.

**John Finley** focused on the application of genomics to new opportunities for product development. Food products must be approached from the consumer perspective. In the development of new products for personalized nutrition, we are seeing a merger of the food-supplement industry and science.

Opportunities exist in improving health through the identification of potential benefits from all of the "omics." "Nutrigenomics" includes proteomics and metabolomics, and, particularly, how small molecules interact with genes. Also, nutrigenomics as a field of endeavor includes everything from gene arrays to how metabolism is affected, each aspect of which is affected by food. Food influences DNA expression, the synthesis of proteins and the activity of enzymes.

Functional foods run the gamut from stopping the growth of some cancers to suppression of the inflammatory response. A common thread is clear with all diseases linked to inflammation: anti-oxidants are beneficial. In the case of arthritis, for example, alleviation of pain occurs.

Criteria for delivering anti-inflammatory ingredients in food include GRAS (or potentially GRAS) and the final product having an attractive flavor. If the product has an unpleasant taste, consumer preference will be for a pill. In some cases, masking of the unpleasant flavor of the therapeutic ingredient is necessary.

Many anti-inflammatory compounds are known. If knowledge of these compounds is combined with understanding of how they down-regulate genes that cause inflammation, it should be possible to make synergistic combinations to produce foods with anti-inflammatory characteristics.

In a recent clinical trial with overweight subjects, the most vulnerable group showed substantial reduction in seriactive protein as did the medium-at-risk group, resulting from a diet that included foods with anti-inflammatory ingredients. In contrast, the most-fit group showed no such reduction. The subjects were screened also for glutathione peroxidase activity as an indicator of oxidative stress; nineteen of twenty-two subjects showed an average 6% increase in the activity of this enzyme, the hoped-for beneficial effect. ♦

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*http://nabc.cals.cornell.edu/pubs/WCIBB2007_proc.pdf*

# III. Redesigning Life from the Ground Up: Applications and Implications of Synthetic Biology

Jay Keasling (University of California, Berkeley; Lawrence Berkeley National Laboratory)

This definition of "synthetic biology" was provided by **Jay Keasling**: *The creation of novel biological functions and tools by modifying or integrating well characterized biological components into higher-order systems using mathematical modeling to direct the construction towards the desired end-product*. Synthetic biology differs from genetic engineering in that it involves development of foundational technologies that make it easier to engineer biology, including core components that can be used in combination reliably and tools for hiding information and managing complexity. Like a computer, synthetic biology involves an operating system, devices that make the operating system work and parts that make the devices work.

Keasling and colleagues, including those at MIT, have created what they call a registry of standard biological parts: biological components available for purchase via the Internet (http://parts.mit.edu/registry/index.php/Main_Page) that anyone can order. Each component—with standard connections in and out—are offered as open source, royalty free.

What has changed to indicate that the time is right for this new concept? Why now?

The numbers of DNA bases synthesized / sequenced per person per day has grown exponentially since the late 1980s. Even for long molecules of DNA, our speed of synthesis is now approaching the biological limit. We have incredible power in terms of DNA synthesis; although it remains expensive, it is now possible to order the synthesis of very long DNA. On the other hand, the costs of sequencing and synthesis are falling exponentially. Before long, the cost will be pennies per base. In the 1960s, it took several PhDs—20 years—to determine the first protein structure. Now it's down to days or even hours, lending power not only to understand nature but also to rebuild nature. Over 2,500 protein crystal structures are available.

But, there's a downside. The March 22, 2007, issue of *Science* reported *de novo* synthesis of infectious poliovirus by chemical-biochemical means from the nucleotide sequence that is available on the Internet. Long DNA-synthesis foundries now operate commercially in many countries.

On the other hand, Keasling is working on the microbial synthesis of the anti-malarial drug artemisinin. Of the 1–3 million people who die of malaria yearly, ~90% are under the age of 5. Some 300–500 million people are infected at any one time, decreasing the GDP of affected countries by up to 50%. The source of the drug, the plant *Artemisia annua*, has been a herbal remedy for various ailments for >2,000 years. The active ingredient was isolated in 1972. Treatment, however, is expensive at ~$2.50, and—although it is a cure—multiple treatments are needed per year as a result of continuous reinfection, an aspect that is deteriorating with global warming. The World Health Organization has estimated the worldwide need at 700 tons per year; current supply is nowhere near that amount.

Keasling believes that production of artemisinin in a bacterium can be achieved with a reduction in cost of an order of magnitude. The system needed some forty parts, none of which were "off the shelf" and many of which were mutually incompatible. It took 6 years to achieve a gain in productivity of seven orders of magnitude. The research, development and delivery team comprise Keasling's laboratory, Amyris Biotechnologies and the Institute for OneWorld Health (the world's first non-profit pharmaceuticals maker). It is hoped that the drug will be available to those who most need it by 2010.

The $CO_2$ level in the atmosphere is at an unprecedented level, causing climate change globally. There is enormous potential for production of renewable energy from sunlight via biomass, particularly from plants that are tolerant of environmental stresses and need little fertilizer. Synthetic biology offers novel ways of building new microbes for the production of novel fuels.

Keasling and his colleagues—at the University of California-Berkeley, the University of Illinois at Urbana-Champaign, Lawrence Berkeley National Laboratory—have come together to create the Energy Biosciences Institute, recently funded by BP. He looks forward to using synthetic biology for significant advances in renewable energy.

There is a plan to create the Joint BioEnergy Institute, pooling the resources of the Lawrence Berkeley, Sandia and Lawrence Livermore National Laboratories and the Universities of California-Berkeley, -Davis, and Stanford. The objective is to capture energy from the sun and turn it into transportation fuels.

The Synthetic Biology Engineering Research Center includes faculty at the University of California-Berkeley and -San Francisco, MIT, and Harvard and Prairie View A&M Universities. This is a linkage of synthetic biologists to solve some of the world's most challenging problems.♦

## IV. How Forward-Looking States are Growing Biobased Economies through Infrastructure and Value-Chain Development

David Fleischaker (Secretary of Energy, State of Oklahoma), Mitch Irwin (Director, Michigan Department of Agriculture), Adrian Polansky (Secretary, Kansas Department of Agriculture)

A recent survey revealed that Oklahoma is most famous for football, cowboys and indians, the bombing of the Murrah federal building, and oil and gas. **David Fleischaker** stated that oil-wells are operational even on the grounds of the state capital. Oklahoma is the third largest producer of natural gas and the fourth largest producer of oil in the United States, with 36,700 and 82,500 wells, and daily production of 4.4 billion $ft^3$ and 167,000 barrels, respectively, which amounts to ~$11 billion of revenue. Five years ago, with oil at $28 per barrel and gasoline at $2.80 per gallon, the gross production tax amounted to $300 million and today it is >$1 billion. Accordingly, the people of Oklahoma are thriving as a result of oil and gas prices.

The number of ethanol plants in Oklahoma? Zero.

The number of E85 stations in Oklahoma? Zero.

The number of biofuel plants in Oklahoma? Two, producing 5–8 million gallons per year.

The large majority of the giant oilfields in the United States were discovered before 1940. Oil production in the United States peaked in 1971 and, except for a temporary rise when Prudhoe Bay came online, has been declining since. In contrast, oil consumption continues to grow, mainly to supply transportation. In turn, the transportation sector relies 97% on oil and only 3% on other fuel types, making it vulnerable. In 1970, of the oil consumed in the United States, 70% was produced domestically and 30% was imported, whereas in 2006 the ratio was reversed at 40:60, with almost a $1 billion per day ($41 million per hour) going overseas to countries that are variously unstable and unfriendly.

As the global demand for energy grows—most markedly from China and India—the price of crude will increase commensurately. The average price in 2006 was $66 per barrel, which was good for Oklahoma. On the other hand, oil production in Oklahoma peaked in the early 1980s and has decreased steadily to ~40% of that level—60 billion barrels per year—the same production as in 1913.

On September 11, 2001, the United States woke up to the reality of national vulnerability, not only to terrorist attack, but also in terms of maintaining access to oil. Dependence on foreign sources of oil impacts national security and economic security. And dependence on hydrocarbons for energy, chemicals and materials threatens the environment. Biofuels are part of the national solution in terms of:

- reducing our dependence on unstable and often-hostile governments,
- reducing our funding of international terrorist organizations,
- revitalizing rural economies, and
- enhancing environmental protection.

Accordingly, Oklahoma has established an initiative to advance the biofuels industry and bioenergy research, with the Oklahoma Bioenergy Center. Although Oklahoma doesn't produce much corn, potential benefits to the state are:

- diversification of the economy,
- contribution to revitalization of rural areas, and
- creation of a new, broad industry
  - new jobs
  - an internationally recognized research center
  - new opportunities to retain youth.

The Oklahoma Bioenergy Center is a consortium of the University of Oklahoma, Oklahoma State University and the Samuel Roberts Noble Foundation, with $40 million of state financing over 4 years. It will conduct research and deliver practical solutions to enable the competitive and sustainable production of liquid biofuels in Oklahoma, and will contribute to the national research effort to enable the United States to achieve prescribed levels of petroleum independence. The primary focus is the sustainable, economic production of cellulosic ethanol from perennial prairie grasses, and the secondary focus is addressing critical steps in production of biodiesel and ethanol from non-cellulosic sources.

Development of a biofuels industry in Oklahoma must be approached from the research side. However, Oklahoma has advantages that cater to this situation. Many prairie-grass species are native and grew broadly before replacement with wheat (which led to the dust-bowl). There are excellent research facilities and four operating refineries with pipeline infrastructure that passes through the state. And growers are anxious and excited at the prospect of new sources of income.

Michigan is uniquely positioned to help lead the transfer of energy source from the Middle East to the Midwest, according to **Mitch Irwin**. Michigan produces 200+ agricultural commodities—second only to California—and has 18 million acres of timberland and 95,000 square miles of freshwater. There is a strong foundation in R&D, largely because of the manufacturing base. With 360+ international-class public and private laboratories and institutes, Michigan ranks second in the nation in R&D expenditure; 3,000+ patents are filed yearly. With several world-renowned universities, it has the fourth largest high-tech workforce in the United States: 560,000. Michigan's business climate is very favorable. It was ranked second as the most business-friendly state (by *Site Selection Magazine*), third for new capital investment and corporate expansion, and first in the growth of life-science industries. Agriculture is a $60 billion industry, second only to the $106 billion automotive industry.

Michigan has a history of innovation. Some of the most successful companies pioneered biobased products over a century ago, including Herbert Dow the biochemist inventor with over 100 patents for innovations like the electrolytic "Dow Process" of bromine extraction. Henry Ford's Model-T originally ran on ethanol and contained 60 lb of soybean in its paint and molded parts. Therefore, as far as Michigan is concerned, it's "back to the future."

Competitive edge results from Michigan's integration of rich resources and a world-class manufacturing base with hi-tech capability. Growth is encouraged in the key sectors: biofuels, biobased chemicals and biobased materials. There is strong support—from R&D through commercialization—of new products and for sustainable feedstock availability. Incentives are provided for producers, retailers and consumers, recognizing that many commercial opportunities will exist along the value chain of the biobased economy. State leadership is aggressive, fast-track permitting and inspections are available, legislation is being enacted to maximize growth, and a carbon-credit trading program has been initiated (under the auspices of the Chicago Climate Exchange).

Manufacturing and agricultural strengths are leading to excellent partnerships such as the Renewable Fuels Commission, a bipartisan effort within the Michigan Department of Agriculture to encourage the production and use of biofuels, increase the viability of state agriculture and reduce the nation's dependence on foreign oil. A collaboration between Wayne State University and NextEnergy, Inc., to research the manufacture of biodiesel and encourage its use, includes innovative methods to produce oil crops and establishment of ASTM-backed biodiesel standards.

Michigan has the largest public / private fund in the United States for the commercialization of biobased technology and products. Monies from the tobacco settlement were turned into a bond program. Thirty tax-free "renaissance" zones have been created, for ag-processing, forestry development and for renewable energy. In her recent state-of-the-state speech, Governor Granholm announced that $100 million will be invested in renewable energy. Consumer tax-breaks have been instituted: 36% on ethanol and 20% on biodiesel blends. Infrastructure grants are available for retailers to encourage installation of biofuel pumps, and tax credits are available for businesses involved in R&D and manufacture of alternative sources of energy.

Eleven ethanol plants are operational, under construction or proposed, representing a production capacity of 880 million gallons per year, utilizing 314 million bushels of corn. Four biodiesel plants are operating, under construction or proposed, representing 60 million gallons annually.

Kansas produces more wheat, grain sorghum and beef than any other state in the nation. **Adrian Polansky** reported cash receipts for farm marketings of $10 billion in 2005—sixth in the United States. And Kansas is seventh in farm-product exports, valued at $2.7 billion in 2005. It ranks second among the fifty states in cropland as well as prime farmland. As important as plant and animal agriculture are there, Kansas is a perfect fit for industrial biotechnology and bioprocessing. The 2004 Economic Growth Act created the Kansas Bioscience Authority (KBA) to develop a plan for growing the biosciences with an investment of $580 million for the coming 10 to 15 years.

The KBA, building on long-standing public and private strengths in the biosciences, guides and funds research and commercialization and workforce-development programs. Of particular relevance are Kansas State University's Bioprocessing and Industrial Value-Added Program and its Biosecurity Research Institute; Kansas University's Higuchi Biosciences Center and its biomedical research focusing on proteomics; Pittsburg State University's Kansas Polymer Research Center; and Kansas is home to many private bioscience ventures. In 2006, Battelle Science and Technology International listed 362 bioscience companies in Kansas employing 9,600 and growing; Edenspace—a leader in the use of plants for environmental protection and renewable fuels—announced the previous week the relocation of its HQ and operations to Kansas. Battelle reported that the Kansas private sector is focused on discovering and delivering new treatments for disease, food safety and animal health, new products from local farm crops, refining agricultural wastes and improving crop quality.

In September 2006, Ventria Bioscience selected Kansas for the site of a bioprocessing facility and for production of pharmaceutical rice for the treatment of diarrhea and dehydration, particularly in children.

Kansas Department of Agriculture's plant health officials are trained to conduct field inspections on behalf of USDA's Biotechnology Regulatory Services, for effective oversight of plants that contain pharmaceutical or industrial compounds mutually beneficial to the public and private sectors.

Some 250 million gallons of ethanol are produced in the state yearly. Production of a billion gallons is expected in the near future. It was announced recently that Abengoa Bioenergy will receive up to $76 million from the US Department of Energy to build a prototype plant in Kansas that will produce 11.4 million gallons of ethanol from cellulosic feedstocks. Kansas has the capacity to grow large amounts of biomass crops. Black & Veatch Clean Energy Technologies, a long-time Kansas company, is poised to be an industry leader in the development of renewable energy solutions derived from the gasification of carbonaceous material.

Looking ahead, Polansky suggested that the National Bio and Agro-Defense Facility should be located in Kansas. Significant percentages of US hog and cattle production occur in that part of the country. Furthermore, the region's academic institutions and private businesses are known worldwide in the arenas of animal health, infectious diseases, vaccines, livestock and crop production, and food safety. BioVentures is a proposed regional biosciences accelerator involving the:

- Kansas Bioscience Authority
- Kansas Technology Enterprise Corporation and
- Kansas City Area Life Sciences Institute

It will provide guidance to bioscience start-ups to better prepare them to raise private capital and achieve product commercialization. Partnering organizations are expected to include:

- Stowers Institute for Medical Research
- Midwest Research Institute
- Kansas State University
- University of Kansas
- University of Kansas Medical Center

The future of humanity depends on bioscience.♦

# *V. The Bush Administration's Initiatives to Spur Commercial Development of Cellulosic Ethanol*

## Alexander Karsner (Assistant Secretary, United States Department of Energy)

**"Andy" Karsner** thanked Brent Erickson for his leadership and for the excellent relationship between the Biotechnology Industry Organization and the US Department of Energy. Because of Erickson's advocacy, outreach, education of lawmakers on Capitol Hill and in the administration, Karsner's job is made easier.

Karnsner began working with President Bush during the 2004 re-election campaign as a clean-energy policy advisor, therefore he understands the president's dedication to industrial biotechnology. There has never been a stronger supporter of industrial biotech in the White House than George W. Bush. Energy Secretary Bodman believes that success in creating a biobased economy will be the most important part of his legacy.

Karsner has spent most of the year that he has been at the DOE trying to alter the national portfolio to a more forward-leaning posture in terms of the government's role, not just in terms of awareness of the tripartite challenges—energy security, global competitiveness, climate change—but also for the strategies that will be needed to confront them. During that week, the president renewed his call to Congress to send legislation for his signature to put into law a new far-reaching alternative-fuel standard that will address modernization of corporate average fuel economy (CAFE) standards and vehicular efficiency, with the goal of reducing gasoline consumption by 20% within the decade. The "Twenty in Ten" plan is the most far-reaching, quantitatively ambitious policy to be tabled by this or any other nation. It is hoped that a 20% reduction in gasoline by 2017 will be achieved by a 75% replacement with alternative fuels and 25% through improved fuel efficiency. This approach will get us out of the "tired old trenches" of improved efficiency *vs.* altered supply. And it tells the nation that we are serious about a bipartisan discussion, and instead of repeating national aspirations that have been affirmed since the earliest oil embargoes, we are seeking achievement in a 10-year timeframe.

Cellulosic ethanol means more than energy security; it is also the most effective path by which to address climate change, having the potential to reduce tail-pipe emission of greenhouse gases by as much as 87% relative to gasoline.

Although the "Twenty in Ten" plan and the other target that is in place—30% displacement of gasoline by 2030—are ambitious, they are attainable. These goals require equally ambitious planning, timetables, deliverables and milestones. In other words, these goals should not be viewed as visionary but as a business plan, one with implications for many in attendance. It is not beyond reach, it is not only possible it is quantifiable. Continuous input via feedback loops will be needed to keep planning for goal achievement.

