Biotechnology
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Industrial Biotechnology: A Unique Potential for Pollution Prevention

Executive Summary

A decade ago, in 2007, a U.S. Environmental Protection Agency (EPA) report, "Bioengineering for Pollution Prevention," found that industrial biotechnology and biobased manufacturing are more energy efficient, cleaner and make use of sustainable renewable resources. The report reviewed the state of the science and recommended future research priorities.

Since 2007, companies have commercialized products that demonstrate industrial biotechnology's unique ability to reduce pollution, achieving measurable improvements in biomass sustainability, energy efficiency and carbon re-utilization.

The U.S. Department of Energy (DOE) has analyzed the technical feasibility and costs of developing a biomass supply sufficient to displace 30 percent of the nation's fossil fuel use. Concurrently, biotech companies have developed technology to improve crop management, first-of-a-kind sustainability inititatives, and new crops with environmental performance benefits.

Industrial biotech companies have begun commercializing processes that use methanotrophs and algae to capture CO2 and convert it to renewable chemicals, averting carbon and other pollutant emissions as well as displacing fossil fuels.

Manufacturers are using enzymes commercially to produce pharmaceuticals and other chemical compounds, food ingredients, detergents, textiles, paper products and biofuels, avoiding use of toxic feedstocks and process reagents, which in turn minimizes toxic waste and byproducts.

At the same time, companies have made substantial progress in improving cellulosic biomass conversion, microbial genetic engineering techniques, biorefinery operations, and life-cycle sustainability, addressing the challenges identified by the EPA report.

Companies have commercialized enzymes for producing cellulosic ethanol from agricultural waste and are operating cellulosic sugar production biorefineries at demonstration-scale. DOE's bioenergy research centers are researching and developing biomass pretreatment technologies.

Over the past decade, researchers and companies have made significant progress in engineering microbes, using synthetic-biology to optimize microbial metabolic pathways to produce new renewable chemicals and biofuels.

Companies that have built and operated commercial-scale biorefineries during the past decade have gained experience and provided insight on the challenges of large-scale biorefinery engineering in addition to bioprocesses.

And the biotech industry continues to utilize life cycle analysis to establish both the sustainability and cost-effectiveness of new biobased products, renewable chemicals and biofuels.

This progress illustrates industrial biotechnology's unique potential to reduce carbon, waste and energy use, while displacing fossil fuels. A new state of the science assessment could document the potential of emerging applications and set a roadmap to support continued commercialization. It could additionally examine the cost savings associated with energy efficiency and reductions in pollution.

Introduction

In January 2007, the U.S. Environmental Protection Agency's (EPA) National Center for Environmental Research published a report examining industrial biotechnology's unique potential to reduce pollution. According to the report, "Bioengineering for Pollution Prevention," biobased manufacturing minimizes the environmental impact of producing consumer goods because industrial biotech processes are more efficient, cleaner and make use of renewable resources; industrial biotechnology is inherently consistent with the principles of green chemistry.[1] The report authors reviewed industrial biotechnology's state of the science in 2007 and suggested future research priorities, including improvements in bioplastics and polymers, biofuels, and biorefinery technologies.

Over the past decade, the industrial biotechnology sector has quantifiably demonstrated its unique ability to reduce pollution in the manufacture of renewable chemicals, biobased products and biofuels. In many cases, the energy efficiency gains and reduction in byproducts translate into cost savings for manufacturers. Companies commercializing new biotechnology applications have achieved measurable improvements in biomass sustainability, energy efficiency and carbon re-utilization. Researchers have made significant progress in addressing the challenges originally identified in EPA's state of the science report. The biotechnology industry is optimistic that with rapid technological development it will achieve solutions to remaining challenges and attain additional environmental and cost saving benefits.

The federal government should launch a new examination of the state of industrial biotechnology science to set priorities for the next decade. In particular, the potential for cost savings in connection with pollution prevention and energy efficiency should be studied.

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[1] Ahmann, D. and Dorgan, J.R. (2007) "Bioengineering for Pollution Prevention through Development of Biobased Energy and Materials: State of the Science Report." Washington, DC: National Center for Environmental Research, EPA/600/R-07/028, Jan. 2007.

Biotechnology's Unique Contributions

EPA's 2007 state of the science report identified four unique aspects of biotechnology that contribute to pollution prevention, which include:

1. use of renewable biomass;
2. potential capture and reuse of waste carbon that would otherwise enter the atmosphere;
3. the ability to improve the efficiency of chemical transformations used in manufacturing; and
4. reduction of hazardous wastes.

Industrial biotechnology continues to improve in these aspects as it commercializes new products.

Biomass Sustainability

In 2005, the U.S. Department of Energy (DOE), in cooperation with the U.S. Department of Agriculture (USDA), analyzed the U.S. agriculture, forestry and waste management sectors' technical feasibility to supply one billion tons of biomass on an annual basis to build a domestic biofuel and biobased product industry.[2] This biomass supply – sufficient to displace 30 percent of the nation's fossil fuel use – was a key feature in EPA's 2007 analysis and conclusion that industrial biotechnology holds potential to reduce pollution.

DOE twice updated the analysis of biomass supplies in the United States, adding to subsequent reports cost calculations for harvesting the resources and delivering them to biorefineries. In 2016, DOE estimated that from 1.2 billion to 1.5 billion tons of biomass could be produced on an annual basis by 2040 at an average $60 per ton; more than half of this could be delivered to biorefineries at less than $84

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[2] U.S. Department of Energy. 2005. Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply. R.D. Perlack and B.J. Stokes (leads). ORNL/TM-/66. Oak Ridge National Laboratory, Oak Ridge, TN. 78p.

dollars per ton.[3] In addition to agricultural and forest biomass, the 2016 Billion-Ton Report identified 142 million tons of currently recoverable and reusable waste and, for the first time, assessed algae biomass and other energy crops for the future. DOE also assessed environmental indicators – such as soil carbon, water and air quality, greenhouse gas emissions and biodiversity – for these biomass resources, finding they held additional potential to reduce pollution.

At the same time, biotechnology has made measurable contributions to agricultural sustainability. Genetically engineered seeds enable farmers to produce more crops with fewer inputs. Farmers planting biotech crops with insect resistance traits have reduced pesticide spraying by 618.7 million kilograms (8.1 percent) over the past two decades (1996-2015), according to PG Economics. Reduced use of pesticides and herbicides saved fuel use, lowering carbon emissions by 26.2 billion kilograms of carbon dioxide over that same time period. Farmers using conservation tillage practices with biotech crops are estimated to have decreased greenhouse gas emissions and soil carbon loss equivalent to 227.2 billion kilograms of CO2 equivalent. If genetically engineered seeds had not been available to the millions of farmers who used them in 2015, maintaining that year's global production levels of corn, soy, cotton and canola would have required millions of additional acres of land – equivalent to 11 percent of the arable land in the United States.[4]

PG Economics' conclusions were replicated by researchers at Cargill and Purdue University, who used Global Trade Analysis Project (GTAP) models to project agricultural biotechnology impacts on food prices and availability as well as land use change and associated emissions. The researchers conclude that farmers' use of genetically engineered crops forestalled conversion of 3.1 million hectares of pasture and forest (2.5 million and 0.6 million hectares respectively) to cropland, relative to 2013 land use. Averting land use change saved 0.9 billion tons of

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[3] U.S. Department of Energy. 2016. 2016 Billion-Ton Report: Advancing Domestic Resources for a Thriving Bioeconomy. M.H. Langholtz, B.J. Stokes, and L.M. Eaton (leads). ORNL/TM-2016/160. Oak Ridge National Laboratory, Oak Ridge, TN. 448p.

[4] Brookes, G. and Barfoot, P. (2016) "GM Crops: Global socio-economic and environmental impacts 1996-2015." Dorchester, UK: PG Economics Ltd., June 2017.

additional greenhouse gas emissions. Looking at it from another angle, the authors calculated that if farmers in the rest of the world adopted biotech seeds (soybeans, cotton, and corn) at the same rate as U.S. farmers, cropland use would decrease, returning 0.06 million hectares to forest and 0.74 million hectares to pasture. Greenhouse gas emissions would decrease by 0.2 billion tons of CO2 equivalent.[5]

Over the past decade, industrial biotech companies pioneering cellulosic biomass technologies have developed first-of-a-kind sustainability initiatives. DuPont signed a memorandum of understanding with USDA's Natural Resources Conservation Service to develop conservation plans with each farmer providing corn crop residues to its Nevada, Iowa biorefinery to ensure sustainable harvest of the feedstock.[6] And POET-DSM established a Responsible Stover Harvest program that limits stover collection to 20-25 percent of above ground residues.[7]

Biotech companies continue to commercialize new energy crops that can optimize the carbon lifecycle for bioenergy, biofuels and bioproducts. In 2015, Canadian seed producer Agrisoma Biosciences contracted farmers in western North Dakota and eastern Montana to grow at least 6,000 acres of Brassica carinata, a type of mustard whose seeds are crushed for oil to produce jet fuel. In 2015, NexSteppe produced more than 25,000 acres (10,000 hectares) of its Palo Alto biomass sorghums in Brazil, demonstrating that the crop could be produced at commercial scale. In 2016, NexSteppe entered into an agreement with Longping Hi-Tech Arable Land Remediation Technology Company, a subsidiary of Chinese seed company

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[5] Mahaffey, H., Taheripour, F., and Tyner, W.E. (2016) Evaluating the Economic and Environmental Impacts of a Global GMO Ban. *Journal of Environmental Protection,* 7, 1522-1546. http://dx/doi.org/10.4236/jep.2016.711127.