Automakers and petroleum suppliers will be asked to come aboard, not only for downstream retail delivery but also to incorporate vehicular efficiency and flexible-fuel platforms more aggressively. To achieve 35 billion gallons of ethanol, five times the current level of bio-derived fuels, we must go beyond the 6 million flexible fuel vehicles currently on the road to 100 million. Accordingly, we will need tens of thousands of service stations delivering E85, rather than the thousand stations now providing it.

All of this means, above all, that we must get out of the realm of business as usual. It will require a new kind of national conversation. It will require new profit models. It will require new commercial paradigms and contractual frameworks to move biofuels more aggressively into the mainstream. Karsner expressed approval of the commitment that large auto companies have expressed to increasingly large proportions of their North American sales with flex-fuel capability, which will create demand-pull. However, we need more uniform geographically distributed and predictable markets for domestically produced biofuels; "stepping stones" of E15 and E20, *etc*., may be needed. We have to go further and we have to go faster and we must address feedstock deployment, conversion technologies, distribution, storage, terminal facilities and downstream retail—and we must have this conversation holistically and assertively in the coming months.

Success ultimately rests on a three-legged stool. DOE has focused for many years on the science and technology leg of that stool. At a time when our science is progressing sufficiently to move to rapid commercialization, we need to envision the end-game: durable, predictable, long-term policies for the market to deliver the goods the nations seeks to stimulate the third leg, catalyzing commercial markets to provide unprecedented levels of sustained capital formation to ensure market penetration of biobased fuels. DOE is working diligently with innovators, entrepreneurs, industry, states and other federal agencies and stakeholders to make this vision a reality. Investments are being rebalanced. The 2008 budget for biofuels and biomass is 20% higher for 2007 and double than for 2006. It is important to keep in mind that the government is not the exclusive source of funds in this field, which may not be obvious to those inside the Washington Beltway. It may not even be the most important source, but it is an indispensable contributor to the end-game, to making the concept of biofuels in a biobased economy more mainstream.

Soon, the results of DOE's ethanologen efforts will be announced. And, even as ethanologens are developing at a rapid pace, DOE wants acceleration to commercial readiness, to bring down the cost of cellulosic ethanol for price parity with the existing market.

DOE will work increasingly with the US Department of Agriculture and land-grant universities to identify regional cellulosic feedstocks. The logistics of biomass handling urgently need to be addressed: management, transport, storage and delivery to enable the biobased economy.

DOE funding is going also to capital investments. At the National Renewable Energy Laboratory, for instance, they are funding the integrated biorefinery facility—$20 million in 2007—which will enable collaborative studies of multiple cellulosic ethanol conversion technologies at pilot scale. This will present greater capacity to engage with scientists in the private sector.

In sum, Karsner has spent his year at DOE trying to change the dialog in the United States about a silver bullet replacing the petroleum economy, helping people understand the role of industrial biotechnology in bringing the future that we seek, and helping people grasp the correct proportionality of the government's role and the urgency to make changes that will stimulate the transformation. Failure—with the problems the nation faces in terms of energy security and of climate change—is not an option. ♦

# Breakout Sessions

## *Biofuels and Bioenergy*

### Between the Tropic of Cancer and Capricorn

**Hideaki Yukawa (Research Institute of Innovative Technology for the Earth)**
**Brad Burton (US Department of Energy)**
**Carlos Gamboa (Votorantim)**

The Research Institute of Innovative Technology for the Earth is a non-profit organization under the Japanese Ministry of Economy, Trade and Industry, focusing primarily on biorefineries and geological sequestration of $CO_2$. A novel bioprocess uses growth-arrested *Corynebacterium glutamicum* to produce ethanol from cellulosic biomass; compounds that inhibit bacterial growth do not affect ethanol-producing pathways. These bacteria, which have been genetically engineered to utilize C5 and C6 sugars when growth-arrested, will be tested in a pilot plant setting in 2008.

In the United States, the president has set a goal of reducing gasoline usage by 20% within a decade. In order to get beyond the 17 billion gallons of ethanol that corn may be able to provide yearly, the US Department of Energy (DOE) is taking steps to hasten the commercialization of cellulosic ethanol. Among several programs, two bioenergy centers of excellence are being established and Section 932 biorefinery grants have been awarded to several companies, totalling $385 million over 4 years. Budget increases in 2007 and 2008 have allowed the DOE to set up a new loan-guarantee program, designed to lower the cost of debt and get commercial projects started; it is crucial to start getting "steel in the ground."

In Brazil, Votorantim New Business has made significant investments in development of sugar-cane for food and fuel needs. Brazil has high growth potential for renewable energy and as a base for a new biobased chemical industry. These advantages include ideal conditions for sugar-cane production, low labor costs, a desirable scale of production, and scientific leadership in sugar-cane agronomy and biotechnology. Research at Alellyx Applied Genomics and CanaVialis has resulted in the discovery of several new genes of interest and creation of over 1.5 million sugar-cane seedlings from over 800 crosses in the development pipeline. A new venture will focus on the conversion of biomass (bagasse in particular) to biofuel. However, even with further development, Brazil is not expected to meet its forecasted internal need for 22 billion liters of ethanol by 2013, and thus is unlikely to export ethanol to the United States.♦

### Cellulosic Ethanol in California

**Anna Rath (Ceres)**
**Rick Zalesky (Chevron Technology Ventures)**
**Ana Halpern-Lande (Tellurian Biodiesel)**
**John Cuzens (Bluefire Ethanol)**

In California, current environmental policy is expected to contribute to the next wave of economic growth. Regulations are focused on a market-based approach in order to drive technology development. The state legislature has passed several bills to encourage biofuel and bioproduct production in tandem with goals of reducing greenhouse-gas emissions. Important bills are AB1493, which limits tailpipe emissions; AB1007, which moves from a fuel-neutral policy to one supporting fuel diversity; AB1190, which rescales excise taxes based on a the carbon content of a fuel; and AB32, which limits greenhouse-gas emissions and establishes a low carbon-fuel standard. AB32, with far-reaching goals, is managed by the Air Board, which will monitor and report all greenhouse-gas emissions with a goal of reduction to 1990 levels by 2020. The California Climate Action Team is using a wedge approach to drive down overall greenhouse-gas emissions; reductions include 40% from transportation, 20% from electric power, 23% from industrial sources, 8% from agriculture and 8% from other sources.

To help meet some of the AB32 mandates, cellulosic ethanol will be a major low-carbon fuel. Fortunately, California is one of the richest states when considering biomass available year-round and has the ability to support a $300 billion energy industry with 507 trillion BTUs available from biomass feedstocks annually. However, technology and feedstock selection for developing cellulosic ethanol is important since not all biomass fuels are necessarily low carbon, and the "winner" needs to have an overall carbon balance that is either negative or only a fraction of that produced by gasoline. Bluefire Ethanol has selected to license the Arkenol process, which relies on concentrated-acid hydrolysis as a pretreatment. It has lower production costs than competing technologies with higher profits per gallon of ethanol produced. BlueFire Ethanol will use inexpensive feedstocks—urban trash, wood waste, rice and wheat straw and other agricultural residues, *etc*.—in their biorefineries.

When considering development of cellulosic ethanol, use of dedicated energy crops is important due to higher yields (projected 10 tons per acre vs. 2 tons for agriculture residues) and subsequent reduction in transportation costs to the biorefinery. California's current 28 million acres of agricultural land is used mostly for crops (39%) and range or pastureland (52%); however, additional land could be used for dedicated high-yield biomass crops. For this to occur, several important issues must be addressed, including growing practices, harvest and transport logistics, seed quality, varietal development, and most importantly, yield improvement. With high-yielding varieties, California will meet a significant portion of its transportation fuel need with 15 million acres, or less, of land.

World energy demand is projected to rise by 50% in two decades, putting significant strain on global resources. There is pressing need for a variety of clean fuels and technologies. Improving energy efficiency will also be important. Similar to the petroleum industry, infrastructure, technology, and large, concentrated feedstock supplies will be important for the biofuels business. Oil companies are not opposed to these technologies since they believe that all the energy we can produce will be needed in the future. Chevron, in cooperation with the state of California, is studying the life-cycle effects of E85 in cars. Biofuel technologies will need to be given time to advance as they must offer tangible benefits both to the consumer and to the environment.♦

## Biomass Pretreatment and Processing

Guido Zacchi (Lund University)
Frank Haagensen (Novozymes)
Nancy Ho (Purdue University)
Johan Van Groenestijn (TNO)

Dilute-acid steam explosion pretreatment of biomass does not require concentrated acid or other caustic chemicals. In general, the process is batch-based with steam injected to raise the reactor temperature to 160–230°C within 10 seconds. The reaction is allowed to proceed for 1–30 minutes followed by rapid steam release. The reaction conditions depend on feedstock composition and what downstream process the slurry will be fed into. Treatment severity is an important consideration: too low and downstream digestability is poor, whereas excessive severity results in creation of degradation products that can inhibit fermentation or reduce process efficiency. For pine, a 190°C pre-treatment for 5–10 minutes has produced sugars at near-theoretical yields. Using simultaneous saccharification and fermentation, researchers at Lund University have achieved over 80% efficiency (based on C6 sugars) in their process with a loading rate of 15 filter paper units per gram cellulose.

Development of cellulosic ethanol processes, requires evaluation of pretreatment options. One important parameter is cellulose accessibility, or the completeness of cellulose hydrolysis. There are two ways of measuring accessibility: chemical and enzymatic. Enzymatic accessibility is more important for cellulase-based hydrolysis. Another important parameter is substrate reactivity, or the overall completeness of the reaction. One benchmark is the time and enzyme dosage required to reach 80% cellulose conversion. These parameters are related since high yields require more time while high dosages reduce conversion time but increase cost. In order to optimize these processes, an enzymatic approach was used to screen a large number of samples for high accessibility, followed by determinations of substrate reactivity on a much smaller number of samples. Thus, Novozymes has been able to achieve a six-fold reduction in enzyme loading requirements on corn stover.

Early efforts to develop cellulosic ethanol were hindered by inadequate knowledge of cell-wall composition as well as an inability to ferment C5 sugars. To overcome the latter obstacle, Purdue scientists developed a strain of yeast capable of fermenting C5 and C6 sugars (xylose and glucose). Genes have been moved to the chromosome to enhance stability and reduce strain-maintenance requirements. Tests in industrial settings have shown good robustness: the organism can digest both glucose and xylose even after twenty cycles. Currently, ethanol concentrations up to 5% are tolerated, but research efforts are currently being directed towards increasing this tolerance to 8–10%.

The biosulphurol process uses acid to hydrolyse cellulose to its constituent sugars. Therefore, uncertain future costs of enzymes are avoided by pretreating biomass in 70% sulphuric acid at a moderate temperature and pressure. Hydrolysis then occurs by adding water and diluting the acid, which is later recovered through anion-selective membranes and standard chemistry. This process uses a novel impregnation reactor with trickle-down and recirculation processes to uniformly treat biomass with sulphuric acid. Overall cost is €0.20–0.30 per liter of ethanol, and the process is robust and non-specific for a variety of biomass types.♦

## Advanced Enzymatic Hydrolysis of Lignocellulosic Biomass

Bin Yang (University of California-Riverside)
Colin Mitchinson (Genencor)
Kevin Gray (Diversa)

The Consortium for Applied Fundamentals and Innovation (CAFI) is evaluating pre-treatment for lignocellulosic biomass processes. Pre-treatment impacts all other downstream processes and represents about 18% of total processing costs. Technologies studied include aqueous ammonia recycle, water-only dilute acid hydrolysis, ammonia fiber explosion (AFEX), controlled pH, and lime pre-treatments. Some pre-treatments, such as dilute acid and controlled pH, released sugars whereas AFEX released mostly monoglucose and monoxylose. Pre-treatment effectiveness was variable, depending on feedstock type. On corn, the addition of xylanase to the enzyme mixture after pre-treatment resulted in 12–31% increases in xylan conversion, depending upon enzyme loading. With poplar, AFEX was found to be less effective than $SO_2$ and lime pre-treatments for good conversion efficiency at lower enzyme-loading rates. Important factors that may affect pre-treatment effectiveness include degree of polymerization, availability of reducing ends, β-glucosidic bond accessibility and crystallinity index.

Enzyme performance is critically important in cellulosic ethanol production. Therefore, Genencor International has been working on optimizing cellulases for better performance at higher temperatures in enzyme cocktails. Using enzymes from *Trichoderma reesei*, they have engineered more thermostable CBH1 and CBH2 enzymes, both of which are important for cellulose breakdown. For CBH1, a full molecule random mutagenesis approach was used, whereas consensus-sequence based recruitment of interesting sites and site-directed mutagenesis was used for CBH2. Over 100,000 clones mutant for CBH1 were analyzed, and from twenty-four that exhibited better thermal characteristics, thirty-seven sites of potential interest were identified. The final result was an improved CBH1 with good activity at 75°C (a 10.9°C melting temperature increase over wild type). For CBH2, thirty-nine site-directed mutants were made and 5,600 clones were screened, leading to an overall $T_m$ increase of 4.3°C.

The complexity of the cell wall is a major obstacle to obtaining high cellulose-to-sugar conversion efficiency. The cellulose is embedded in a matrix of hemicellulose and lignin, making digestion difficult. The hemicellulose component is variable and more structurally complex than cellulose due to complex branching. Hemicellulose utilization is required for high efficiency of conversion of cell-wall material to ethanol. Interestingly, while hemicellulases do not act on cellulose, they have been found to act synergistically with cellulases as mixtures containing hemicellulases have higher glucose yields. Therefore, hemicellulases are a vital component of any enzyme cocktail. Xylose yields to date have been 50–80%, depending upon feedstock. Thus, feedstock composition has a strong impact on the economics of biomass-to-ethanol conversion, and compositional variations and their effects on conversion efficiencies need elucidation. ♦

## Pathways for Introducing New Biobased Transportation Fuels

Dhananjay Ghonasgi (ConocoPhillips)
Richard Marinangeli (UOP)
Al Darzins (National Renewable Energy Laboratory)

From a fossil-fuel refiner's perspective, it is too early to judge biofuels as "winners" or "losers" since both ethanol and biodiesel have significant drawbacks. Biodiesel, for instance, has a good cetane number and lubricity, whereas pour point, acidity, stability, and cold-weather handling are concerns. Ethanol is high octane, but requires a separate transportation infrastructure, has significant blending and reformulation issues, and while supply is in the central United States, demand is primarily along the coasts. Regardless of what biomass fuel is ultimately used, efficient conversion technology is an absolute requirement. Producing renewable diesel from biomass using a petroleum refinery is one option that eliminates chemistry concerns with traditional biodiesel and takes advantage of refinery scale and costs. As a whole, the refinery industry does have some experience in using biomass feedstocks and more time should be allowed to let the market determine overall biofuel winners.

Renewable fuels should be considered for production in refineries for a variety of reasons. They are expected to make up a larger share of the future fuels pool due to mandates, energy-security issues, *etc*. A significant advantage is the ability to take advantage of the existing refinery infrastructure and fuel-distribution system while allowing the refinery to control quality of renewable blending components and generating future $CO_2$ credits. UOP and Eni are working together to develop a process route to convert vegetable oil to high-quality diesel by reaction with hydrogen. The goal is to develop a stand-alone green diesel unit for refineries in order to avoid costs associated with co-processing. The product has low sulphur (<1 ppm) and high cetane, as well as good stability and cloud-point properties. Also, this process has less environmental impact since it avoids methanol usage.

Although many processes may be used in the future to create diesel fuels from biomass, the overarching problem of supply and demand cannot be ignored. Currently, oilseed crops and waste oils—even if used in their entirety—could provide only a fraction of current US diesel needs. To provide more, a novel technological breakthrough is required. One potential solution is using algae to produce the triglyceride (vegetable oil) feedstock. The advantages include using a non-food source for fuel, very high productivity (>50% of dry weight as triglycerides), use of non-productive land, growth in saline water, and usage of waste $CO_2$ streams such as from power plants. Algal feedstocks would consist of shallow, stirred ponds and could be up to fifty-fold more productive than traditional crops. Situated in areas of high insolation, they could produce hydrogen, lipids, hydrocarbons, carbohydrates and biomass. To realize potential yields of >10,000 gallons per acre, significant R&D investment will be required, but the rewards could be enormous.♦

## Enzyme Advances for Bioethanol

**Kenneth Barrett (Diversa)**
**Thomas van Rueden (DIREVO Biotech)**
**Blake Simmons (Sandia National Laboratory)**
**Jan de Bont (Royal Nedalco)**

Diversa scientists recently turned their attention to developing a better α-amylase that would have higher performance in a dry-mill corn-ethanol plant. Specifically, an enzyme was needed with optimal activity at high temperatures (>195°F) and low pH (<5), causing rapid viscosity reduction. Using proprietary culture methods and directed evolution, Fuelzyme LF was developed. The original enzyme was derived from an extremophile archaebacterium and over 21,000 variations were analyzed. This enzyme has a unique mechanism and is effective at lower enzyme-loading rates than are currently possible, which allows better plant control and operational flexibility.

DIREVO is a partner in a project titled *New Improvements for Lignocellulosic Ethanol* (NILE), to improve the overall cost efficiency of the cellulose-to-ethanol process. They have focused on developing better enzymes, including cellobiohydrolases, endoglucanases, and β-glucosidases by improving enzyme activity, thermostability, and reducing sensitivity to inhibitory compounds. Multi-parameter miniature high-throughput screening and application-relevant assays have resulted in enzymes with improved properties in all three areas. They are now engaged in commercial process development programs and in developing mixed feedstock technologies, and are interested in finding partners for collaboration.

Sandia National Laboratories is rapidly expanding its efforts in the biosciences arena. Part of the focus is on developing robust enzymes for integration with pre-treatment effluent, which can have a pH range from acidic to basic depending on the process. *Sulfolobus solfataricus* is being examined as a source for acid-tolerant and thermostable enzymes. Of the three cellulase-type enzymes present in this organism, current research is targeting endoglucanase SSO1949, which has pH and temperature optima of 1.8 and 80°C. Focus is on improving binding and releasing properties, by mutating specific residues. Future work will include additional enzymes and scale-up of purification and activity analysis of SSO1949.