[6] USDA Natural Resources Conservation Service. (2013). USDA announces new conservation collaboration with DuPont to promote sustainable harvesting of bio-based feedstocks for cellulosic ethanol. March 29, 2013. Retrieved from USDA Natural Resources Conservation Service: http://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/newsroom/releases/?cid=STELPRDB1086233.

[7] POET Biomass. (2014). Responsible Stover Harvest. April 25, 2014. Retrieved from POET-DSM.com: http://poet-dsm.com/resources/docs/responsible-stover-harvest.pdf.

Longping Hi-Tech, to distribute, market and sell NexSteppe's Palo Alto biomass sorghum hybrids in China.

Nearly one-fifth (19 percent) of China's arable land is polluted with heavy metals such as cadmium, arsenic and nickel, according to the Chinese government. The root systems of biomass sorghums can extract heavy metals from contaminated soil. The sorghum can provide biomass feedstock for biopower as it remediates the soil to safe levels for food and feed production.8

Carbon Capture and Utilization

Microbes exhibit a multiplicity of metabolic processes for producing energy and chemicals, including photosynthesis, fermentation and respiration. Microbes also can use a wide variety of potential feedstocks – in addition to sugars – such as gases, including carbon oxides, and both organic and inorganic compounds. Industrial biotech companies have made progress in commercializing processes that use methanotrophs and algae in combination with alternative feedstocks.

USDA, EPA and DOE estimate that as of 2014 the United States had more than 2,000 operational biogas systems using manure, landfill gas and water recovery biosolids as feedstocks. The agricultural sector emits more than 200 million tons and landfills emit approximately 100 million tons of CO2 equivalent pollution each year. Annual methane reductions from the landfill, livestock and wastewater sectors could range from 4 million to 54 million metric tons of greenhouse gas emissions by 2030, according to the agencies.[9]

Companies utilizing captured carbon oxides (CO and CO2) to produce renewable chemicals also avert carbon and other pollutant emissions by displacing

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[8] Youngman, R. "Scale and speed: China's USP in cleantech. Tackling China's polluted arable land problem." CleanTech Group. Nov. 11, 2016. https://www.cleantech.com/scale-and-speed-chinas-usp-in-cleantech-tackling-chinas-polluted-arable-land-problem/

[9] U.S. Department of Agriculture, U.S. Environmental Protection Agency, U.S. Department of Energy. (2014) *Biogas Opportunities Roadmap: Voluntary Actions to Reduce Methane Emissions and Increase Energy Independence.* Washington, DC: August 2014.

petrochemicals. In a review of lifecycle assessments for carbon capture and utilization/sequestration schemes, researchers from the University of Manchester School of Chemical Engineering and Analytical Science found that production of dimethylcarbonate from captured CO2 reduces carbon emissions by 4.3 times and ozone layer depletion by 13 times compared to conventional dimethylcarbonate production.[10] The same authors found a large difference in the lifecycle assessment results for producing fuels (specifically biodiesel) from algae, depending largely on the assumed method of utilizing the resulting algal biomass. Lifecycle assessments for carbon capture and sequestration schemes outperformed those for utilization, since sequestration is assumed to permanently remove carbon from the atmosphere. However, another international team of researchers note that carbon can leak from sequestration; they propose that microbial systems can aid in detecting leaks and enhancing storage.[11]

Industrial biotech companies continue to make progress in commercializing processes that ferment methane or industrial off-gases into useable products. LanzaTech, with headquarters in Illinois, has piloted and demonstrated production of ethanol and 2,3-butanediol – a building block for plastics – from industrial off-gases, biomass syngas and syngas from gasified municipal solid waste. The company's industrial off-gas demonstration facilities are co-located at steel mills in China and Taiwan, with a pilot plant in Georgia, USA. At the Georgia site, ethanol produced from the China demonstration plant was used as feedstock to produce several thousand gallons of low-carbon aviation fuel The company is currently building its first commercial facilities in China and Belgium. LanzaTech won a

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[10] Cuéllar-Franca, R.M., Azapagic, A. (2015) Carbon capture, storage and utilisation technologies: A critical analysis and comparison of their life cycle environmental impacts. Journal of CO2 Utilization. 9, March 2015, pp. 82–102. http://dx.doi.org/10.1016/j.jcou.2014.12.001

[11] Hicks, N. et al. (2016) Using Prokaryotes for Carbon Capture Storage. *Trends in Biotechnology.* October 3, 2016. DOI: http://dx.doi.org/10.1016/j.tibtech.2016.06.011