Royal Nedalco recently announced the intention of building a second-generation bioethanol plant with a €150 million investment in their Sas van Gent facility. It will have a capacity of 200 million liters per year and initially will use wheat bran as a feedstock. Currently, two important challenges are faced: developing industrial enzymes or cocktails that quickly and efficiently release both C5 and C6 sugars from the cellulose and hemicellulose streams as well as finding yeast strains capable of fermenting both C5 and C6 sugars into ethanol. In collaboration with Wageningen University and enzyme companies, they are developing and testing new industrial enzymes. The second problem—fermenting sugars—is being addressed in collaboration with Bird Engineering and Delft University of Technology where they are developing new yeast strains; one strain is capable of fermenting glucose and xylose within 25 hours, and further development to include fermentation of arabinose is expected. ♦

## Improvements in Fermentation for Ethanol Production


### Lisbeth Olsson (BioCentrum)
### Stephen Hughes (US Department of Agriculture)
### Thomas Jeffries (US Forest Products Laboratory)


All cellulose-to-ethanol scenarios rely on an efficient cellular factory capable of efficiently fermenting multiple sugar streams into ethanol. At BioCentrum's Center for Microbial Biotechnology at the Technical University of Denmark, efforts have been directed at improving ethanol yields from yeast. One of the central problems is the redox constraint. Research has been focused on changing the use of redox equivalents during ammonium assimilation in order to decrease the amount of NADH used, which, in turn, increases the availability of NADPH. Deletion of the GDH1 pathway, a major sink for NADPH, was combined with over-expression of GDH2 and GS-GOGAT pathways. The resultant strain had an 8% increase in ethanol yield and a 40% decrease in glycerol yield in laboratory conditions, and a 4% increase in ethanol yield and 20% decrease in glycerol yield in simulated industrial conditions. Work on improving tolerance to industrial conditions by improving the strain background is underway.

The USDA has developed core high-throughput systems capable of rapidly producing, transforming, and screening genetic mutants for production of optimized enzymes for biorefinery applications. This system automates PCR, transformation, culture growth, and plasmid preparations using 96-well plates. Initial work with this system has been focused on improving the Lyt-1 protein that is toxic to the corn ear worm. A new protein was engineered, capable of killing over 25% of the ear worms over 2 days in a plate-culture assay. Although some problems, such as introduction of PCR artifacts, into the process may need better control, these high-throughput systems will be beneficial for optimizing enzymes used in biorefineries.

*Pichia stipitis* is a naturally occurring yeast capable of efficiently fermenting glucose and xylose to ethanol. Found in the gut of beetles infesting white-rotted wood, these organisms have evolved essentially in a continuous culture of partially digested wood. In combination with a novel pre-treatment technology that uses diethyl oxalate, which selectively removes hemicellulose and results in very little sugar degradation, recent research has shown that *P. stipitis* can effectively metabolize glucose, arabinose, xylose, galactose and mannose. Furthermore, DNA sequencing has shown that this organism has a well developed complement of genes for cellulose and hemicellulose degradation, making it an attractive target for further R&D. ♦

## Integrated Corn-Cellulose Biorefinery


### Mark Stowers (Broin)
### Don Higgins (Novozymes)
### Susanne Kleff (MBI International)


Some of the early cellulosic ethanol facilities may be add-ons to existing plants, such as a dry-grind corn-ethanol facility. Broin Companies envision the development of an integrated corn-cellulose biorefinery that incorporates new fractionation technology at the front end of the process coupled with a bolt-on process for cellulosic ethanol. To help make this vision a reality, they have developed two new technologies: Broin Fractionation (BFRAC) and Broin Project X (BPX). BFRAC automatically removes the corn hull and fiber for processing in a cellulosic ethanol facility, while BPX allows simultaneous saccharification and fermentation in the corn-starch process. Project LIBERTY involves the transformation of an existing corn-ethanol facility to a commercial scale biomass-to-ethanol plant, where 27% more ethanol from an acre of corn is expected while reducing water usage by 24% and fossil-fuel consumption by 83%.

In order to successfully run an integrated corn-cellulose biorefinery, development of better enzyme mixtures will be necessary for cellulose and hemicellulose streams to be used effectively. In particular, hemicellulose presents technical challenges in trying to obtain high sugar yields. Composed mainly of xylose and arabinose, corn hemicellulose presents a complex challenge since it includes galactose side chains in addition to the usual arabinose, acetate, and acidic side chains. Since pre-treatment affects the amounts and types of enzymes needed, co-development of pre-treatment and hydrolysis processes is crucial. By comparing acidic, neutral, and alkaline pre-treatments, scientists at Novozymes have found that unoptimized mixtures of cellulases and monocomponent hemicellulases have increased sugar yields by up to 30%, depending upon enzyme dose and pre-treatment type. This information will be further used in development of integrated corn biorefineries.

In addition to ethanol, integrated biorefineries could also produce value-added speciality chemicals such as butanetriol from C5 sugars and succinic acid from C5 and C6 sugars. Work at MBI International has demonstrated succinic acid production and scale-up to 1,000 gallons, and has identified ways to further reduce cost in the hope of making it a useful way to produce this commonly used chemical intermediate. *Actinobacillus succinogenes* has been modified for increased yields and reduced by-product formation in order to efficiently create chemical intermediates from cellulose streams. If incorporated into a 16 million bushel per year integrated biorefinery, 20–75 million pounds of succinic acid could be produced, depending upon which sugar stream (C5 or C5 + C6) is used, using corn bran as feedstock. Plans involve optimizing butanetriol production and demonstrating succinic acid production from other biomass sources. ♦

## Seed-Oil Processing: Strategic Applications of Biotechnology, Implications for Biodiesel Production

Nelson Barton (Diversa)
Chris Dayton (Bunge)
Derek Masterson (Crown Iron Works)

Soybean, canola, and sunflower produce high-phosphorus oils, *i.e.* containing significant amounts of phospholipids, which must be removed in a degumming step to avoid downstream-process or end-use problems. Degumming generally results in a yield loss of 2–4%. This problem may be avoided by first removing the phospholipid head group and thus separating the water and oil-soluble components and reducing any oil that is lost in the gum fraction. Diversa scientists have developed a unique phospholipase C, Purifine, which removes the head groups of phosphatidylcholine and phosphatidylethanolamine. This enzyme is active in conditions of low moisture (2–4%) and moderate temperatures (40–70°C), and has a pH range of 5.5–9; furthermore, it is compatible with chemical refining processes and increases overall yields by almost 2%.

An alternative to conventional degumming and chemical processes is being implemented worldwide by Bunge. Caustic processes are being replaced with enzymatic degumming based on phospholipase A, allowing recovery of the oil normally trapped in the gum. They gain back one molecule of oil for every two of phospholipid. The process produces no soapstock or waste wash-water, and reduces overall chemical requirements. Oil yields have been increased by 97% with phosphorus removal as from conventional processes. Furthermore, life-cycle assessments per 1,000 tons of refined oil show energy savings of 413 GJ, $CO_2$ reduction of 44 tons, and significant reduction in $SO_2$ (518 kg), $PO_4$ (376 kg), and ethylene (18.6 kg) equivalents.

Formally, biodiesel is defined as mono-alkyl esters of long-chain fatty acids that originate from vegetable oils or animal fats and meet the ASTM D6751 standard. Additionally, it can be blended with conventional diesel in any amount, and in mixes up to 20% (B20) it has the cold-pouring characteristics of regular diesel. In the United States, approximately 150 million gallons were produced in 2006 and about 300 million gallons are expected in 2007, lagging behind Europe's 2006 production of 1.8 billion gallons. The US diesel market is around 65 billion gallons, and even a low B2 standard would require half of the entire US soy-oil production. Not enough fats are available to displace anything more than a small percentage of conventional diesel, and new technologies will be required. There are many important process steps in producing biodiesel including removal of phosphorus, fatty acid, and water, starting with a high-quality feedstock. Other key factor for a successful operation include good process control, an adequately equipped laboratory, and trained management and personnel. ♦

## Development of Superior Energy-Crop Varieties

Steven Thomas (Ceres)
David Bransby (Auburn University)
Twain Butler (Samuel Roberts Noble Foundation)

The feedstock used for ethanol production in the United States is corn, which is problematic for two reasons: it is a source of food and feed, and there is insufficient to substantially reduce need for gasoline. Cellulosic ethanol, on the other hand, has the potential to replace 30% of US liquid-fuel consumption based on assumptions made in the "billion ton report" created by the US Departments of Energy (DOE) and Agriculture in 2005. There is a variety of cellulosic feedstocks including forest products; crop residues; and herbaceous energy crops such as switchgrass, sugar cane, *Miscanthus*, and giant reed. Switchgrass is the most promising herbaceous energy crop, even though its yields are lower than for other species. It can be planted from seed, it requires minimal fertilizer after establishment, and it can be mowed with existing farm equipment, and then either baled or chopped in the field.

DOE has identified switchgrass as a potential dedicated energy crop due to its desirable traits. A major limitation is successful establishment, therefore Ceres and the Noble Foundation are conducting a breeding project to create cultivars with improved germination and vigorous early growth. The project includes conventional breeding, marker-assisted breeding, and trangenics, and the main objective is to create genomic intellectual property. Since biomass density is critical for biomass-to-ethanol conversion-process economics, the goal is to create a crop with yields of 10–15 tons per acre. In addition to improving yields and establishment success, Ceres is working on developing switchgrass varieties of high nitrogen-use efficiency and tolerance of drought and cold.

There are over 200,000 plant species, any of which can be used to produce ethanol, and the important question is which are best suited as feedstocks. DOE has identified perennial switchgrass as an ideal energy crop based on its high yield, adaptability to a wide range of soil types and environments and low-production cost. The major disadvantage of switchgrass is establishment. Therefore the Noble Foundation and Ceres plan to address this challenge through breeding (described above) and agronomy research to elucidate optimal management guidelines. ♦

## Regional Feedstock Partnerships: Producing the Billion Tons of Biomass

Terry Nipp (Sun Grant Association)
Mark Downing (Oak Ridge Laboratories)
Jim Doolittle (South Dakota State University)
Kelly Tiller (University of Tennessee)

President Bush announced a national goal to make cellulosic ethanol production practical and competitive by 2012, and to replace 20% of US gasoline consumption with ethanol in the next 10 years. The Sun Grant initiative, legislated in the 2002 farm bill and the 2005 highway bill, is a regional collaboration of five land-grand universities involved in R&D of biobased industries. The purpose of Sun Grant is to enhance US national energy security through development, distribution, and implementation of biobased energy technologies, based on the US Departments of Energy and Agriculture's "billion ton report." Sun-Grant centers look at regional needs and opportunities and involve industry people, academics, nonprofits and citizen-interest groups with the goal of spurring the establishment of a domestic biobased industry.

The Sun Grant initiative completed two regional workshops in 2006, one in the southeast center of excellence located in Knoxville, TN, and one in the north-central center located in Sioux Falls, SD. Three additional workshops are scheduled for 2007: at the south-central center located in Oklahoma City, OK, the northeast center located in Ithaca, NY, and the western center located in Portland, OR. These meetings foster working partnerships among industry, academia, and other interested parties to propose policy and communication guidelines to facilitate rapid development of biobased industries and utilization of bioenergy crops. One issue that arose during the first two workshops concerned the projection in the "billon ton report" that there will be a 50% improvement in bioenergy-crop yields. Workshop participants emphasized the need for R&D in breeding, genetics, and crop-production systems to realize such improvement.

Sun Grant Bioweb (Bioweb.sungrant.org) is an online encyclopedia containing information about biomass feedstocks, biofuels, biopower, bio-products and biorefineries. The information on Bioweb is maintained by the Sun Grant; it is comprehensive, peer-reviewed, and easy to navigate. ♦

## Woody Crops for Biorefineries

Edwin White (SUNY College of Environmental Science)
Marilyn Buford (US Forest Service)
Don Riemenschneider (US Forest Service)
Nathan Ramsey (ArborGen)

In order to fulfill President Bush's goal of domestic production of 35 billion gallons of liquid biofuels by 2017, we will need to take advantage of woody crops as feedstocks. National net annual growth of forest-woody biomass exceeds removals by approximately 50% on 500 million acres in the United States, mostly due to the closure of pulp and saw mills, providing large amounts of potential feedstock for biofuel production. Advantages of woody crops compared to other agricultural sources include year-round availability; large and positive net energy ratios; sustainable management is possible; physical-chemical characteristics of wood are consistent from source to source; and the forest-products industry has already developed technical and engineering management strategies.

Short-rotation woody crops, such as poplar and willow, are invaluable resources as feedstocks for the production of biobased products including biofuels. Research and development on short-rotation woody crops has been conducted for many years, focused primarily on genetics, management systems, harvest and transportation, and sustainability. Genetic research includes traditional breeding, genome sequencing and mapping for modification and breeding; this research has the potential to create enhanced species designed specifically as bioproduct feedstocks. Management systems, harvest and transportation, and sustainability research need to optimize resource utilization along with minimizing impacts on the environment.

To utilize trees as an energy source, we need to understand growth of the crop including limits of productivity, operational tasks and their costs, and how management decisions affect costs. Costs of woody-crop production include land, site preparation, weed control (generally only in the initial years of stand establishment), fertilization, management, harvesting and transportation. Unfortunately, there has been a substantial drop in research capacity in the US Forest Service, but it is still feasible to overcome obstacles and create a new forest-product biobased industry.

ArborGen is dedicated to developing trees that grow faster and produce more on less land, thus conserving the diversity and complexity of native forests while furthering biofuel-feedstock development. Biotechnology can be used to improve traits (*e.g.* reduced lignin content) for dedicated woody energy crops in managed forests, thus benefiting the emerging biobased industry. Woody biomass as feedstock makes sense in the southeast since infrastructure—logging, transportation, initial processing—is already in place. This region is poised to be a major producer of bioproducts from forest feedstocks.♦

## Perennial and Annual Grasses as Cellulosic Feedstocks

Lynn Wright (University of Tennessee)
Neal Gutterson (Mendel Biotechnology
Geoff Thomas (SorBio Energy Systems)

POLYSIS—an agricultural-policy, linear-programming, simulation model—was utilized in 2006 to assess the potential of various regions to provide sufficient amounts of model feedstocks (*e.g.* hybrid poplar, willow, switchgrass) for large-scale production of cellulosic ethanol. The model was tied to the US Department of Agriculture's baseline projections that assumed little change in total crop-land use, ethanol-use increase, and increase in corn prices with a maximum of $3.00 per bushel. Results indicated that higher yields of energy crops (*e.g.* switchgrass) will be essential for farmer profitability and displacement of corn-land use, and that initial agricultural opportunities will be on lower-value land in areas with lower crop income.

The maximum production capacity of corn ethanol will fall short of the goal that President Bush articulated recently of 35 billion gallons of ethanol by 2025; therefore, there is widespread interest in cellulosic-ethanol production. Perennial grasses (*e.g.* switchgrass, *Miscanthus*) have advantageous traits including efficient use of water and nutrients, accumulation of significant below-ground biomass, and regrowth for approximately 10 years. But, in order for perennial grasses to be commercially successful as feedstocks, high yields are essential. *Miscanthus gigantus* already yields above 10 tons per acre, but can be propagated only from rhizomes, which is too labor-intensive for large-scale production. Mendel Biotechnology has a breeding program to create a high-yielding *Miscanthus* variety that can be grown from seed.

Sorghum is another species with potential to be a commercially successful feedstock for cellulosic ethanol production. Most sorghum species are annuals, but some are highly productive, producing up to 27 tons of biomass per acre in 120 days. Sorghum, a C4 grass, is related to sugar cane and corn; it is well adapted to a wide variety of conditions from the tropics to high elevation deserts; and it would work well in combination with winter cereals. One major advantage of sorghum over perennial grasses is that large amounts of seeds are already available for cultivation.♦

## Sustainable Availability of Biomass

Manoj Kumar (DSM)
Jamie Fingueret (Centro de Tecnologia Canavieria)
Alfred Mutsaars (Shell)

Global energy consumption is predominantly from oil, coal, and gas with renewables making up only 3.4% (in 2004); however, this relatively small number is making an important impact on feedstock use. The expected rapid global increase of production and use of biomass for energy production must be balanced by minimizing ecological, social and economic risks. The development of sustainable biomass production will depend on science and technology to ensure low greenhouse-gas emissions and preservation of biodiversity, while governments and corporations will be responsible for maintaining human rights and proper management of the environment. The exploitation of biomass for energy production must be managed sustainably.

Thirty-six percent of global bioethanol is produced in Brazil, where all automotive fuels contain at least 25% ethanol. Brazil exports approximately 3 billion liters of ethanol per year, using sugar cane as its sole feedstock for ethanol production. Bagasse is burned for electricity production, which is sold. The Brazilian ethanol industry is moving towards the use of the entire sugar-cane plant. Researchers are characterizing the bagasse and straw to determine how fermentable they are. Since the bagasse is already present at the ethanol plant and has been pretreated for sugar extraction, its hydrolysis could potentially add substantial amounts of feedstock, though bagasse use for ethanol would compete with bioelectricity production.

Although its core business is oil, gas, and fossil fuels, Shell is developing and investing in renewable forms of energy. There is particular interest in cellulosic biofuels due to their advantages over first generation biofuels, including greenhouse-gas abatement. Eco-ethanol and BtL (biomass to liquid) will first be blended with and later replace gasoline and diesel respectively. Full-scale commercial plants are expected within the next 5 years. ♦

## Biofuels: Feedstocks of the Future

Bruce Ferguson (Edenspace)
James McLaren (StrathKim)
Jim Sears (Solar Democracy)

Edenspace Systems has developed improved varieties of corn for ethanol production and is working on crops for cellulosic ethanol. One interesting approach in the development of cellulosic feedstocks it to insert microbial cellulolytic enzymes into plants to avoid the cost of adding enzymes for pre-treatment. It is possible to grow plants containing cocktails of up to four enzymes; however, complete hydrolysis of lignocellulose requires more enzymes than this.