Presidential Green Chemistry Challenge Greener Synthetic Pathways Award in 2015 for its technology.[12]

Newlight Technologies commercialized a process to ferment methane and CO2 to plastics, producing AirCarbon™ plastics at a facility in California. Newlight produces almost 30 billion pounds of product per year for manufacturers, including IKEA, Dell, Hewlett Packard and other companies. EPA's Green Chemistry Challenge program recognized the plastic as net carbon negative.[13] And AirCarbon™ achieved a Bronze level award in the Cradle to Cradle Certified™ products program administered by McDonough Braungart Design Chemistry.[14] California-based Intrexon has formed a joint venture with Dominion Energy to develop engineered microbes that ferment methane to farnesene and isobutanol, chemicals that have applications in fuels, cosmetics, and solvents. A second joint venture for Intrexon Energy Partners is exploring the same technology to produce 1,4-butanediol, a building block used in polyester and other plastics.

Calysta, a Menlo Park, California-based company, is developing sustainable feed ingredients for fish, livestock and pet nutrition. The company opened a small-scale facility in Teesside, England, in September 2016 to begin market introduction of single cell protein from methane. In April 2017, NouriTech™ – a partnership between Calysta and Cargill – broke ground on a 37-acre facility in Memphis, Tennessee. When completed in 2018, the facility will produce 200,000 metric tons per year of Calysta's FeedKind® protein for animal nutrition. NouriTech will employ 160 full-time workers.[15] An assessment of the environmental footprint of Calysta's production method indicates a reduction in use of water and land use in comparison

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[12] https://www.epa.gov/greenchemistry/presidential-green-chemistry-challenge-2015-greener-synthetic-pathways-award.

[13] Environmental Protection Agency. "EPA Honors Winners of the 2016 Presidential Green Chemistry Challenge Awards." June 13, 2016. https://www.epa.gov/newsreleases/epa-honors-winners-2016-presidential-green-chemistry-challenge-awards-0.

[14] http://mbdc.com/newlight-technologies-aircarbon-achieves-bronze-level-cradle-to-cradle-certification-award/.

[15] http://calysta.com/2017/04/calysta-cargill-officially-break-ground-on-nouritech-a-new-feed-production-plant-in-memphis/

to protein from agricultural crops, and the potential to reduce carbon emissions through use of biogas as the methane feedstock.[16]

Algae and cyanobacteria naturally possess the ability to metabolize carbon dioxide to chemicals and biomass. Companies continue to develop algae strains and production systems that capture carbon emissions and utilize them for new products. Algenol, a Fort Meyers, Florida based company, received a Presidential Green Chemistry Challenge Climate Change Award in 2015 for its development of cyanobacteria and photobioreactors that capture carbon dioxide from industrial emitters and convert it to ethanol and biomass. The algae biomass can be further processed to green crude.[17]

Energy Efficiency

Catalysis is an essential part of green chemistry. Catalysts lower energy requirements, increase reaction rates, and can reduce the number of process steps necessary to make chemical transformations compared to stoichiometric reactions.[18] Biocatalysis is another of industrial biotechnology's unique contributions to pollution prevention.

Biocatalysts – enzymes – possess unique properties that make them preferable to other organic and inorganic catalysts – metals and acids – in green chemistry. Enzymes are selective, specific and have a high catalytic rate; they are more efficient, producing chemical products with higher purity and fewer byproducts or waste.[19] With higher purity, manufacturers can separate end products from a production process with less solvent and fewer steps. Enzymatic processes –

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[16] Cumberlege, T., Blenkinsopp, T., Clark, J. (2016) Assessment of environmental impact of FeedKind™ protein. UK: Carbon Trust, April 2016.

[17] https://www.epa.gov/greenchemistry/presidential-green-chemistry-challenge-2015-specific-environmental-benefit-climate.

[18] Delidovicha, I., and Palkovitsa, R. (2016) Catalytic versus stoichiometric reagents as a key concept for Green Chemistry. Green Chem., 2016, 18, 590-593 DOI: 10.1039/C5GC90070K.

[19] Op. cit. Ahmann, D. and Dorgan, J.R. (2007)

because they are biological and natural – work under mild conditions, further reducing energy inputs and costs. Enzymes are biodegradable and can be improved through genetic engineering. Additionally, in contrast to metal catalysts, manufacturers typically produce enzymes through a fermentation process.

All biomanufacturing processes – whether enzymatic or microbial – share the unique characteristic of avoiding use of toxic feedstocks and process reagents, which in turn minimizes toxic waste and byproducts. Manufacturers must manage byproducts of bioprocesses to prevent pollution.[20]

Manufacturers use enzymes commercially to produce pharmaceuticals and other chemical compounds, food ingredients, detergents, textiles, paper products and biofuels.[21] The main energy use in the laundry process is heating the water. The average American family completes nearly 400 loads of wash per year, and only 21 percent of those loads are in cold water. By lowering the temperature from 60°C/140°F to 30°C/86°F, energy cost savings of 60 percent can be achieved. Enzymes deliver performance in low temperatures, saving energy in the wash process. They also reduce toxicity for local water bodies by replacing phosphates.[22]

In 2005, Novozymes introduced Polarzyme®, the first commercial enzyme to effectively remove stains in cold water. Using enzymes as detergent ingredients means manufacturers can reduce use of petroleum-based ingredients. In addition, wastewater that goes down the consumer’s drain from an enzyme-based cleaner is biodegradable, helping minimize the impact on public waterways. If everyone in the United States lowered their washing temperature from hot to warm and warm to cold, it could save the planet 7.4 million tons of CO2, which is equivalent to the annual emissions from 5 million cars or $1 billion in energy bills every year.

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[20] Junker, B. (2010). “Minimizing the environmental footprint of bioprocesses.” BioProcess International, Oct. 1, 2010. http://www.bioprocessintl.com/manufacturing/facility-design-engineering/minimizing-the-environmental-footprint-of-bioprocesses-303905/.

[21] Phillips, T. (2016) “Enzyme Biotechnology in Everyday Life.” The Balance, Oct. 13, 2016. https://www.thebalance.com/enzyme-biotechnology-in-everyday-life-375750.

[22] Nielsen, A., Neal, T., Friis-Jensen, S., and Malladi, A. (2010) “How Enzymes Can Reduce the Impact Of Liquid Detergents.” Happi, September 2010.

Industrial biotech companies continue to make progress in commercializing enzyme applications. University based genetic engineering researchers also continue to improve enzymes and enable novel chemical reactions. For example, researchers at the California Institute of Technology recently engineered a novel enzyme that can catalyze a carbon-silicon bond, something unknown in nature despite the relative abundance of the two elements.[23] This research holds potential for improved efficiency in drug development.

Codexis, headquartered in northern California, earned a Presidential Green Chemistry Challenge Greener Synthetic Pathways award in 2012 for its development of a novel enzyme that can produce the cholesterol lowering drug Simvastatin in a more efficient process than previously, reducing costs and waste products.[24]

Overcoming Challenges

In its 2007 state of the science report, EPA enumerated four challenges for biotechnology to realize its full potential for pollution prevention, including:

1. increased conversion rates for biological processes, including biomass saccharification;
2. selecting, improving and engineering microbes for conversion processes;
3. building and optimizing biorefineries; and
4. standardizing sustainability measurements, such as through life cycle analysis.

Companies and universities have achieved substantial research and development progress to address these challenges, warranting optimism that ongoing biotechnology research and development can overcome them in the future. EPA

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[23] Kan, S.B.J., et. al. (2016) "Directed evolution of cytochrome c for carbon–silicon bond formation: Bringing silicon to life." Science 25 Nov 2016: 1048-1051.
[24] https://www.epa.gov/greenchemistry/presidential-green-chemistry-challenge-2012-greener-synthetic-pathways-award.

should launch a new examination of the state of industrial biotechnology science to help industry set research and development priorities for the future.

Biomass to Sugar Conversion Rates

Biomass recalcitrance is a well-known and well-characterized challenge. Biomass' carbon content includes cellulose, hemicellulose and lignin. Lignin is difficult to degrade with enzymes but can be transformed into aromatics through chemical or thermochemical methods. Cellulose and hemicellulose yield different sugars, which are fermented by different naturally occurring microbes. Companies can separate cellulosic sugars from both hemicellulose and lignin through a variety of biomass pretreatment methods.[25] Researchers continue to improve the energy efficiency and cost effectiveness of integrated biorefinery processes that separate and transform biomass carbon streams, overcoming biomass recalcitrance.

In 2010, Novozymes introduced the world's first commercially viable enzyme mix, Cellic® CTec2, for producing cellulosic biofuel from agricultural waste. The product resulted from years of research – supported by DOE awards – aimed at bringing cellulosic ethanol's production cost below $2.00 per gallon for initial commercial-scale plants. Several pilot- and demonstration-scale facilities around the world now use the Cellic® enzyme mix to convert corn stover, wheat straw, wood chips, sawdust, waste and sugarcane bagasse to fuel. [26] In 2013, Novozymes also introduced a trio of enzymes – Spirizyme Achieve, Avantec and Olexa – that

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[25] McCann, M.C., and Carpita, N.C. (2015). Biomass recalcitrance: a multi-scale, multi-factor, and conversion-specific property. J Exp Bot 66 (14): 4109-4118. https://doi.org/10.1093/jxb/erv267.