Sorghum has potenial for fuel-ethanol production. The research strategy at StrathKim is to optimize ethanol production from this crop with enhanced germplasm. Sorghum is currently grown mostly the midwest—west of the corn-belt—and in 2006, 5 million acres of grain were harvested. It grows well in regions too dry for corn and is already used in commercial ethanol production. Therefore, the process does not need to be altered a great deal. Researchers examined 1,200 genetic lines from breeding programs and selected seventy genotypes and elite hybrids. Evaluation of selected varieties revealed relatively little variation in ethanol yields and conversion efficiencies.

Algae—solar-powered chemical plants that feed on atmospheric $CO_2$—are an exciting new potential feedstock for biodiesel production. They are fast growing and can produce approximately 7,000 gallons per acre per year of biodiesel. In addition to being a feedstock for biodiesel, algae contain valuable chemicals such as omega-3-fatty acids. Production strategies for growing the algae consist of small photobioreactor ponds that allow light penetration and control of temperature.♦

## Forest Biorefinery: Reshaping the Future of the Pulp and Paper Industry

Arthur Rasgaukas (Georgia Institute of Technology)
Kendall Pye (Lignol Innovations)
Virginie Chambost (École Polytechnique)

In a biobased economy renewable raw materials are converted to products and energy in a sustainable manner. Although the US forest-products industry is an important segment of the nation's economy, it has not earned its cost of capital in a decade. A solution to this problem is to introduce new products to provide significant growth while protecting the core of the industry. In order to leverage the existing forest-products industry to reinvent itself, the following will be necessary:

- develop sustainable forest productivity,
- extract value streams from products prior to pulping, and
- create new value streams from residuals and spent pulping liquors.

New biorefinery technologies for woody biomass are being developed to increase profits, while attempting to make use of existing Kraft and sulphite pulp mills. Lignol's solvent-based pre-treatment technique is unique in that it removes all lignin from the cellulose and hemicellulose fibers, thus creating a more easily digestible substrate for hydrolytic enzymes, and the lignin is recovered as a valuable co-product. Potential markets for the lignin include surfactants, concrete additive, industrial carbon fibers and animal-feed additives.

The pulp and paper industry is in economic stalemate and it is time to think and act differently. A potential survival strategy is to diversify the core business with marketing and technological partners, and make the most of the existing value chain by migrating to a new business paradigm. Necessary key factors for future success of this industry include:

- a rescue strategy containing product innovation,
- a diversification strategy,
- a market-driven mentality,
- ongoing product-centric R&D,
- process flexibility, and
- a focus on sustainable business practices.♦



## Plant Cell-Wall Structure and Modification

James Seiber (USDA/ARS)
Ronald Hatfied (USDA/ARS)
William Orts (USDA/ARS)
Bonnie Hames (Ceres)
David Lee (Edenspace)

Alfalfa has the potential to be a biofuel feedstock since its hexose and cell-wall contents are comparable to those of switchgrass and corn stover. It would be advantageous to reduce lignification through genetic manipulation in order to increase digestibility, and research has shown that a lignin reduction of 3–4% can increase digestibility by 5–7.5%. It is important to remember that no single feedstock solution exists for biofuel production; we must design sustainable methods that take account of the total agricultural system along with the biofuel-production process.

In order for bioethanol production to be commercially successful, design of "athletic" biorefineries—those that can handle all types of feedstocks year-round—will be necessary. New hydrolytic enzyme cocktails will include cellulases, hemicellulases and other novel enzymes that break lignocellulosic bonds (*e.g.* ferulic acid esterase). And it will be necessary to design processes that can handle mixed streams [*e.g.* municipal solid waste (MSW) and straw]. Use of MSW as a feedstock will be advantageous due to close proximity of landfills to populous areas.

Current means of characterization of biomass feedstocks are based on traditional wet-chemical methods developed for wood, pulp and paper, which are labor-intensive, expensive, and time-consuming. Rapid analysis methods using spectroscopic techniques that can be used for genomic and biotech applications are being developed at Ceres, Inc. These methods are initially calibrated using wet-chemical analyses, and multivariate statistical analyses transform the spectroscopic data into compositional data that can be applied to research concerning feedstock development for biobased industries.

One goal at Edenspace Systems is to increase yields of cellulosic ethanol by engineering into plants microbial enzymes involved in cell-wall hydrolysis. They have initiated this work with tobacco and corn, and proceeded by inserting promising enzymes (*i.e.* those that do not adversely affect plant growth and have good cellulolytic activity) into switchgrass and sorghum. They plan to further characterize enzyme impact on the plant cell wall using electron microscopy and atomic-force microscopy, and toinvestigate potential xylanase/glucanase synergisms.♦

### The Evolving Biorefinery

**Richard Hess (Idaho National Laboratory)**
**David Russell (Renessen)**
**Kiran Kadam (Pure Vision Technology)**

Storage and transport of biomass feedstocks for biorefineries are critical and complex issues that require R&D for the commercialization of biobased products. We will need to look at regional feedstock supply systems and compare scenarios (dry versus wet storage, bales versus silage, *etc*.) in order to properly design and locate biorefineries. Regional climate conditions and harvesting scenarios also will affect storage and transportation, and will result in different solutions for biorefineries in different locations.

Along with the development of higher-quality corn grain for animal feed, Renessen scientists are working on improving the efficiency of corn-ethanol production. They are developing improved feedstocks and a new processing system that produces additional marketable co-products. Ethanol producers using this technology will be able to process an enriched starch raw material with increased yields of fuel-ethanol and of high-protein, dry-distiller grains for inclusion in animal feed.

Pure Vision Technology is interested in designing flexible biorefineries that can produce ethanol as well as pulp and paper products. A two-stage pre-treatment process (hot water followed by alkali) removes and separates lignin from the carbohydrate portion of biomass feedstocks. This allows production of low molecular weight lignin (along with ethanol or pulp and paper products) that can be marketed for lignin-based products such as adhesives and phenol substitutes. ♦

## *Chemicals and Biomaterials*

### The New Bio-Economy: Novel Product Opportunities Based on Small-Acreage Crops

**Jack Grushcow (Linneaus Plant Science)**
**Jeff Martin (Yulex)**
**Paul Dribnenki (Agricore United)**

Plant-derived oils hold great promise for the biofuels industry, but face the residual effects of more than 100 years of inexpensive, petroleum-based oil. To begin replacing petroleum-based oil, increased oil production from plants is being sought through genetic engineering. Plant oils offer a cost-competitive alternative to petroleum-based fuels and, in preliminary tests, cars run on vegetable oils have shown greater fuel efficiency.

Natural rubber is the second largest import to the United States after oil. Derived from the tree *Hevea brasiliensis*, production of natural rubber in tropical countries is labor-intensive and has the major drawback of causing latex-allergy disease. As an alternative, guayule (*Parthenium argentatum*), a species that grows in the United States, can be used to supply rubber with the advantages of not causing an immune response and its bagasse has potential as a feedstock for bioethanol production.

Research is in progress to develop flax with greater health and nutrition benefits. Currently, flax is used in the production of seed oil, paint, ink, varnish and food products. Agricore has developed a new strain of flax (Linola) with a high content of omega-3 fatty acids, which are used for their health benefits as food additives. Linola is also useful for production of oils low in saturated fatty acids and meal protein. ♦

### Impact of Genomics and Systems Biology on Biobased Materials

**Aindrila Mukhopadhyay (Lawrence Berkeley National Laboratory)**
**Glenn Nedwin (Dyadic International)**
**Blake Simmons (Sandia National Laboratories)**

Cellular stress responses were discussed in the context of using microorganisms as sources of bioproducts. Stress responses are elicited from cells as a consequence of processing conditions (high temperature or extremes of pH) and as a function of altering biochemical pathways to improve product yields. In order to understand how such pathways can be modified to optimize production, a global approach that incorporates genomics, proteomics and metabolomics must be utilized. For example, the isoprenoid pathway of *E. coli* can be modified to produce the anti-malarial compound, artemisinin.

The cost of making cellulosic ethanol is approximately $2.25–2.75 per gallon. One way to lower production cost is to reduce the price of enzymes to less than $0.10 per gallon. Dyadic is beginning to address this challenge with fungal strain C1, optimized to produce five critical enzymes necessary for biomass degradation. In a low-cellulase background, the enzymes are secreted in a ratio optimal for hydrolysis. The company has also developed a high-throughput screen for use with the C1 fungi that makes use of a special telomeric vector. The vector contains two human telomeric sequences, two fungal selection markers, and an origin of replication, affording stable incorporation and high transformation efficiency of DNA libraries into the fungi.

Research in the area of highly ordered and self-assembled biomaterials, involving calcite and silicate, was described. There is intense interest in the genes required for the complex formation of nanostructures from silica in diatoms. Little is understood of what stimulates, regulates or terminates structure formation. Silicatein is an enzyme involved in the formation of spicules in diatoms. Its catalytic domain has been used in conjunction with various metal oxides to make nanostructures with potential use in the production of photovoltaic cells. ♦

## Novel Biological Generation of Chemicals

**Jérôme Maury (Denmark Technical University)**
**Yaman Boluk (Alberta Research Council)**
**Stevens Brumbley (Austrialian Institute for Bioengineering)**
**Eric Seewald (Bayer CropScience)**

Yeast can be used as a cell factory for the production of isoprenoids, which have many applications including pharmaceuticals such as taxol and the anticancer compound licopene. Chemical synthesis / extraction of these compounds is costly and yields are often low. To circumvent these problems *Saccharomyces cerevisiae* is being used for the production of a class of isoprenoids, sequesterpenes. The production strain was modified in the gene ERG9 of the mevalonate pathway to reduce the amount of farnesyl pyrophosphate (a precursor of sequesterpenes) side products. In addition, the *Escherichia coli* methyl-erythritol phosphate pathway was heterologously expressed in the yeast strain to circumvent native regulation.

In the chemical industry, 90% of chemicals are produced from hydrocarbon-based sources, whereas only 10% are from natural renewables. Hemicelluloses have the potential to replace many hydrocarbon-based compounds. They differ from hydrocarbon feedstocks in being heterogeneous, amorphous and of high molecular weight and they can differ dramatically depending on source. Various hemicellulose extraction methods exist, including alkali and acid treatments. Polymers precursors / chemicals that can be obtained from hemicelluloses include furfural, xylitol, ethanol, 1,3-propanediol and succinic acid.

Sugar cane has been genetically engineered to increase production of polyhydroxybutyrate (PHB). The three main genes involved—those for PhaA (ketothiolase), PhaB (acetoacetyl CoA reductase) and PhaC (PHB synthase)—were engineered with a plastid-targeting sequence, such that after the proteins are synthesized, they are localized in plastids. The genes are constitutively expressed during the life of the plant and the system allows early detection of high PHB-producing plants.

Plants have potential as biofactories for the production of new materials. They synthesize many compounds that have potential industrial uses, including waxes, oils, fibers, proteins and carbohydrates, specifically latex, detergents and cosmetics. ♦

## New Uses of Co-Products: Lignin and Glycerin

**Božena Košíková (Slovak Academy of Sciences)**
**Nicolaus McCready (Iowa State University)**
**Jim Millis (Cargill)**

Many plastics are composed of synthetic polyalkylenes that require the addition of chemical stabilizers to prevent degradation during use. However, after addition of stabilizers, the plastics continue to remain stable even in landfills. Addition of lignin to plastics at concentrations greater than 10% by weight led to greater stability of the plastic and increased degradation of the material under conditions that mimicked a landfill. The polymers were more effectively degraded when exposed to higher temperatures, UV light, and white-rot fungus.

There are more than 2.3 million miles of road in the United States of which 96% are surfaced with asphalt. Hot-mix asphalt (HMA) contains a binding component that, over time, becomes brittle and leads to cracking and pothole formation. Chemical performance-enhancers can be added to extend road life but are costly and not used for low-volume thoroughfares.

Lignin (unpurified, 10–12% by weight) left over from cellulosic ethanol production was added to HMA and found to improve resistance and increase stiffness. The lignin-asphalt blend was also found to be more effective in warm climates.

With the expansion of the biodiesel industry, a 1.4-million ton growth increase is predicted for the world supply of glycerine by 2010. At current projections, a yearly surplus of 1.1 million tons will be created, requiring development of new applications and markets. Glycerine markets are in personal-care products such as toothpaste and cosmetics in addition to coolant, animal feed, and the production of 1,3-propanediol. In the future, markets are expected to expand in the area of epichlorohydrin and glycol production. Cargill is focusing on using glycerine for production of propylene glycol. ♦

## Current Developments in Industrial Biotech in Germany: Biopolymers and Biorefineries

Manfred Kircher (Degussa)
Dietrich Scherzer (BASF)
Birgit Kamm (BIOPOS)
Joerg Rothermel (German Chemical Industry Association)

Biotechnology makes up only 7% of the world's chemical industry, but there is huge potential for increasing the niche. The use of white biotechnology is competitive with chemical methods because there are many products for which chemical synthesis is lengthy and costly or because the compounds can be produced only by biotech methods, such as fermentation to produce sphingolipids (use in cosmetics). White biotechnology will be driven to expand by advances in systems biology (genomics, proteomics, metabolomics) and process technology (enzyme catalysis, whole-cell catalysis).

Targeted gene transfer to organisms is being used for the production of biochemicals and renewables. Native systems provide guides for the production of novel compounds. Fungal proteins are being made to modify the surface properties (water resistance) of various materials, spider-silk proteins are being studied for use in novel textiles, and skin proteins are being applied to create better cosmetics. In addition, microorganisms are also being modified for the production of plastics such as polylactic acid (PLA), polyhydroxyalkanoates, and polyhydroxybutyrate.

The ultimate goal of the biorefinery is to transfer the efficiency and logic of fossil-fuel based industry into a biobased system. Biorefineries will contribute to the development of a strong biobased economy, which is composed of three pillars, biopower / energy, biofuels, and biobased products. One product that is suited to biorefinery production is PLA because of its relative ease of synthesis from starch and its high value to the chemical, pharmaceutical, and cosmetics industries.

The German chemical industry ranks #1 in Europe and #4 in the world in production; however, only 4.6% utilizes renewable feedstocks. There is optimism for progress in biorefinery development in Germany. The evolution of the biorefinery has been such that only naturally occurring compounds have been extracted and used (oils and lubricants) in known markets. Now production is expanding to the generation of biomolecules—proteins, sugars, oils, *etc*.—for use in biotech processes. Biorefineries are expected develop to utilize renewable resources like biomass to produce the majority of basic chemicals. ♦

## Application of Advanced Metabolic Engineering Tools for the Production of Chemicals by Fermentation

Stephen Van Dien (Genomatica)
Philippe Soucaille (Metabolic Explorer)
Kevin Madden (Microbia Precision Engineering)

One of the major goals of biotechnology is to develop microorganisms that can utilize renewable feedstocks to produce desired compounds with minimal by-products. Genomatica is using an approach that couples *in-silico* modeling of microbial pathways with wet-lab studies. For this system, a program called OptKnock was designed to analyze a given organism's cellular metabolism and define novel pathways (other than central metabolism) in which product formation could be coupled with cellular growth. The algorithm alleviates sites of bottlenecks, by-product formation, and inhibition and the organism can be engineered to continuously produce a given compound in order to grow. The engineered organisms are then used in wet-lab experiments where adaptive evolution is used to select strains with optimized growth and compound production. This method was used to optimize succinic acid production in *Escherichia coli*.

*E. coli* was manipulated to produce monopropylene glycol (MPG) in a continuous anaerobic process. The first step in the process was rational strain design using software (Metavista) that analyzes how changes in central metabolism affect product formation. Designer strains are then evolved using *in vivo* evolution in chemostat culture. The evolved population is then analyzed and compared with the original designer strain to determine which enzymes are involved in peak MPG production; in this case the enzymes were NADPH-dependent.

In many instances, optimal compound production is obtained by modifying non-obvious metabolic targets. In microbial precision engineering, strains are developed by first compiling metabolic / genomic profiles using several methods including microarrays, mass spectrometry, and 2-D gels. Once a standard profile is developed for a given set of conditions, it is used to compare strains grown under various conditions. These tools allow analysis of genes relating to metabolic diversity resulting from mutations or environmental conditions, such as those involved in the fungal micro-pellet phenotype (failure to form filaments) and in carotenoid formation in *Yarrowia lipolytica*. ♦

## Breakthroughs in Biobased Plastics

**James Barber (Metabolix)**
**Marek Kowalczuk (Polish Academy of Sciences)**

Plastics are a $350 billion per year market with a large fraction derived from fossil-fuel sources. In an effort to reduce greenhouse-gas (GHG) emissions and to alleviate the growing solid-waste problem, natural plastics derived from corn starch are being utilized. "Natural plastics" reduce petroleum usage by 80% and GHG emissions by 66% as compared to the petrochemical products they replace. A joint venture between Metabolix and ADM has led to the construction of a 110 million-lb per year natural plastics plant in Clinton, IA, slated to begin production in late 2008.

Polyhydroxyalkanoates (PHAs) are key components of plastics, particularly for packaging. They can be made from renewable resources using fermentation, genetically modified organisms, or *in vitro* enzyme systems. In order to compare the utility of these production methods, it is necessary to develop a means of analyzing the PHA product for uniformity of structure and the presence of contaminants. Mass spectrometry was found to be an effective means of characterizing the molecular structure and purity of biopolyesters. ♦

## Industrial Materials from Agricultural Lipids and Co-Products

**Sevim Erhan (USDA/ARS/NCAUR/FIO)**
**Daniel Solaiman (USDA/ARS/ERRC/FOAC)**
**Ray Miller (DuPont)**

Industrial products (lubricants, surface coatings, inks, *etc*.) are being developed from vegetable oils at the National Center for Agricultural Utilization and Research (NCAUR). Ink formulations typically contain a large fraction of petroleum components. Soy-based inks are cleaner and have a lighter color such that they require the addition of less pigment (costly component). Scientists at NCAUR are also researching the replacement of hydraulic fluids with vegetable-based oils.