[26] "New Novozymes Enzymes for Cellulosic Ethanol Enable Production Cost Below US$2 Per Gallon." Green Car Congress, February 16, 2010. http://www.greencarcongress.com/2010/02/cellicctec2-20100216.html

collectively increase starch ethanol yields by up to 5 percent, corn oil extraction by 13 percent, and energy efficiency by 8 percent.[27]

Companies have employed and tested a wide variety of biomass pretreatment methods and catalysts over time, including concentrated acid, sulfur dioxide, hydrogen peroxide, steam explosion (autohydrolysis), ammonia fiber expansion (AFEX), wet oxidation, lime, liquid hot water, carbon dioxide explosion and organic solvent treatments. A recent review of research shows that effective biomass pretreatment must increase the accessible surface area of the cellulose structure, decrystallize cellulose, separate the polymer structure of cellulose and hemicellulose, solubilize the hemicelluloses and/or the lignin, modify the lignin structure, and maximize the enzymatic digestibility of the pretreated material. Companies must accomplish all this while minimizing the loss of sugars, especially preserving the pentose (hemicellulose) fractions but limiting the formation of components that are toxic to or inhibit growth of fermentative microorganism.[28]

Three DOE-funded bioenergy research centers – the BioEnergy Science Center at Oak Ridge National Laboratory in Tennessee, the Great Lakes Bioenergy Research Center at the University of Wisconsin–Madison and Michigan State University in Lansing, and the Joint BioEnergy Institute at the Lawrence Berkeley National Laboratory in California – have reported progress on all the challenges associated with cellulosic and other advanced biofuels.[29] The centers have focused on AFEX, dilute acid, and ionic liquid pretreatment methods. Researcher also continue to

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[27] Keller, R. New enzymes increase ethanol production 5%. Farm Journal's AgPro, June 10, 2013. http://www.agprofessional.com/news/New-enzymes-increase-ethanol-production-5-210866971.html.

[28] Maurya, D.P., Singla, A., and Negi, S. (2015). An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. *3 Biotech*, *5*(5), 597–609. http://doi.org/10.1007/s13205-015-0279-4.

[29] Department of Energy. (2016) "Department of Energy-funded Bioenergy Research Centers File 500th Invention Disclosure." March 7, 2016. https://www.energy.gov/technologytransitions/articles/department-energy-funded-bioenergy-research-centers-file-500th.

develop enzymes that can tolerate extreme heat, ionic liquids, or other pretreatment catalysts to achieve simultaneous saccharification and fermentation.[30]

A number of companies also have made substantial progress in commercializing cellulosic sugar production. DOE's Bioenergy Technologies Office recently identified 22 companies that could supply kilograms or metric tons of cellulosic sugars and lignin to support biorefinery research.[31] Renmatix, based in King of Prussia, Pennsylvania, operates a demonstration scale plant in Georgia with a capacity to make three tons of sugars per day. It also operates a feedstock processing facility in Rome, New York. The company's PlantRose® process uses "supercritical hydrolysis" – high temperature and high pressure water – to separate a pure stream of cellulosic sugars from the hemicellulose in wood chips. The company recently received a new round of investment from Bill Gates and petroleum company Total.[32] Renmatix was presented a Presidential Green Chemistry Challenge Small Business Award in 2015.[33] Two other identified suppliers – Leaf Resources and ZeaChem – recently formed a joint venture to demonstrate Leaf Resources' Glycell™ process in the United States.[34] ZeaChem has built and operated a demonstration biorefinery in Boardman, Oregon.

Selecting, engineering and optimizing microbes

Over the past decade, researchers and companies have made significant progress in engineering microbes to produce new renewable chemicals and biofuels. A

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[30] Khare, S.K., Pandey, A., Larroche, C. (2015) Current perspectives in enzymatic saccharification of lignocellulosic biomass. Biochemical Engineering Journal. doi:10.1016/j.bej.2015.02.033.

[31] http://energy.gov/eere/bioenergy/cellulosic-sugar-and-lignin-production-capabilities-rfi-responses.

[32] Fehrenbacher, K. (2016) "Bill Gates Backs Biofuel Company Renmatix." Fortune, Sept. 15, 2016.

[33] https://www.epa.gov/greenchemistry/presidential-green-chemistry-challenge-2015-small-business-award

[34] Leaf Resources, "Joint Venture agreement with and Investment in US based biorefinery company, ZeaChem Inc." Australian Securities Exchange Announcement, Feb. 29, 2016.

number of companies now provide organism design and DNA synthesis services, such as Gingko Bioworks, Intrexon, and Twist Biosciences.[35] These companies and other researchers use synthetic-biology approaches and other modern biotechnology tools to optimize both microbial hosts and metabolic pathways. Researchers have developed gene editing techniques to improve the speed and precision of genetic engineering. Despite some successes and the commercialization of a few renewable chemicals, companies still face challenges for optimizing microbes for new molecules.[36] An updated state of the science report could guide companies and researchers in overcoming those challenges.

California-based Verdezyne has successfully commercialized a microbial production process, producing butanediol in partnership with BASF. The company is currently building a plant in Malaysia to produce dicarboxylic acid chemical intermediates such as adipic acid, sebacic acid and dodecanedioic acid (DDDA), using a yeast fermentation platform that received a Presidential Green Chemistry Challenge award.[37] DDDA is a building block for nylon 6,12, which is used in engineered plastics that require special properties. Verdezyne's process converts lauric acid, a twelve carbon fatty acid, derived from vegetable oil to DDDA through ω-oxidation by a genetically engineered *Candida sp.* yeast. Verdezyne scientists engineered the yeast to enable rapid, high-yield production while minimizing accumulation of pathway intermediates.

Selecting and engineering microbes remains challenging because microbial systems must use the carbon in feedstocks both to generate the desired chemical or intermediate and to perform necessary metabolic functions, such as producing

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[35] "The State Of Synthetic Biology: Investors Placing Bets in Food, Biofuel, Healthcare, And More." CBInsights Blog, March 15, 2017. https://www.cbinsights.com/blog/synthetic-biology-top-sectors/.

[36] Peralta-Yahya, P.P., et. al. (2012) Microbial engineering for the production of advanced biofuels. Nature 488, 320–328 (16 August 2012). doi:10.1038/nature11478.

[37] https://www.epa.gov/greenchemistry/presidential-green-chemistry-challenge-2016-small-business-award.

enzymes. The additional metabolic functions genetically engineered into a microbe create additional needs for carbon.[38]

Moreover, naturally occurring microorganisms often invade large-scale bioprocesses, taking nutrients away from the genetically engineered microorganisms designed for the system. Scientists at MIT and Cambridge, Massachusetts-based startup Novogy, engineered several common microbes – *E. coli, S. cerevisiae, and Y. lipolytica* – to depend on essential nutrients like nitrogen and phosphorous from atypical or inorganic sources. This change enables the engineered strains to outcompete invading microbes but limits their ability to survive outside the production environment, in the event of an unanticipated release.[39]

Renewable chemicals producers face ongoing challenges in establishing optimal protein expression levels to maximize target chemical production. While genetic engineers have increased exponentially their ability to read, write and edit genetic code, they have not developed a systematic approach to optimizing microbial metabolic pathway regulation. Researchers are developing dynamic pathway regulation as a tool to prevent the formation of toxic metabolic intermediates and proteins. Additionally, the production environment or system often is considerably different at commercial scale, when compared to lab, pilot and demonstration projects.[40] Manufacturers must understand the production environment when selecting and engineering microbial hosts.

EPA could help guide efforts to overcome these challenges by producing a new state of the science report.

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[38] Hollinshead, W., He, L. and Tang, Y.J. (2014) Biofuel production: an odyssey from metabolic engineering to fermentation scale-up. Front. Microbiol., 09 July 2014 | http://dx.doi.org/10.3389/fmicb.2014.00344.

[39] Shaw, J.A. et al. (2016) "Metabolic engineering of microbial competitive advantage for industrial fermentation processes." Science, Aug. 5, 2016, p.583-586. DOI: 10.1126/science.aaf6159.

[40] Chubukov, V., et. al. (2016) Synthetic and systems biology for microbial production of commodity chemicals. Systems Biology and Applications 2 (16009). doi:10.1038/npjsba.2016.9.

Building and optimizing biorefineries

In reviewing the state of industrial biotechnology 10 years ago, EPA defined several challenges for optimizing bioprocesses within biorefineries. Those challenges included designing new bioreactors; engineering new processes to separate desired end products from the fermentation process; and recycling or reusing solids and sugars remaining after the fermentation process. Researchers continue to study the challenge of designing large-scale fermentation processes, understanding that they can limit microbes' access to nutrients and feedstocks.[41] Companies that have built and operated commercial-scale biorefineries during the past decade have gained experience and provided insight on the challenges of large-scale biorefinery engineering in addition to bioprocesses.

An October 2016 workshop hosted by the DOE's Bioenergy Technologies Office (BETO) focused on the challenges of storing and handling biomass feedstocks, thoroughly testing processes prior to scale-up to achieve cost-effectiveness, and finding economic value or additional co-products in waste streams.[42] The workshop reviewed BETO's support of more than 35 first-of-a-kind integrated biorefinery projects – ranging in size from pilot to pioneer plants – over a decade's time. BETO identified several ongoing challenges for biorefineries to address in the future, including several connected with biomass, such as the inherent variability of many proposed feedstocks – both in size and in source – and the operational difficulty of handling them in a production process.