Sphingolipids—a key component of surfactants, skin-regeneration products, and novel oleochemicals—are made by combining sophorose and fatty acids. Their production has been best characterized in *Candida bombicola*. Rhamnolipids are being studied in the opportunistic pathogen *Pseudomonas aeruginosa* for use in biocontrol agents, biosurfactants, and inkjet pigment dispersal. Technology is being developed to produce these compounds in high yields in non-pathogenic bacteria.

DuPont has several new polymers on the market, produced with the aid of genetically engineered organisms, including Sorona, which has properties desirable for textile / fabric production including durability, softness, stress recovery, UV / chlorine resistance, and stain resistance. The polymer is currently being used for the production of chemical-free stain-resistant carpeting. Another polymer, Cerenol, has applications in inkjet ink, hydraulic fluids, cosmetics, thermoplastics and autofinishers. ♦

## Industrial Biotechnology in China

**Zhenjun Sun (China Agricultural University)**

With a growing population of over 1.3 billion, there is concern in China over whether there is enough biomass feedstock and land to support significant biofuel production. Because of the limited space for crop production, China must be creative in its sources of feedstocks. Under consideration are sweet sorghum, sugar cane, sweet potato, oil-bearing trees (*Jatropha* and *Humilis*), energy grasses and microalgae (*Chlorella protothecoides*). It is thought that roughly 23 million ha of sweet sorghum could be developed in China to produce an estimated 130 billion liters of bioethanol, with enough crop left over for animal feed. China is undertaking a State Project for Bioenergy, which seeks to lower $CO_2$ emissions, increase production of bioethanol, bioplastics and biodiesel, and to increase income for rural farmers, all by 2020. ♦

## Sequencing and the Use of Genomics to Improve Biological Conversion of Biomass

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Improved low-cost, high-throughput genomic sequencing capabilities have permitted novel investigations into natural biomass-conversion processes. Filamentous fungi are important bioprocess organisms that produce a variety of valuable products, are capable of growth at low pH and of high product titers, and utilize hexoses and pentoses. A major challenge is increasing the production of valuable compounds, while decreasing biomass growth and production of byproducts. This issue can be addressed using functional genomics and metabolic modeling. Genomics aids and accelerates research; a large number of filamentous fungal genome projects are in progress. With an available genome, it is possible to rapidly determine which pathways and enzymes are native to an organism and hypothesize enzyme activities. However, one cannot learn from genomes the appropriate growth conditions or kinetic constants. Thus, functional genomics, modeling and hypothesis-driven research studies are essential.

The genome of a high-butanol-producing strain of *Clostridium beijerinickii* has been sequenced and compared to those of other clostridia. A microarray was used to profile the genes in *C. beijerinickii* and it was most related to *C. acetobutylicum*; however, *C. beijerinickii* has no native cellulosome genes. Post-genomic analysis included transcriptional examination of the global shift in gene expression from acidogenosis to solventogenesis and a comparison of the high-butanol strain and the wild-type strain. Subsets of genes were investigated. There was increased activity of solventogenic genes in the high-butanol-producing mutant. Sporulation-gene activity was reduced in the mutant compared to the wild type, suggesting that sporulation defects are associated with enhanced solvent production. Substrate studies showed that although *C. beijerinickii* prefers glucose, it can utilize mixtures of hexoses and pentoses. The organism was also grown on hot water and ammonia fiber exploded distiller dry grain hydrolysates and there was comparable growth and solvent production.

Termite-gut systems are highly efficient bioreactors for the breakdown of lignocelluosic materials. Termite families are divided into lower and higher termites with the distinction that the higher termites' gut systems lack protozoan communities. Both types of termites are capable of digesting wood, but in the higher termites, protist symbionts cannot be responsible, therefore the hindguts of the higher termites were analyzed for bacteria with cellulase activities. For this study, a *Nasutitermes* sp. was obtained from Costa Rica and the P3 section of the gut system was selected for analysis. Since it is difficult to culture more than 99% of microorganisms, metagenomics—sequencing of the entire community rather than one strain (genomics)—was performed. Sequences were cloned from a variety of different organisms. Spirochetes were dominant, but there were also Fibrobacteres. Over 600 glycosyl hydrolase domains were found. Free $H_2$ was a key intermediate, but the hydrogenases appeared to be sensing, not necessarily catalytic, and only one formate dehydrogenase was found. ♦

## Innovative Approaches to Microbial Strain Improvement

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For metabolic engineering the genetic possibilities are almost infinite, making selection difficult. The use of "-omics" makes it possible to eliminate hypothesis selection, resulting in an unbiased approach, but good experimental design is key for this strategy to be successful. Multivariate data analysis (MVDA) can be used to translate differences in metabolomes into phenotypic differences, by emphasizing the strength of correlation between factors rather than the magnitude of a response. The usefulness of a combined metabolomics / MVDA approach to optimize industrial bioprocesses is illustrated by phenylalanine production in a designed and patented strain of *Escherichia coli*. In the *E. coli* experiments, it was found that for many of the intermediates in the phenylalanine pathway there was a positive correlation with intermediate concentration and phenylalanine yield. By over-expressing the genes for the intermediate converting enzymes, the patented strain was improved by 50%. This gain illustrates that a metabolomics / MVDA approach is useful for rapid strain improvements by extracting relevant information from large data sets.

A current issue with the "-omics" approach is that there can be high rates of error. For example, in the public genome of *Bacillus subtilis* 168 there could be approximately 2,000 sequencing errors. Over 700 of these errors have been validated. In a 2-D-gel proteomics study of *E. coli*, over 1,000 proteins were identified, a third of which appeared to be erroneous. The error rate in genomic and proteomic information could be even greater for less-well studied organisms. Despite this drawback, there has been success with high-throughput genomic sequencing technologies. Several products have been developed based on cloned *Aspergillus niger* enzymes and pyrosequencing technology of the proprietary undomesticated *B. subtilis* BSP1 has led to the discovery of a new protein, BsmA, that is related to biofilm formation. With the availability of metagenomics and gene shuffling, it is possible to identify new products and pathways, thus the available information for the synthetic biology toolbox has increased. Experimental data integration into mathematical models as constraints is increasing the meaning of those data. Although the data derived from "-omics" technologies can appear to give a complete picture and be used in mathematical models, it is important to validate those models. Proper experimental design is key for system biology to be useful for biotechnological applications.

An integrated metabolic engineering platform requires metabolic modeling and experimental technologies. This platform makes it possible to explore a range of potential metabolic pathways and to rapidly engineer and evolve stable organisms. A collection of integrated technologies has been developed to comprise this platform. These include: SimPheny, which is a software platform for computational systems biology, a metabolic model collection containing more than twenty genome-scale models for bacteria, single-cell eukaryotes, single mammalian cells, microbial co-culture systems, and a BioPathway predictor. The BioPathway predictor explores the entire space of possible biochemical routes to a product. Some of these routes will already exist in the organism of interest, some may require heterologous insertion of enzymes or it may be necessary to evolve the enzymes. In a 3-hydroxypropionic acid case study, there were nine known pathways to produce 3-hydroxypropionic acid and 296 novel pathways were identified. Filters were applied to the potential pathways to find the thermodynamically feasible approach. OptKnock strain design and evolutionary engineering laboratory methods were used to address one of the primary issues of bioprocess engineering, the trade-off between biomass production and product yield, by coupling product formation with cell growth. ♦

## Advances in Biocatalysis with Emphasis on Pharmaceutical Manufacturing

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One of the most popular strategies for producing homochiral building blocks for organic synthesis is enantioselective ketone reduction. Enzymes are capable of catalyzing carbonyl reductions and are often used as an alternative to chemical methods. Historically, whole yeast cells have been used to mediate alkene reductions and a large reductase library has been established. Single-enzyme-mediated reductions can also be implemented. For example, old yellow enzyme, a flavoprotein that was isolated in 1995, reduces activated alkenes and nitroalkenes. Enoate reductases, which are found in clostridia, and old yellow enzyme, which is found in fungi, bacteria, and plants, are both flavin-containing reductases. A library of alkene reductases has been created from multiple organisms. Studies have been performed to compare these enzymes with multiple substrates including 2-cyclohexenones, citral, and perillaldehyde. It has been found that a straightforward model allows one to predict the outcomes of most old yellow enzyme-mediated enone reductions; the selectivities of enzymes correlate with sequence subgroups, and non-flavoprotein enone reductases have activities different from those of the old yellow enzyme homologs and may be useful new biocatalysts.

MolecularBreeding is directed molecular evolution by DNA shuffling. Gene variants are shuffled to create a library of novel genes and then high-throughput screening is used to identify genes with improved properties. Screening metrics include substrate specificity, enantioselectivity, stability and expression. The desired process is designed first and then the biocatalyst is evolved to enable that process. To illustrate this method, TBIN, a key intermediate for the drug atorvastatin, was selected. The original production step of TBIN involved stoichiometric borohydride reduction of boronate complex, which required cryogenic conditions, vacuum distillation, and created a stoichiometric borate waste stream. The proposed reduction step would involve catalytic transfer hydrogenation under ambient conditions, with a benign waste stream and easy downstream processing. Seven rounds of evolution were employed to achieve productivity targets and improve the process. Another technology for discovering new reactions is the use of diagnostic panel plates. This involves the use of 96-well plate screening of enzyme activity with increasing stringency to find optimal enzymes for specific substrates.

The advantages of biocatalysis for synthesis of single enantiomers of chiral intermediates are increased enantioselectivity and regioselectivity, mild temperature, pressure and aqueous conditions, and the possibility to immobilize and reuse enzyme catalysts. Many important chiral pharmaceutical intermediates are produced in this way for antiviral agents, anti-diabetic drugs, anticancer agents, and anti-anxiety and anti-Alzheimer's drugs. Strategies for production of these pharmaceutical products include the use of wild-type organisms (*e.g. Rhodococcus* sp.), genetically modified *Escherichia coli* with enzymes from bacteria and yeast, or immobilized enzymes. Several examples illustrate these strategies. To create the antiviral drug Atazanavir, used in the treatment of AIDS, two enzymatic strategies are used including a fed-batch fermentation of wild-type *Rhodococcus erythropolis* and a separate step with modified *E. coli* containing a leucine dehydrogenase from *Thermoactinomyces* sp. and a formate dehydrogenase from *Pichia pastoris*. The creation of an anti-diabetic drug also uses the formate dehydrogenase from *P. pastoris* and a phenylalanine dehydrogenase from *Thermoactinomyces sp*. cloned into *E. coli* for reductive amination. Another step in this process uses immobilized lipase B from *Candida antarctica* to perform enzymatic ammonolysis. An anti-anxiety drug requires microbial reduction of 6-ketobuspirone to produce (S)-6-hydroxybuspirone and (R)-6-hydroxybuspirone and involves reductases from *Hansenula polymorpha* and *Pseudomonas putida* and dehydrogenases from *Saccharomyces cerevisiae* and *P. pastoris*.

Phage Biocatalysis is a technology that uses bacteriophages with attached enzymes to create consolidated bioprocesses. These lytic phages can be added directly to the fermentation vessel to infect bacteria and then the resultant crude lysate can react directly with added substrate allowing the user to eliminate intermediate steps of cell collection and lysis, enzyme purification, and transport and storage before use. In a comparison study between a commercial α-amylase and a T7 bacteriophage with a fused α-amylase, the phage-fused α-amylase had equivalent kinetic properties to the free α-amylase and a comparable temperature range. Phage-linked enzyme activity was also high in crude cell lysate when compared to glycine buffer. Tandem enzyme activities, where α-amylase and xylanase A were linked in both possible configurations to the phage, and dual enzyme activities, where the amylase and xylanase were fused in different locations on the phage, were also achievable. Phage Biocatalysis was tested for application to the starch liquefaction process in the ethanol industry and it was found that the total time to produce enzymes was extremely low, purification was not necessary, and results equivalent to those required in the industry were achieved without optimization. ♦

## Health and Nutrition


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In taste signaling, ion channels control salt and sour signaling, while G-protein coupled receptors (GPCRs) control sweet, bitter and umami (savory) taste sensations, caused by binding of tastants to the GPCRs, triggering a $Ca^{+2}$ response. Experimentation with knockout mice led to the discovery that if the Trp M5 channel is absent they could not taste sweet, savory or bitter flavors. It is postulated that an amplifier of the Trp M5 channel will amplify the sweet taste in soft drinks or the savory flavors in soups. Blocking the Trp M5 channel may reduce the bitter taste of medicines. Due to growing interest in reducing the sugar content of food products, a molecule with no flavor could be valuable as an additive that will enhance the taste of a nutritive sweetener. High-throughput screening using a fluorescence imaging plate reader has been used to identify inhibitors and enhancers. Addition of one of the prototype enhancer compounds results in a five-fold amplification of the tastant signal. Whole-cell electrophysiology studies show that the effects of the enhancer are reversible.

Lactic acid bacteria span approximately twenty genera, over half of which are considered useful for food technology including fermented foods and probiotics, *i.e.* cultures of live microorganisms that confer health benefits to the host. Improved sequencing technologies have allowed the publication of multiple probiotic species' genomes; *Lactobacillus acidophilus* NCFM is one such organism. Functional analysis of this genome using gene-knockout systems and microarray analysis of transcription enabled the reconstruction of the carbohydrate utilization pathway. This strain also exhibits adherence to human epithelial cells and numerous surface proteins have been identified. The probiotic effect on human gene expression of NCFM was evaluated using a DNA chip with 38,550 genes. The expression profiles showed increased gene expression in the co-cultures of human cells and NCFM when compared to the profiles of human cells mixed with NCFM culture supernatant or a control.

Genetic, diet, and lifestyle profiles are now being made available to consumers, so that they may generate personalized information regarding gene-environment interactions to achieve improved health. Consumer attitudes toward the use of genetics are equally favorable for appraisal of prescription drugs and nutrition / diet-related recommendations. An established ethics platform is important to maintain this positive view. This includes responsibly communicating genetic information other than medical diagnoses, determining what consumers feel are the appropriate contacts or channels for communication of personal genetic information, and ensuring protection of privacy. The process of providing these profiles to consumers begins with identifying functional sequence variations, which are frequently single-nucleotide polymorphisms. For a "nutrigenetic" gene to be identified as informative for a consumer it must include common variations (not rare diseases) and have a documented effect on health. An example is the MTHFR gene, which normally has a C at position 677, whereas 20% of the population have a T at this location. This substitution is associated with elevated homocysteine levels, which can be normalized by a folic acid supplement. In clinical studies it was found that individuals given personalized nutrigenetic information showed increased compliance with the goals of maintaining appropriate blood levels of glucose, homocysteine and LDL. ♦

## Enzyme Biotechnology for the Production of Value-Added Oils and Nutraceuticals

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Lipases are useful in the modification of healthful lipids because of the mild reaction conditions required and their selectivity. One application is in the development of structured lipids (SLs), triacylglycerols or phospholipids that have been modified to contain both long-chain and medium- and/or short-chain fatty acids in the same molecule. SLs are referred to as nutraceuticals or functional lipids, depending on structure. Physiological fatty acids can be used to maintain a balanced omega fatty acid ratio (n-3 / n-6), which is important for production of anti-inflammation compounds. Enhanced absorption of omega-6 fatty acids was observed in cystic fibrosis patients when the fatty acid was esterified to the sn-2 position of the SL, indicating that the position of the fatty acid on glycerol is key for absorption. Lipase-catalyzed lipid modification is performed in a packed bed bioreactor with immobilized enzymes and a low-pressure short-path distillation to produce *trans*-free SL margarine from canola oil. To create cold-spreadable products, butterfat and vegetable oils are blended, but experimental results showed that a butterfat-SL blend is superior to both pure butter and butter-canola blends, thus canola-based SL could be a potential substitute for canola oil. Another application of SLs is the use of gamma linolenic acid (effective *in vitro* against cancer cells), which is important for infant nutrition and development and reduces symptoms associated with atopic dermatitis.

Concern is increasing over formation of *trans* fatty acids during partial hydrogenation of oils and fats. Enzymatic inter-esterification (IE) of oils and fats has been studied as an alternative to partial hydrogenation and chemical IE processes. The primary goal is to produce semi-solid fats with mechanical properties and softening points comparable to those of dairy spreads, with low or zero *trans* fatty acids, and physical properties similar to partially hydrogenated oils. The production process needs to be optimized and potential applications of the resulting products identified. Three different immobilized lipases and five different oils were compared in orbital and magnetic agitation. Magnetically stirred reactors reached equilibrium first. Reactor types were compared; the mini-packed bed reactor was more efficient than the larger batch reactor and continuous operation was more effective than batch operation. Functional characterization of the products showed that above 15°C IE products are softer than the corresponding physical mixtures. The biocatalysts were stable, with 90% and 100% residual activities after fifteen reaction cycles.

Phosphatidylcholine (PC) was successfully modified by phospholipase A1-catalyzed acidolysis, with fatty acids obtained by saponification of fish oil. The modified PC product contains high levels of eicosapentaenoic, docosapentaenoic and docosahexanoic acid residues. This modified PC can be incorporated into food products as emulsifiers and stabilizers and will provide the health benefits of n-3 polyunsaturated fatty acids (PUFAs). The reaction system was initially a batch vacuum reactor to eliminate the presence of water. The phospholipase A1 was then immobilized in a packed bed reactor and the effects of pH, temperature and substrate ratio assessed. Optimum conditions for enzyme adsorption to the support were pH 7 and 50°C. The effect of water activity was also assessed. Water was found to have a positive effect on incorporation of the n-3 PUFA into PC and a negative effect on yield of the modified PC product. ♦

## Genomics for Industrial Fermentation

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A genome-scale metabolic model showing gene-protein-reaction associations has been constructed for *Lactobacillus plantarum* WCFS1. Uses of this model include data interpretation / visualization, for example in the analysis of anaerobic fermentation data with a complex medium; a metabolic map gives insight into which compounds influence ATP production. It was determined that valine catabolism is a transhydrogenase activity involving NADH / NADPH balancing rather than ATP. The model has also been used to assess the problem of growth delay in aerobic *L. plantarum* cultures. Data from before and after the growth delay were incorporated in the metabolic model and it was discovered that many reactions involving $CO_2$ were up-regulated after the growth delay ended. Using this information, it was hypothesized that flushing with $CO_2$ would improve the fermentation process and remove the delay, and when tested the hypothesis was found to hold. The model can be used to predict metabolism. For example, the prediction was made that *L. plantarum* would grow on an unusual and inexpensive substrate. Initial growth on this substrate was poor, but after adaptive evolution the predicted performance was achieved. Although these models are incomplete (kinetic data are limited) these models can become powerful as a result of better integration of "-omics" data and establishing better constraints on metabolism with respect to kinetics, physiochemical properties and population dynamics.

In *Saccharomyces cerevisiae* metabolism and physiology, growth rate and cell-cycle progression are tightly coordinated with the fermentation environment. In aerobic fed batch reactors with low sugar and slow growth, *S. cerevisiae* shows high biomass production. The produced cells are then applied commercially in anaerobic fermentation with high sugar to raise dough. There is an adaptation period between these two types of growth and to improve the commercial product, it is important to understand the genetic controls of the adaptation phase. To test the genetic response, oxygen-respiring conditions were replaced with nitrogen and a glucose pulse was given. The global genetic response was then analyzed using DNA microarrays; it was biphasic with massive genetic reprogramming occurring 30 minutes after the pulse was administered. Further genomic studies with *S. cerevisiae* have allowed production of a xylose-fermenting, high-ethanol-producing strain. *Aspergillus niger* CBS 513.88 is another important industrial organism and with the completion of a genome sequence it has been possible to establish metabolic functional categories for the organism and to perform comparative genomics and synteny studies to other *Aspergillus* species. Knowledge of the genome of this strain has made it possible to engineer the organism to be an optimal citrate producer.

The association between growth and product formation in industrial microorganisms is a limiting factor for increasing product formation, therefore it would advantageous to decouple product formation from growth in non-biomass-driven applications. This goal could be achieved by creating a state of zero-growth product-formation using genomic approaches. Zero growth is defined as a metabolically active, non-growing state of a microorganism in which product-formation capability is maintained. This state is fundamentally different from starvation and resting states. Zero-growth experiments have been performed with *L. plantarum* and *S. cerevisiae*, in batch cell suspensions and retentostats (reactors with cell-recycling and a constant rate of substrate addition). Glycolytic intermediates were analyzed in cultures of *L. plantarum* producing sorbitol. In growing cells, phosphate intermediates accumulated, whereas in resting cells pyruvate increased. Soluble ATPase were cloned into cells to artificially increase ATP demand and although growth was not affected, sorbitol production decreased. Prolonged retentostate cultivation was performed with *L. plantarum* and *S. cerevisiae* with over 80% viability, in terms of metabolic activity, in both species. Microarray results indicate that cell-wall integrity and glycogen genes are up-regulated in retentostats. It was also observed that retentostat growth led to heterogeneity in the *L. plantarum* population. ♦

## Novel Enzymes for Carbohydrate-Based Foods and Drugs


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The use of fibrous plant materials in ethanol production has created a need for value-added by-products. Using enzyme-assisted extraction, it is possible to obtain antioxidants from press residues or "slimming" fibers from potato pulp. Cardio-protective antioxidants are found in the skin and seed fractions of dark fruits and wine-press residues. Red-wine pomace could be a feedstock for extracting these compounds. The hypothesis was put forth that the phenolic compounds of interest are locked up inside the cell-wall matrix and may be released by enzymic hydrolysis of polysaccharides. Fruit skins have both primary and secondary cell walls, which require multiple enzymes for degradation. It was determined experimentally that the phenols were bound randomly in this complex matrix, so all of the matrix should be digested. Using a mixture of pectinase and protease, 40–50% hydrolysis was achieved. The resulting antioxidants were tested on human low-density lipoproteins and results indicated that potency may be dependent on the enzyme treatment. Potato pulp is another potential feedstock. Enzymatically "solubilized" Potex fibres, tested on a small group of human subjects, acted as dietary fiber. The slimming effect was explored further in rats, and digestion results indicated that there was increased intestinal fermentation in those fed the solubilized fiber. These solubilized fibers show potential for food functionality in reduced-fat liver paté and wheat bread.

Enzymatic engineering of glucose polymers can result in the creation of a variety of useful products, *e.g.* glycogen. Two new methods of enzymatically synthesizing glycogen have been developed. The first requires sucrose phosphorylase, glucan phosphorylase, and branching enzyme and uses sucrose as the substrate. The second method involves isoamylase, branching enzyme, and amylomaltase and uses starch as the initial material. The advantage to the first method is that only one step is required and branching is highly controlled, but yields are low. The second method has a high yield and offers control over the molecular mass of the product, but requires two steps and has decreased control of branching. Both glycogen products have similar properties to glycogen from natural sources, but are purer and have more-uniform characteristics than the natural compound. Immunological benefits of natural glycogen have been proposed, but conclusive evidence has not been available because of impurities and non-uniformity. Synthetic glycogen can, therefore, be used to evaluate immunological activity. It was shown that glycogen does have anti-tumor activity, which can be attributed to the enhancement of the functions of macrophages. The degree of stimulation is related to molecular mass.

Bread becomes stale with age because of starch retrogradation, which can be diminished by using Novamyl amylase. A life-cycle assessment comparing low and high doses of Novamyl indicated that high doses would decrease fertilizer need and farming-equipment usage for wheat production, heating and electricity for milling and baking, packaging and transportation, and wasted stale bread; 1 kg of Novamyl could save 3,800 kg $CO_2$. Protein engineering was applied to the Novamyl enzymes for use in cakes the properties of which differ from those of breads. One major limitation to using Novamyl variants was sucrose inhibition. It was determined that hexasaccharide and maltose fit into the active site of the enzyme, whereas sucrose docks in a slightly different location. Random micro-titer plate screening and site-directed mutagenesis were employed to improve sucrose binding. The resulting enzyme was five-fold more effective for cakes than the initial enzyme. ♦

## Bio-Solutions for the Detergents Industry


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Detergent enzymes provide a broad spectrum of performance benefits. In the conventional view of functionality, an enzyme was considered a specific "power" ingredient, but today an array of enzymes—cellulases, lipases, amylases, proteases, *etc*.—are considered "basic" ingredients. There is demand for detergents effective at lower wash temperatures, because of higher energy prices. Experiments evaluating wash performance and wash temperature for different amounts of surfactant and enzyme blends, showed that the best performance was achieved by decreasing surfactants. Studies with cost-neutral formulations showed that using enzymes to replace conventional ingredients resulted in the same wash performance at 20°C that the reference formulation achieved at 40°C. By reducing wash temperature from 40°C to 30°C a household can save approximately 30% on electricity per wash. If all of Europe made this adjustment, ~4 billion kWh (the equivalent capacity of two power plants) could be saved. The development of new enzyme products will continue and assay design will be a key to success. Improved expression technologies, better understanding of structure-function relationships and high-throughput screening systems will expedite the development of new products. For example, to improve the storage stability of a pectate lyase, enzyme variant libraries were developed and high-throughput screening determined that stability was improved two- to three-fold with a single amino acid substitution, and multiple substitutions resulted in a 100-fold improvement.

To minimize impacts of price increases of petroleum-based raw materials in line with trends toward environmental sustainability and energy conservation, natural and alternative feedstocks for laundry applications are being explored. Palm oil stearine (POS) has potential as a replacement for sulphonates and methylestersulphonates in surfactants. Other C12–C14 sources are possible, in addition to tropical oils. Algae are a potential source of oil, because of their high lipid content. *Cuphea*, a plant indigenous to the Americas, is another possibility; some species produce mid-chain-length triglycerides. Much work needs to be done for *Cuphea* to be competitive. Seed genetics must be elucidated and yields and oil-recovery processes optimized. Biosurfactants could also be sourced from microbial fermentation. There is a broad range of possible biosurfactants with properties similar to synthetic surfactants, therefore functionality may be the same. The limiting factor for the adoption of biosurfactants is cost. Availability of glycerine—another important component of laundry products and a byproduct of biodiesel production—is increasing as the biodiesel market grows. Glycerine could be converted to other products such as acrylic acid and propylene glycol. However, there are cost concerns with glycerine from biodiesel; it is difficult to ensure a continuous low-cost supply and if subsidies are removed the market may collapse. Biopolymers, the third category of compounds of interest for laundry applications, include polyamino acids, starches and polysaccharides.

Surfactant-based cleaning products have traditionally been improved by chemical modification. A new strategy for improvement is the addition of non-toxic Class 1 microorganisms. Grout and pores in commercial kitchen floors are difficult to clean and populations of unwanted bacteria thrive there. A three-tiered approach has been taken to develop the Novo DeepClean series, which combines chemical, microbiological, and enzyme technologies. The surfactant component effectively removes surface soil and the bacterial component breaks down the residual soil in pores and crevices. BioS 3112 organism is capable of degrading long-chain volatile fatty acids, which eliminates odors. The added bacteria replace the existing bacterial population. The third component, enzymes, hastens the initial cleaning action and improves effectiveness of the microbial activity.

Multifunctional enzymes, such as SGNH-hydrolases with acyltransferase (AcT) activity, could be useful for replacement of detergent chemicals. Mining of the SGNH-hydrolase enzyme family, resulted in the selection of AcT, an arylesterase with acyltransferase and perhydrolase activities and glycine C-acetyltransferase, which has acyltransferase activity. These enzymes are active across broad temperature and pH ranges, do not require cofactors, have long shelf life and can be successfully produced at cost-effective titers. Acyltransferases are capable of *in situ* generation of bleach from peroxide, surfactants from alcohols, and fragrances from terpene and benzyl alcohols. The production of fragrances is a particularly useful application, because a drawback of lipases is that the release of free fatty acids generates odor. With acyltransferases it is possible to generate a fragrant ester that would remain bound to the residual soil on the fabric. Identification of the unique structural characteristics of enzymes with AcT activity has made it possible to engineer lipases with AcT activity, which will resolve an unmet need in the laundry industry. ♦

## Industrial Biotechnology for Oil and Mineral Recovery

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Roughly two thirds of the oil discovered in the United States is unrecoverable using existing technologies, and although this has driven research into alternative fuels, it is still important to recover this resource to develop energy independence. New surfactants and microbial recovery methods have been developed, but both have drawbacks. Surfactants with ultra-low interfacial tension qualities also have poor high-temperature stability, which limits their usefulness. Microbes also have temperature and time limitations. A new technology combines a specific class of surfactants, branched alcohol propoxylate sulphate surfactants, with low-molecular-weight proteins. The resulting product shows greater reduction in interfacial tension than with the surfactant alone, and also exhibits increased heat stability. Field studies with this technology showed that this protein-surfactant combination converts oil to surfactant-like materials that further increase oil mobility and recovery. This effect, seen under sterile conditions, is increased in the presence of indigenous bacteria. Another observation is that the proprietary proteins drive the indigenous bacteria to produce more $CO_2$ because the proteins facilitate the uncoupling of oxidative phosphorylation. The increased oil production and operating efficiency resulting from this technology will more than offset the chemical costs and be a cost-effective method of enhanced oil recovery.

For enhanced oil recovery (EOR), the use of microbial gums provides several advantages including lack of dependence on climatic conditions for product quantity, the ability to alter product composition and properties through the production process, and the polysaccharide products are extracellular, making recovery simple. Xanthan gum in particular has potential for oilfield EOR, due to unique rheological properties. It is nontoxic, has high molecular weight, is water-soluble, has high plasticity and viscosity at low concentrations and is compatible with metallic ions. Xanthan gum can be commercially prepared by anaerobic submerged fermentation of the plant-pathogenic bacterium *Xanthomonas campestris*. Experiments were performed on two strains, C1 and C15, to produce xanthan gum from cane-sugar juice, which is abundant in Brazil. Fermentation was conducted at approximately 28°C with aeration and agitation, then the temperature was raised to 85°C to precipitate the cells, and ethanol was added to precipitate the xanthan gum. Rheological studies were performed on the gum products at 25°C and 50°C. Strain C15 produced xanthan with viscosities high enough to be used for EOR. Increasing temperature reduced viscosity, but at 50°C was still high enough for use in EOR applications. ♦

# *Business Development, Infrastructure and Public Policy*

## The Emerging Defense Department Bioenergy Market

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Milissa Pavlik (US Air Force Research Laboratory)

The US Department of Defense (DOD) is the world's single largest consumer of energy, at ~2% of overall US usage, *i.e.* $11 billion annually or 62% of that utilized by the federal government. Jet fuel constitutes the largest fraction at 70%. Some 50% of the fuel consumed by the army is by non-combat forces; it spends $200 million annually on fuel and $3.2 billion on its distribution. Under the president's executive order 13423, signed in January 2007, *Strengthening Federal Environmental, Energy, and Transportation Management*, there is new emphasis on energy-saving, mostly through conventional means—conservation, improved efficiency of use and increased awareness—and by utilizing alternative sources. A number of army, navy and air force agencies are involved in biotechnology research. Total DOD spending on biotechnology increased by 45% between 2000 and 2004. Although current research on improved energy efficiency is non-biotech in nature, stronger linkages with the US Departments of Energy and Agriculture and the Environmental Protection Agency are contributing to growing interest in biological and hybrid approaches. The Defense Department is committed to alternative / renewable energy. Its priorities include bio-batteries and -refineries for expeditionary warfare. In a 30-day period, the average special operations force A-team uses 4,000 batteries of thirty-two types at a cost of $330,000. And since fuel is the second largest tonnage on the battlefield, the use of waste from mess / ration breakdown is being examined as a means of generating fuel on-the-go; there is potential to produce 300 gallons of ethanol per day from 1.2 tons of waste. Research in cooperation with the DOD has many advantages, *e.g.* the Defense Advanced Research Projects Agency (DARPA) funds research that commercial entities will not support. However, the DOD is a large and complex organization encompassing many R&D entities each with unique requirements.

The United States—the world's largest consumer of crude (~25%)—imports ~65% of its oil requirement. Global competition is increasing, particularly from India and China. Worldwide refining capacity is at ~97% of demand, therefore supply and demand are tenuously balanced. A $10 per barrel increase in oil increases the amount that the DOD spends on fuel by ~$1.4 billion per year. The typical forward-operating base, in Iraq for example, requires 10,000 gallons of diesel per day (excluding aircraft) of which 10% is needed for vehicles and 90% for power production, of which 95% is needed for air conditioning. Three truck-supply lines into Iraq, from Kuwait, Jordan and Turkey, carry 890,000, 200,000 and 200,000 gallons of fuel per day. Six truck-supply lines into Afghanistan carry 50,000–115,000 gallons per day for a total of 700,000 gallons per day. Half of the transportation fuel is consumed by the truck convoys, which are vulnerable and take thirty attacks per day in Iraq. Within the army, major activities in bioenergy research include:

- Edgewood Chemical Biological Center
  - biobatteries / tactical biorefineries, biosensors, biomanufacturing,
- Natick Laboratories
  - food / biofeedstock materials, soldier support,
- Tank-Automotive Research, Development and Engineering Center
  - alternative fuels, engines, vehicles.

With respect to energy in particular, a confluence is occurring in terms of national priorities, military strategic importance and tactical relevance. Although low-tech solutions may be brought to bear, there is need for advanced technology with contributions from academic, commercial and national laboratories. The cost of fuel should be measured not only in dollars but also in the lives of military personnel.

The US Air Force—the largest consumer of energy in the federal government—is serious about being a global leader in facility energy conservation and renewable energy supply and is committed to a transition to alternative and renewable fuels. Plans are in place to push for the production and purchase of domestically produced synfuel from plants that use coal, natural gas, or other derivations that incorporate greenhouse-gas reduction processes to provide the right fuel. Advanced energy technologies must meet environmental expectations, and compliance requirements to achieve full acceptance and integration. Current efforts include aircraft conversion for use of Fischer-Tropsch fuels. Certification of the B52 fleet was achieved within a year, and it is projected that the whole fleet will be similarly certified by 2010. Also the ethanol / biodiesel blend (8% / B20) produces a 28% offset in petroleum consumption, with superior shelf-life and low-temperature performance, and reductions in NOx, CO and smoke. In 2006, the air force used 7 million gallons of B20 (three times more than the navy and seven times more than the army). However, performance with B20 was variable; in extreme cases, engines were ruined for reasons yet to be fully explained. Effort is on-going also to convert plastics to syn fuels at forward-operating bases to reduce need for fuel convoys. Several such plants are already in commercial operation in Australia, Europe and Japan. The air force intends to increase use of alternative fuels by 10% per year and to achieve a 20% reduction in petroleum usage by 2010 and a 50% synthetic off-set by 2016. ♦

## Hot Policy Topics: Recent US Policy Initiatives


**Graham Mathews (Dickstein Shapiro)**
**Anne Steckel (American Farm Bureau Federation)**
**Neel Kashkari (US Department of the Treasury)**


The likelihood of adoption of legislation addressing climate change has increased dramatically over the past year as public opinion has shifted as a result of severe weather, drought and wildfires in the west, reports of melting polar icecaps, Al Gore's *An Inconvenient Truth* and the November 2006 elections. Many House and Senate committees are examining the issues and several bills are in the pipeline with similarities and differences in terms of phased reductions in greenhouse-gas emissions and market-based cap-and-trade systems. In general, regulations will be imposed "upstream" (on producers, processors, refiners, *etc.*) with all sectors of the economy affected (electric power generation, industrial / commercial entities, refiners of imported oil, *etc.*). The Sanders-Boxer-Waxman bill is projected to have the strongest effect on emissions, with a 75% reduction over the current level by 2050. The "Holy Grail" of phase 1 is reduction in $CO_2$ emissions to 1990 levels by 2020, potentially achievable under the auspices of the Sanders-Boxer-Waxman, Kerry-Snowe, Oliver-Gilchrist, and McCain-Lieberman bills. The Energy Policy Act of 2005 mandated a renewable fuel standard of 7.5 billion gallon of ethanol by 2012, whereas the president's 2007 "state-of-the-union goal" was 35 billion gallons of "renewable" and "alternative" fuels by 2017. Implications for US agriculture and the biotechnology industry include:

- a corn-demand increase by 50% by 2008,
- the highest corn acreage in 60 years at ~87 million acres in 2007,
- the surge in corn production will continue to be driven by demand for ethanol (2.2 billion bushels in 2007 will increase to ~3.2 billion bushels in 2008),
- corn prices are more than double those of a year ago,
- increased feed prices are causing increased prices of beef, pork, and poultry to consumers, and
- an ethanol "backlash" on Capitol Hill from meat-producers' associations.