In addition to the remaining technical challenges, BETO identified several economic challenges. While companies have gained experience in scaling biobased processes from the lab to the commercial stage, they have not always achieved economic success. Outside economic factors, such as a high cost of capital for first-of-a-kind

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[41] Op. cit. Hollinshead, W., He, L. and Tang, Y.J. (2014)

[42] U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office. (2016) "Summary report from the October 5–6, 2016, Biorefinery Optimization Workshop in Chicago, Illinois." Washington, DC: DOE/EE-1514, December 2016.

bioprocesses combined with the costs of creating new markets and value chains for biobased products can make it difficult to smoothly scale-up projects.

Complete life-cycle sustainability

EPA's state of the science report recognized the need for consistent measurement of product sustainability, which enables comparisons of biobased products with petroleum-based products and with each other. Industry continues to utilize life cycle analysis to establish both the sustainability and cost-effectiveness of new biobased products, renewable chemicals and biofuels. According to the Organization for Economic Cooperation and Development, "Because of the interdependencies in processes involved in growing, harvesting, manufacturing, distributing and disposing of a product, sustainability requires a lifecycle ('cradle-to-grave') systems analysis encompassing the whole value chain."[43]

Industrial biotechnology makes measurable contributions to the life-cycle sustainability of other industries, such as animal agriculture. Within the past decade Novozymes, through a global alliance with DSM, introduced commercial enzymes that significantly improve the digestibility and nutritional value of animal feed. Improved nutrient uptake leads to better feed utilization and helps reduce environmental impacts.

In 2008, Novozymes introduced RONOZYME Proact®, a protease enzyme that improves the digestibility of protein for poultry. The enzyme reduces the nitrogen content of the manure by 3 to 4 percent, which reduces emissions of ammonia, nitrous oxide and nitrate. With widespread adoption of the enzyme, the United States could reduce chicken manure's nitrogen content by up to 80,000 tons, reducing emissions of ammonia by more than 48,000 tons per year. That could also reduce greenhouse gas emissions by 924,000 of CO2 annually, equal to taking 180,000 cars off the road. In 2011, Novozymes introduced RONOZYME HiPhos®, a

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[43] OECD (2011). Future Prospects for Industrial Biotechnology, OECD Publishing. doi: 10.1787/9789264126633-en

phytase enzyme that nearly eliminates inorganic phosphorous supplements in swine feed, with a potential global savings of approximately 1.7 million megatons of phosphate annually. The more phytate phosphorus the animals digest, the less phosphorus they excrete into the environment.[44]

There is an ongoing need to standardize sustainability certification systems, particularly at an international level, since some standards prohibit use of biotechnology products and processes in certified products. Forum for the Future has proposed voluntary standards for industrial biotechnology, challenging companies to commit to closed-loop manufacturing processes that maximize the value of biomass feedstocks and various waste streams.[45] Forum for the Future's suggested voluntary standards for industrial biotech companies include support for regulatory structures that put public interest (societal and environmental benefits) and private gain (business benefits) on equal footing, and promote extensive stakeholder engagement. The most stringent requirement for companies and researchers is to avoid any risk of gene transfer in the open environment.

Conclusion

In its 2007 assessment of the state of the science, EPA identified the unique potential for emerging industrial biotechnology innovations to prevent pollution across the life cycle of manufacturing consumer goods. Over the past decade, companies have commercialized industrial biotech applications and processes that achieved measurable environmental benefits. Through ongoing research and development, companies continue to address remaining challenges in

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[44] Glitsoe, V., Ruckebusch, J-P., and Knap, I. (2015) Innovation in enzyme development. DSM & Novozymes. White paper presented at the 36th Western Nutrition Conference, September 2015, Winnipeg, Manitoba, Canada. https://www.dsm.com/content/dam/dsm/anh/en_US/documents/DSM%20Whitepaper_Enzymes_CTA2.pdf.

[45] Porrit, J. (2013) Sustainable Returns: Industrial Biotechnology Done Well. London: Forum for the Future, January 2013.

commercializing cellulosic biomass conversion and building biorefineries. New genetic engineering tools and gene editing techniques can help speed solutions to remaining challenges.

At the same time, researchers continue to develop new applications that were not envisioned a decade ago, such as CCU technologies that utilize carbon monoxide and carbon dioxide directly as inputs for bioprocessing. A new state of the science assessment could document the potential of emerging applications and set a roadmap for successfully addressing challenges to continued commercialization. It could further document the cost savings associated with energy efficiency and reductions in byproducts in manufacturing. Industrial biotechnology continues to hold unique potential to generate environmental benefits that include carbon reductions, reduction of waste and energy use, and displacement of fossil fuels.