There is growing recognition on Capitol Hill that corn is not the whole story, that ethanol feedstocks must be diversified to include biomass crops for the production of cellulosic ethanol. This will require additional R&D to integrate pre-treatment, hydrolysis and fermentation processes.

Broad benefits are expected to accrue from significant adoption of renewable fuels, including rural revitalization, enhanced energy and national security, mitigation of climate change and creation of new markets for agricultural products. The 2002 Farm Bill was the first to contain a separate energy title, reflecting policy linking agriculture to energy. As a result, a number of initiatives were launched to boost production of farm-based renewable energy, including ethanol, biodiesel and wind. Although the 2007 Farm Bill will be written with a budget deficit, there is general agreement that the Energy Title of the next Farm Bill will include opportunities for growth in production of renewable fuels, new markets for biobased products, and investments in businesses producing value-added goods. The Farm Bureau believes the focus of the Energy Title of the 2007 Farm Bill should be on farmers, increased economic development in rural America and continued creation of opportunities for agriculture to lead the United States to greater energy and national security. The concept of a "transitional assistance" program to assist farmers in the adoption of cellulosic crops will be appraised, and a loan-guarantee program will be established for cellulosic energy projects, particularly in rural areas. There will be funding for demonstration projects to streamline collection, transport and storage of cellulosic feedstocks and for accelerated development of one-pass harvesting equipment. And workforce-education programs in biofuels technology will be established at land-grant universities and at biofuels research and testing centers. Research will be funded for better utilization of distillers' grains for feed production.

Importation of oil by the United States is increasing more rapidly than consumption as domestic production falls (America ranks tenth in reserves of crude). The United States is the largest consumer of oil (21 million barrels per day in 2005), but China's consumption is growing quickly (7 million). Approximately two thirds of US oil consumption is for transportation, and 97% of transportation energy comes from oil. This dependence on foreign sources of oil presents economic and national security risks. Volatile prices hurt families and businesses, and terrorists view global oil infrastructure as a target with negative consequences for the United States in particular. Iran and Venezuela have been emboldened by high oil prices, with diplomatic, political and military implications. President Bush's "Twenty in Ten" plan is a bold effort to reduce, by 2017, national consumption of gasoline by 20% (5% from improved efficiency, 15% from alternative fuels); it includes doubling of the strategic petroleum reserve. Development of alternative transportation fuels is the critical factor; scientists, entrepreneurs and farmers will be key to their economic production, including corn / sugar ethanol, cellulosic ethanol from variety of feedstocks, biodiesel, butanol and other biofuels, electricity and hydrogen. The US Departments of Energy and Agriculture are aggressively supporting development of biofuels. The Advanced Energy Initiative has funding of $2.7 billion ($179 million for biofuels). The DOE is establishing a loan-guarantee program, has selected six cellulosic plants with funding of up to $385 million and is providing funding of $250 million to establish two bioenergy research centers. The Farm Bill is expected to provide >$1.6 billion over 10 years for bioenergy research and energy-efficiency grants and $2 billion in loans for cellulosic ethanol plants. ♦

## Industry, University, Government Collaborative Centers for Industrial Biotechnology


### Alan Weimer (Colorado Center for Biorefining and Biofuels)
### Justin Bzdek (Blue Sun Biodiesel)
### Al Darzins (National Renewable Energy Laboratory)
### Rick Zalesky (Chevron Technology Ventures)


Establishment of the Colorado Center for Biorefining and Biofuels ($C_2B_2$)—announced March 19, 2007—will occur July 1, 2007. Its missions are:

- to be a leading center for research and education in biorefining and biofuels,
- to recruit, train and produce the best engineers and scientists in the biorefining and biofuels community, and
- to improve fundamental understanding and to develop new technologies in areas relevant to the commercialization of integrated, sustainable biorefining and biofuels processes and to accelerate their introduction into the marketplace.

The $C_2B_2$ will be composed of University of Colorado (Boulder), Colorado State University (Fort Collins), the Colorado School of Mines (Golden) and the National Renewable Energy Laboratory (Golden) with four corporate and seven small-business members. Its customer-base will be its sponsors (large and small), students and postdoctoral scientists, Colorado tax payers and partner institutions. For yearly sponsorship fees of $50,000 (large companies) or $10,000 (SBIR companies), benefits will include:

- access to expertise and facilities with minimal bureaucracy ("one-stop shopping"),
- a venue promoting inter-disciplinary projects,
- access to exclusive intellectual property for sponsored research,
- a shared-risk environment for exploratory research with access to shared royalty-free IP, and
- a venue for training employees and as a source for trained graduates.

Students and postdoctoral scientists will have access to funding, excellent facilities and research projects, a collegial learning environment, reputable institution affiliation and employment opportunities. By combining faculties and facilities, sponsors will be able to carry out R&D and provide education, training and opportunities for students on a scale impossible for any single institution.

Blue Sun Biodiesel—a founding member of $C_2B_2$—is a market- and technology-driven vertically integrated biofuels company, focused on agricultural R&D, fuels and next-generation technology R&D, ag operations and fuel operations. It's the Rocky Mountain region's leading supplier of premium biodiesel blends made from pure vegetable oils, and recently completed series-A financing to build biodiesel plants, more blending terminals and crush facilities. Blue Sun Fusion premium B20 diesel fuel is marketed through exclusive distributors in ten western states and Canada. Blue Sun farmer cooperatives produce biodiesel oilseed crops commercially. Completion of construction of a Blue Sun biodiesel plant (exceeding ASTM specifications) is expected late 2007. Their patent-pending technologies include biodiesel ultra-filtration and oilseed processing. Strategic partnerships are sought for the development of technologies for processing next-generation biodiesel feedstocks—cellulosic and other plant residues, algae, *etc*.—to phospholipids, Fischer-Tropsch products, paints, adhesives, *etc*., including supercritical $CO_2$ applications for direct conversion of grain to biodiesel.

The $C_2B_2$ is important to the National Renewable Energy Laboratory in providing access to:

- fundamental and applied expertise / equipment not currently available,
- scientist exchanges with $C_2B_2$ institutions and sponsor companies,
- alternative funding sources for biofuels research (seed grants),
- broad collaboration with strong industrial input, and
- the very best students (training rotations) and postdoctoral scientists.

Research thrusts at $C_2B_2$ will involve crop engineering, biochemical engineering, thermochemical engineering, process engineering, product engineering and systems engineering.

Chevron does not provide grants for $C_2B_2$, instead it collaborates directly. Intellectual property can be a difficult issue in private / public collaborations; sometimes universities demand sole ownership. Shared IP, under the auspices of $C_2B_2$, serves all involved equally. Researchers in public institutions tend to underestimate the potential of new projects to come to fruition. At $C_2B_2$ there is appropriate emphasis on commercialization. Short- and long-term $C_2B_2$ projects will be carefully balanced for optimal integration; Chevron's involvement makes good business sense. ♦

## Going Green: Ins and Outs of Growing a Green Start-Up

Andrey Zarur (Kodiak Venture Partners)
Peter Fasse (Fish & Richardson)
Oliver Peoples (Metabolix)

Global climate change is widely accepted as the single greatest threat to humanity's survival. On the other hand, the world's energy demands, in total and *per capita*, are growing at the fastest rate ever. Furthermore, US national security concerns mitigate against continued consumption of traditional energy sources. A clean-energy economy is developing globally, as part of which venture capital must play a role in development of start-up companies. Venture capital (VC) firms are already investing heavily in clean-tech start-ups, particularly in biofuels; the 2005–2006 investment rate was faster than in the early Internet days (1996–1997). However, although most requisites for successful VC investments are present, two important shortcomings have precluded investment by Kodiak:

- inappropriate business models are currently used by ethanol producers and potential company acquirers, and
- overall energy balance is hard to calculate.

The faulty energy-balance budget is the single most common flaw in business plans for start-up companies in renewable energy.

Early development of an overall intellectual-property (IP) strategy in conjunction with legal counsel—an involved strategic partner rather than a mere scribe—is highly recommended. The goals of the IP portfolio should be identified and a global strategy developed along with a budget and filing schedule and identification of the technologies to be protected. The strategy should identify the types (patents, trademarks, copyrights, trade secrets) that will best protect the products. A company can waste time and money with random attempts to protect IP that do not conform to a clear strategy. Since the IP can be the core value of a start-up company, a realistic dollar amount should be budgeted toward protection of all types of IP. Although provisional patent applications are useful, temptation to file cheap provisionals should be avoided. It may save fees initially, but it is more important to describe inventions carefully to support later applications. Trademark-availability and domain-name-clearance searches should be done before selection of a corporate name and embarking on a marketing campaign. Freedom to operate should be verified before designing the commercial product, with time and money budgeted to identify competitors' patents. Employees should not own the IP; as early as possible, patent applications should be explicitly assigned to the company.

Metabolix was founded 1992–1993 with technology licensed from MIT, with the vision of combining bioscience and nature to bring clean solutions to the world in plastics, fuels and chemicals. Between 1994 and 2000, a genome-engineered microbial platform was developed for synthesis of natural plastics. In 1998, a new endeavor was initiated: production of natural plastics in genetically engineered switchgrass. An alliance with ADM was forged in 2005 and Metabolix went public in 2006. More than 80% of the microbial dry weight accumulates as natural plastic. The process has been demonstrated at the industrial scale and can be manipulated to produce a range of plastics. Natural plastics have strong advantages over petroleum-produced counterparts in terms of fossil-energy content and greenhouse gasses emitted. The technology is young and will continue to advance for many years with new opportunities for value creation in line with market forces. Commercialization will require significant capital and secure feedstocks will be essential. Although licensing will undervalue the technology, going it alone would be too expensive, hence partnering offers a viable alternative. The joint venture with ADM will lead to commercial production of natural plastics via fermentation, and Tepha is a spin-off company resulting from collaboration with Boston's Children's Hospital that manufactures medical devices including TephaFLEX absorbable sutures. Further partnering opportunities exist in terms of natural plastic production in switchgrass and biomass biorefinery products, including biobased chemical intermediates.♦

## Making the Big Bet: Success in Developing Cellulosic Ethanol Alliances

William Baum (Diversa)
William Provine (DuPont)
Carlos Riva (Celunol)

Diversa's enzyme and organism discovery and development technologies represent an accelerated path to commercial production of cellulosic ethanol. Choice of biomass source is important because of its contribution to overall process cost, resulting from availability, recalcitrance and digestibility. Ultimately, genetically engineered biomass crops will enable refinement of processing economics. A variety of biomass-pretreatment methods is available, each of which differentially affects the resultant carbohydrate and non-carbohydrate components, enzyme requirements and the choice of ethanol-producing organism. A pre-treatment-compatible cellulase / hemicellulase combination is necessary for saccharification of complex feedstocks. Ethanol yield and cost are directly proportional to saccharification efficiency. Organisms fermenting the mixed C6 / C5 sugars from the saccharification stream must produce ethanol efficiently and at high concentration. Organisms are in development for fermentation of the five sugars in lignocellulosic biomass (glucose, xylose, arabinose, galactose, mannose). Most candidate organisms have relatively low rates of C5 conversion and are variable in tolerance of ethanol and in sugar-utilization rate. Various pre-treatment / saccharification / fermentation options have been envisioned, including:

- separate saccharification and fermentation,
- simultaneous saccharification and fermentation (SSF),
- simultaneous saccharification and co-fermentation (SSCF, fermentation of C6 and C5 sugars in a single reactor), and
- consolidated bioprocessing (CBP, saccharification and fermentation in a single organism).

Diversa has entered several partnerships to address aspects of feedstock type, quality and pre-treatment and to optimize bacterial and yeast ethanologens, while optimizing Diversa enzyme cocktails for saccharification.

DuPont's integrated corn biorefinery (ICBR) concept involves the conversion of all above-ground plant components to ethanol, chemicals and steam / electricity. By conversion of stover and cobs, 375 gallons per acre of ethanol may be produced in addition to 340 gallons from kernels. DuPont has forged formal partnerships with several private and public entities; the concept, initiated in 2003 is projected to see commercialization in 2010. Biofuels—ethanol and butanol are the focus at this stage—present global market opportunities. Current annual consumption rates *vs.* projections for 2020 are:

- North America—5 billion gallons *vs.* 30 billion gallons,
- South and Central America—4 *vs.* 7,
- European Union and Eurasia—1.1 *vs.* 20, and
- Asia and Pacific—1.7 *vs.* 30.

Enzyme pre-treatment of cobs and fiber—at room temperature—produces a mixture of solids (30%) and soluble sugars (235 grams per liter). Metabolic engineering has produced superior ethanologens; a strain of *Zymomonas mobilis* efficiently converts glucose and xylose, simultaneously, to ethanol. The Broin-Dupont collaboration encompasses a cellulosic ethanol demonstration plant in South Dakota, and a 125 million gallon per year plant in Emmetsburg, IA (construction jointly funded by Broin and the US Department of Energy), that will include 25–30 million gallons per year of cellulosic ethanol. Anticipated ancillary benefits include:

- 11% more ethanol per bushel of corn,
- 27% more ethanol per acre of corn,
- an 83% reduction in fossil fuels compared to a similar-sized grain plant, and
- a 24% reduction in water usage.

Celunol is a leader in the emerging cellulosic ethanol sector. Founded in Massachusetts in 1994, it has exclusive rights to breakthrough technology that led to construction of the first pilot plant in the United States, in Jennings, LA. Now under construction in Jennings is a 1.4 million gallon per year demonstration plant and under license is a 1.4 million gallon per year demonstration plant in Japan. Two uniquely designed microorganisms are employed for separate fermentation of C5 and C6 sugars after acid hydrolysis of multi-feedstock biomass. The merger with Diversa meant a combining of complementary platforms, bringing the potential to achieve commercial production of cellulosic ethanol by 2009 rather than by 2011, *i.e.* Diversa's gene-discovery and enzyme-development and -production capabilities with Celunol's operating assets, proprietary processes and project-development capability. Celunol-Diversa's keys to successful commercialization of low-cost fuel-grade ethanol include:

- critical first-mover advantages
  - foundational-process optimization and commercial expertise
  - partners providing complementary state-of-the-art technologies
  - as a magnet for world-class scientific and operational talent,
- multi-feedstock process capability, and
- strategic site selection for choice of land and feedstock.♦

## Lignocellulosic Business Models in Action

Michael Ott (BIOWA)
Scott Kohl (ICM)
George Anderl (Genencor)
David Hogsett (Mascoma)

BIOWA Capital seeks opportunities to invest in the bioeconomy in terms of ethanol and biodiesel plants, new technologies and related infrastructure. Commercial production of cellulosic ethanol has three main challenges:

- harvest, transport and storage of biomass feedstock,
- conversion of feedstock to ethanol and high-value co-products, and
- transportation of ethanol and co-products to end-users.

Regionally specific feedstocks require different pretreatment methods, enzymes and organisms for optimal production of ethanol. Industry is capable of addressing the second challenge, with government help, as evidenced by the number of companies committed to commercial production of cellulosic ethanol: ADM, Broin, Celunol, Iogen, *etc*. The US rail system is nearly overburdened transporting current amounts of ethanol and DDGs. Expansion of the ethanol industry will quickly outstrip rail-transportation capacity. Dedicated pipelines will be necessary, requiring investment of public funds.

Between 1980 and 2000, production of ethanol in the United States accelerated at 75 million gallons per year per year, whereas between 2001 and 2006 the rate of increase was 770 million gallons per year per year, much of it contributed by ICM. From 2006, the rate of increase is projected at 2,950 gallons per year per year, which, at a total production of ~12 billion gallons per year in 2008, would require 4 billion bushels of corn. Clearly, alternative sources of carbohydrate will be required to meet demands for ethanol, *e.g.* other grains, sugar cane, and biomass such as wood, woodchips, stover, switchgrass and paper. Many different options are available for processing biomass, with three main approaches:

- sugar platform
  - largest body of work in the recent literature
  - similar to today's corn-starch industry,
- thermal platform
  - long history with petroleum industry
  - different from the corn-starch industry,
- "hybrid" platforms
  - emerging from current research
  - combine attributes of the sugar and thermal platforms.

Retrofitting existing infrastructure needs careful consideration; new construction may be less costly. Capital costs for first-generation demonstration plants will be higher than projected; however, second-generation plants may be expected to be less costly and consideration may be given to construction on adjacent property, in order to share infrastructure such as roads.

Genencor, a division of Danisco, envisions a future where:

- biotechnology fulfills many unmet needs
  - in development and deployment of industrial enzymes
  - in the production of fuels, chemicals and materials,
- biotechnology helps create sustainable industrial activities, and
- biorefineries take their place alongside oil refineries.

Oil consumption is expected to increase in all geographical sectors over the next two decades. The petroleum usage of China and India could accelerate to ten times that of the United States, which currently leads the world. The upper limit of US agriculture to replace gasoline with corn-starch ethanol is ~10% at ~15 billion gallons per year, hence cellulosic ethanol will be needed. Starch and cellulosic sources have the potential to produce ~50% of US needs for transportation fuel without an overhaul of the agricultural system and significant planting of biomass crops. The major barrier to economic production of cellulosic ethanol has been addressed over the past 5 years: cellulase cost is no longer a bottleneck in biomass conversion. Furthermore, there is much opportunity for continued improvement at the system level. Some fifteen companies have already invested in cellulosic ethanol (or have stated the intention). The winners will be technology integrators who can rapidly demonstrate the utility of their systems, deploy them and build ethanol capacity. Each player must select and optimize four unit operations: substrate, pre-treatment and enzyme hydrolysis appropriate for the substrate, and fermentation. The Mascoma project in Rochester, NY, obtained $15 million in funding from the state to build and operate a biomass-to-ethanol plant to demonstrate cellulose-to-ethanol technology on a commercial scale using locally available agricultural and forestry products. Genencor will expand manufacturing capacity at its existing NY facility to supply enzymes to the Mascoma effort.

Mascoma envisions a world with broad access to sustainable, renewable, and affordable energy, with Mascoma providing a significant portion of that energy through low-cost, efficient and environmentally beneficial use of cellulosic biomass technologies. The company's overall strategy is to develop and deploy a suite of advanced technologies, and, over time, establish defensible intellectual property (IP) for integration into production plants for rapid market expansion. Mascoma will be active in licensing and partnering as necessary to ensure freedom to operate across the cellulosic-ethanol value chain. In 2006, the United States produced a near record 10.7 billion bushels of corn, of which 1.8 billion was used for ethanol production, 17% of the total. There is potential to produce 15 billion bushels per year by 2015, a third of which could be used for 15 billion gallons of ethanol. Corn-starch ethanol production currently is 5.6 billion gallons per year, with capacity for 6.4 billion gallons under construction. President Bush has set a production goal of 35 billion gallons per year by 2017, which will require cellulosic ethanol. The US Departments of Energy and Agriculture have estimated that 1.3 billion tons of cellulosic biomass could be sustainably produced per year in the United States (equivalent to 60 billion gallons of ethanol); in the short term the focus is on wood, corn stover, rice straw, bagasse and corn fiber, and in the long term on high-yielding dedicated energy crops (switchgrass, *Miscanthus*, willow, *etc*.). Mascoma's $20 million demonstration plant in New York was announced in December 2006, with $15 million of state funding. It will have a multi-process design to accommodate up to 20 tons of feedstock per day (paper sludge, hardwood chips, willow chips, corn stover). The first commercial-scale plant is being designed, with discussions on-going relative to multiple sites with partners and feedstock suppliers. ♦

## Forging Bioproduct Synergies: The Illinois-Ontario Example


**Gord Surgeoner (Ontario Agri-Food Technologies)**
**Rich Schell (Illinois Global Partnership)**
**Mohini Sain (University of Toronto)**
**Hans Blaschek (Energy Biosciences Institute)**


At BIO 2006 in Chicago, IL, a memorandum of understanding between the Province of Ontario, Canada, and the State of Illinois was signed by Joseph Cordiano, minister of the Ontario Ministry of Economic Development and Trade, and Jack Lavin, director of the Illinois Department of Commerce and Economic Opportunity. One of the key objectives of the MOU is to promote bioproducts. Plastics production in Ontario—a $2 billion per year industry—serves a diverse market that includes packaging (34%), construction (28%) and the automobile industry (18%). Ontario produces more light vehicles than Michigan. The DaimlerChrysler S-Class automobile now has twenty-seven biobased components weighing 42.7 kg, compared to 24.6 kg in an earlier model, for an overall reduced weight. Impact-bearing polyol foam—for auto upholstery, *etc*.—can be produced from soybean. In comparison with synthetic polyol, it is price neutral, but has superior properties. Thermoplastic starch is rapidly replacing polystyrene foam in auto-part packaging. Single polymer technology is garnering support—an initiative to replace interior glass and multi-plastic parts with a single polymer. Polylactic acid is of limited scope for the auto industry; although its functional properties match those of petroleum-based plastics, production is relatively costly and durability is poor. The outlook for polyhydroxyalkanoates is more promising in that their properties are superior to those of polypropylene with a predicted price of <$1 per lb; commercial production is expected in 2008. Hemp grows well in Canada and holds promise as a biomass crop.

Illinois' agricultural commodities generate more than $9 billion annually, of which corn constitutes $4 billion and soybean $3 billion. Approximately 370 million bushels of Illinois corn are converted in eight plants to 1 billion gallons of ethanol. Twelve more plants are planned. Two biodiesel plants are operational and seventeen are under construction. New collaboration between the University of Illinois and the University of California, funded by BP, is expected to put Illinois in the vanguard of bioenergy-crop production as a participant in the Energy Biosciences Institute; there is particular interest in maximizing productivity of *Miscanthus*. Furthermore, researchers in several disciplines at the University of Illinois are planning a new $20 million research facility dedicated to interdisciplinary bioenergy research. The Integrated Bioprocessing and Research Laboratory (IBRL), completion anticipated in 2009 with funding from the US Department of Energy, will focus on advanced bioprocessing technology for economic production of biomass-based fuels, such as cellulosic ethanol, and other biochemicals. The Illinois state legislature is considering expansion of the services provided by the National Corn to Ethanol Research Center at Southern Illinois University, Edwardsville, to create an Illinois State Bio-Refining Center of Excellence for ethanol training and research. The center is envisaged as the nation's pre-eminent ethanol-related institution, providing state-of-the-art training to the emerging ethanol-based workforce and spurring R&D projects to improve and advance the state's ethanol industry. The center will also serve as a third-party source to review, test and validate research around the country on renewable energy resources. Chicago has been the crossroads of the US railroad industry for more than a century. A third of rail and truck cargo moves to, from or through the Chicago region, making Illinois well placed for transportation of biofuels and other biobased chemicals.

Global consumption of plastics stands at ~200 million tons per year, a $500 billion market; it is growing at 7% per year. Production of crop-based fibers for manufacture of plastics and other structural materials for automobiles is a growing industry; production passed the 1-million-ton milestone in 2006 and is expected to grow by up to 350% by 2020. The Biocar Initiative is a joint effort involving industry and the Universities of Guelph, Toronto, Waterloo and Windsor to increase bioplastic components in cars and trucks manufactured in Ontario. Weight saving per automobile is projected at 10 kg. Replacing 70% synthetic plastics with renewable materials in cars results also in savings in $CO_2$ emissions and energy consumption involved in the manufacturing process. Plans include genetic modification of biomass crops to improve the uniformity of feedstocks. The Ontario-Illinois MOU will provide a research and technology bases to underpin the Biocar Initiative. Also, a state-of-the-art pre-commercialization facility is available for manufacture of auto-parts from reinforced bioplastic-fiber composite (for thermoforming, injection, compression and extrusion). A rapid commercialization strategy has been devised, drawing on strategic collaborations to offset pre-commercialization risks, and the BioAuto Council is a consortium encompassing, a twenty-four member technology and investment committee, government representatives and policymakers, auto manufacturers, parts suppliers, farmers, fiber suppliers and other stakeholders to assist delivery of new products to the marketplace.

The global energy company BP announced in February, 2007, that it had selected the University of California-Berkeley, in partnership with the Lawrence Berkeley National Laboratory and the University of Illinois at Urbana-Champaign, to lead its unprecedented $500 million, 10-year, research effort—the Energy Biosciences Institute (EBI)—to develop new sources of energy and reduce the environmental impact of energy consumption. A research agenda is being developed. The scientists involved will seek total-system solutions to the production of biofuels that are cost-effective and carbon-neutral. Approximately twenty-five thematic laboratories will be assembled to carry out basic and applied research in energy biosciences including:

- feedstock development,
- biomass depolymerization,
- biofuels production,
- fossil fuel bioprocessing and carbon sequestration, and
- socio-economic issues.

Success will be enhanced with a facility housing scientists skilled in chemistry, physics, engineering, materials sciences, genomics and biology, in contiguous workspaces fostering interactive research, and will be propelled by the common purpose of solving a global problem of great magnitude and urgency, the excitement of performing pioneering research and utilizing innovative methodologies, and the goal of expeditiously bringing transformative energy technologies to the marketplace. The EBI is expected to sponsor symposia to inform both the public and the professional community of progress in the various fields of interest. And, because of the level of entrepreneurial activity in the region, meetings will be sponsored also to engage the business community. ♦

## Achieving Bioenergy's Full Potential: A Path Forward for States and Other Public Agencies

Margaret Brennan (Rutgers University)
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To promote a more economic and environmentally responsible energy future, New Jersey has set a goal of reducing projected energy use by 20% by 2020 and meet 20% of the state's electricity needs with renewable energy sources by 2020. To achieve this, the state needed to determine its biomass capacity and the energy potentially derivable using current and near-term technologies, as well as analyses of the economic feasibility of bioenergy options and the development of effective bioenergy policies. Information about a region's biomass resources typically resides across an array of state agencies, educational institutions, and federal research facilities. Rutgers University was given primary responsibility for collecting and analyzing information for the state-energy master plan. The Rutgers Energy Institute (REI) integrates scientific, engineering, economic and policy resources across all academic units to foster both fundamental and applied scientific research, along with policy considerations, to develop sustainable energy production compatible with economic growth and environmental vitality. The EcoComplex is the university's environmental research and extension center, dedicated to moving science from the laboratory to real-world applications in New Jersey's businesses and industries and to promoting NJ as a center for environmental innovation and enterprise. Assessment of NJ's energy-from-biomass potential involved:

- examination of characteristics and quantities of NJ's biomass resources,
- examination of technologies (commercially available, or near) capable of producing bioenergy for electric power and transportation,
- development of a state-wide mapping of waste / biomass resources, and
- development of policy recommendations for moving NJ into the forefront of bioenergy innovation.

These deliverables will create capacity to further develop bioenergy potential for the state. Forty biomass resources (sugars / starches, lignocellulosic biomass, oil crops, solid and other wastes) and twelve technologies were analysed and an interactive database created, allowing a multitude of analyses based on biomass supply and technology variation. Because agricultural feedstocks are in relatively short supply in New Jersey, trash will be used to generate ethanol. Sorting in the home and at the landfill will be necessary to obtain best productivity; economic incentives will be needed. The energy captured will be used for electricity generation. States should consider universities as key partners in the exploration of local opportunities for bioenergy production.

States and regional entities may enhance bioenergy-production capability with a five-step plan:

- document biomass quantity, quality and location,
- assess technologies to determine estimated bioenergy contribution,
- determine infrastructural requirements,
- examine technical, regulatory and legislative impediments, and
- incorporate these into a bioenergy master plan and involve stakeholders.

A feedstock model that aggregates comprehensive biomass resource data provides a good start and can be used to assess energy potential from multiple technologies. To understand technical, infrastructural, and regulatory impediments, it is important to look across the value chain: feedstock resources / collection, transportation / conversion and refining / distribution / markets. For California, Navigant Consulting consolidated over 200 potential actions into ten categories, according to impact and criticality. This process provided an objective, structured methodology for identifying and prioritizing potential state actions. In July 2006, CA issued a bioenergy action plan with targeted objectives:

- maximize bioenergy contribution toward achieving petroleum-use reduction, climate change, renewable energy, and environmental goals,
- establish CA as a market leader in technology innovation, sustainable biomass development, and market development for biobased products,
- coordinate research, development, demonstration, and commercialization efforts across federal and state agencies,
- align regulatory requirements to encourage production and use of CA's biomass resources, and
- facilitate market entry for new applications of bioenergy including electricity, biogas, and biofuels.

Contingent upon this action plan are these bioenergy production targets:

- 20% of CA's redundant power supply mandate,
- 20% of biofuel consumption to be produced in CA by 2010, 40% by 2020; and 75% by 2050.

A comprehensive framework can help policymakers understand bioenergy's potential as well as the gaps that exist in reaching that potential.

A fifth of the 268-million-ton US corn harvest, was used to produce ethanol in 2006. New plant construction has already taken 2007 corn futures as high as $4.35 per bushel, impacting the price of corn in livestock farming, food and exports, and placing the profitability of corn-ethanol production at risk. In contrast, the gasification / fermentation process developed by Bioengineering Resources Inc. profitably co-produces ethanol, electricity and other by-products from feedstocks such as municipal solid waste, biosolids and animal wastes, agricultural residues, used tires and plastics, wood wastes, forest thinnings and food-processing wastes. The BRI technology thermally decomposes these feedstocks through an enclosed, oxygen-starved process (gasification). Then, in a reaction that takes 2 minutes or less, a patented bacterial culture (*Clostridium ljungdahlii*) reconstructs the resulting synthesis gases ($CO$, $H_2$ and $CO_2$) into ethanol, hydrogen and water. The final distillation process creates 99.5% fuel-grade ethanol. The process consumes 95% of any carbon-based feedstock, leaving a final residue of non-hazardous ash, yielding 75–85 gallons of ethanol per dry ton of biomass (twice that volume from tires or plastics). The anaerobic bacterial culture produces no undesirable by-products or human / environmental health hazard. Theoretically, the BRI technology could produce all of the nation's liquid energy requirements from domestically available feedstocks. ♦

## Growing the Biobased Economy


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President Bush has set ambitious goals for biofuels, including making cellulose-based ethanol cost-competitive with corn ethanol by 2012 and reducing US gasoline use (by light-duty vehicles) by 20% by 2017 ("Twenty in Ten") through a 5% reduction from enhanced efficiency (corporate average fuel economy, CAFE) and a 15% reduction from alternative fuels (*i.e.* 35 billion gallons per year). The US Department of Energy has set the longer-term "30 in 30" objective of replacing 30% of US gasoline consumption (by light vehicles, based on 2004 usage) with alternative fuels by 2030. Although biofuels represent only 3% of transportation fuels today—at 5.6 billion gallons per year—production is growing rapidly and total capacity with current and new construction is expected to be 11.7 billion gallons per year by the end of 2008. Corn-starch ethanol will contribute substantially to the "Twenty in Ten" market goal, but more biofuels will be needed. We need to enable production of ethanol from cellulosic biomass, a largely untapped resource in greater supply than corn; cellulosic ethanol, generated from non-food and waste feedstocks, emits ~60% less greenhouse gas than reformulated gasoline. Eventually, much more ethanol will be made from cellulosic biomass than from corn starch. DOE efforts are helping to pave the way for a strong, domestic bioenergy industry—with commercial success possible early in the next decade. Collaborative R&D is producing results both in biofuels and in bioproducts:

- substantial decreases in cost of ethanol production, from over $5 to approximately $2.50 per gallon,
- the first six commercial biorefineries were launched in February 2007,
- organisms have been developed with superior ability to convert mixed sugars to ethanol, and
- high-value plastics, foams, and coatings from oil crops and corn sugar have been developed.

But much remains to be done and achieving the "Twenty in Ten" challenge will demand continuing cooperation and collaboration over the next 10 years and beyond.

Title IX—the "energy title"—in the 2007 Farm Bill (in preparation) represents a new direction that will create opportunities for agriculture and for sustainable energy production. The US Department of Agriculture is drafting guidelines and regulations to implement provision of new opportunities for biobased products, power and other renewables; appropriations will be needed for some of the implementations. It is proposed, for example, that the BioPreferred Program be reauthorized and revised to improve effectiveness, with $18 million invested over 10 years. Also recommended is reauthorization of the Renewable Energy Systems and Energy Efficiency Improvements Loan-Guarantee and Grant Programs; the goals of the latter would be consistent with those contained in the Biorefinery Development Grants Program. Also proposed is a new program to provide $100 million in direct support to producers of cellulosic ethanol, operating like the CCC Bioenergy Program which was funded by the 2002 Farm Bill at up to $150 million for FY 2003–2006.

The 2001 Energy Policy of the European Union promoted renewable electricity generation with plans to increase production from 14% in 1997 to 21% by 2010. Biofuels for transport applications were promoted with measures to replace diesel and gasoline by up to 5.75% by 2010, and with tax exemptions. The European Commission's *Life Sciences and Biotechnology: A Strategy for Europe 2002–2010* laid out how biotechnology has the potential to improve non-food uses of crops as sources of industrial feedstocks or new materials such as biodegradable plastics, how biomass may contribute to alternative energy sources such as biodiesel and bioethanol, and how biotechnology may lead to the development of cleaner industrial products and processes using enzymes. The Commission is carrying out a mid-term review to provide a comprehensive assessment and cost-benefit analysis of the consequences, opportunities and challenges of biotechnology for Europe to update the *Strategy* for the 2007 Spring European Council. The term "knowledge based bio-economy" (KBBE) encompasses the industries and economic sectors that produce, manage and otherwise exploit biological resources (*e.g.* agriculture, food, forestry, fisheries and other bio-based industries); the European bio-economy has an approximate market size of over €1.5 trillion, employing more than 22 million. Refineries are already located throughout Europe, producing ethanol and biodiesel, and industrial biotechnology activities are in progress in a dozen member states.

A bioproduct development survey was completed by Statistics Canada—commissioned by Agriculture and Agri-Food Canada—in 2004, based on 2003 data. Two hundred and thirty companies were examined, initially in terms of province and size (two thirds were "small"), with determinants made in terms of bioproduct innovation and financial success. For many of the larger firms, bioproducts are a secondary activity; two thirds entered the field as a result of internal R&D and forty-four were start-ups (twenty-eight from universities and fourteen from other firms). The majority of the bioproducts were on the market, as opposed to being at the proof-of-concept or R&D stage. Success in achieving external funding increased with firm size (small 75%, medium 83%, large 92%). Small companies were less likely to obtain funding, but those that were successful obtained more of their target and averaged more than large firms. Access to new technologies via licensing was a significant factor in successful product development. A second survey, completed in 2006, will offer more insights into the sector as well as opportunities to appraise trends. ♦