Perspective

Biofpr Biofuels Bioproducts & Biorefining

The impact of biotechnological advances on the future of US bioenergy†

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Abstract: Modern biotechnology has the potential to substantially advance the feasibility, structure, and efficiency of future biofuel supply chains. Advances might be direct or indirect. A direct advance would be improving the efficiency of biochemical conversion processes and feedstock production. Direct advances in processing may involve developing improved enzymes and bacteria to convert lignocellulosic feedstocks to ethanol. Progress in feedstock production could include enhancing crop yields via genetic modification or the selection of specific natural variants and breeds. Other direct results of biotechnology might increase the production of fungible biofuels and bioproducts, which would impact the supply chain. Indirect advances might include modifications to dedicated bioenergy crops that enable them to grow on marginal lands rather than land needed for food production. This study assesses the feasibility and advantages of near-future (10-year) biotechnological developments for a US biomass-based supply chain for bioenergy production. We assume a simplified supply chain of feedstock, logistics and land use, conversion, and products and utilization. The primary focus is how likely developments in feedstock production and conversion technologies will impact bioenergy and biofuels in the USA; a secondary focus is other innovative uses of biotechnologies in the energy arenas. The assessment addresses near-term biofuels based on starch, sugar, and cellulosic feedstocks and considers some longer-term options, such as oil-crop and algal technologies.
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Supporting information may be found in the online version of this article.

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Introduction and key findings

Advanced biotechnology will be crucial to improving biofuel supply chains. Over the next 10 years, most of the progress based on biotechnology research and development will be in conversion processes. Improvements in feedstocks will follow over a slightly longer period.

Potential biotechnology game changers for bioenergy include:

- parallel yield and convertibility improvements in biofeedstocks and residues
- robust, easily convertible lignocellulosic feedstocks and residues with minimal pre-treatment
- predictable agronomic feedstock improvements for yield and sustainability (e.g., low nitrogen and water use and increased soil organic carbon sequestration)
- ability to control rhizosphere (the soil microbial) communities to improve biofeedstock traits
- economically stable bioconversions able to handle biofeedstock variability
- new tools to rapidly and rationally genetically engineer new microbial isolates with unique complex capabilities (e.g., new enzymes and fuels or capability to thrive in harsh conditions such as pH or temperature extremes)
- rational reproducible control of energy fluxes and carbon balance in microbes (e.g., decoupling growth from metabolism)
- cellular redesign to overcome fermentation product inhibition while maintaining yield and rate
- stable, high-rate microalga lipid production in open systems
- expanded compatible biotechnology processes to produce co-products(e.g., from lignin) along with fuels

The developing bioenergy industry faces several broad issues that are critical to accelerating bioenergy deployment but that will be influenced only indirectly by biotechnology:

- Scaling up an industry capable of achieving US and global bioenergy goals requires building a huge supply chain.
- Given the large scale of biofuel production needed, massive market demand for co-products is necessary.
- The low density and decomposition of biomass is challenging for feedstock storage and logistics.
- Land-use and sustainability issues must be addressed. Biotechnological and agronomic approaches can increase sustainability and manage water use.

Also critical to bioenergy, but only indirectly influenced by biotechnology, are policy and economics issues. For example, the Renewable Fuel Standard (RFS) and other government policies directly support the biofuels industry, and uncertainty about their stability directly influences bioenergy investment. Consistent policies are essential for near-term deployment because they affect whether developers can obtain funding for first-generation biorefineries. Policies regarding uses of genetically modified organisms (GMOs) also will have a direct bearing on biotechnology implementation. Use of GMOs within the confines of a biorefinery is generally accepted if biocontainment is practiced; however, the use of GMO plants and algae is a matter of debate worldwide. Public acceptance of GMO plants in the USA depends on their being regulated and thoroughly studied. These restrictions can add years to field testing and eventual deployment; however, biotechnological advances can speed the regulatory processes. Algal feedstock developers presume their GMOs will be closely contained in closed photobioreactors.

Background

The global biofuels industry is growing.[1,2] A cellulosic biofuels industry is emerging with a total projected capacity worldwide of about 195 M gal/year (a modest amount compared with the 20 to 30 B gal/year for first-generation starch- and sugar-based ethanol production). Cellulosic biorefineries have opened in Italy, the USA, Brazil, China, and Spain; and others are scheduled to launch in 2014–2015. Most employ biological processes. Second-generation biofuel refineries have been announced for 2017 and beyond with a projected capacity of about 5 to 6 B gal/year. Global biofeedstocks could produce about 914 million tons of residues by 2030, which could replace half of the gasoline needed by then. The USA remains the deployment and technology leader in biofuels – both conventional (grain-based) and advanced (e.g., cellulosic ethanol, algal biodiesel, drop-in biofuels).However, other nations are gaining (e.g., Brazil, China, the European Union, and Southeast Asia). In 2011, Brazil produced just over half as much ethanol as the US total of ~53 B L/year.[3]

Scaling up the bioenergy industry to meet US and global bioenergy goals will require a supply chain with a volume rivaling the current agricultural and energy industries combined.[4] Although biofuel production has similar key requirements to other energy supply chain networks, it has several unique aspects related primarily to biofeedstocks and the potential use of biology for conversion. Consideration must be given to the *feedstocks* employed (e.g., agricultural residues, dedicated energy crops), *logistics and land-use* decisions associated with the selected feedstocks, *conversion technologies* used to convert the feedstocks (e.g., thermochemical conversion, fermentation) to *products* (e.g., ethanol),and *utilization* of the products produced (Fig. 1). See Supplemental S1 for longer definitions.

This assessment examines the influence of biotechnology on US biofuel production through the lens of the supply chain. Currently, ethanol from corn provides close to 10% of US liquid 'gasoline' fuel supply. Mandates of the US Energy Independence and Security Act of 2007 will require significant increases in feedstock amounts and sources. Thus, future supply chains may include cellulosic feedstocks for ethanol production (currently in deployment) and advanced fungible biofuels based on cellulosic or other biomass feedstocks (e.g., algal oils).

Discussion

Advanced biotechnology can benefit the bioenergy supply chain with near-term developments in conversion and co-products. (Most of our discussion covers this area. Feedstocks have great mid-term potential and receive less attention.) A main current driver for continued improvement in bioenergy is that liquid biofuels remain the only renewable alternative for the diesel and jet markets, which are benefiting from biotechnological improvements.

Table 1 summarizes factors in the biofuel supply chain and highlights how advanced biotechnology might significantly impact them. The following sections consider these factors in sequence – feedstocks, logistics and land use, conversion, and products – and present analyses of the feasibility of advances, risks, barriers to major advances and crosscutting impacts on the supply chain.

Drawing on improved understanding of mechanisms, advanced biotechnologies lead to targeted manipulations using genetic engineering, synthetic biology, directed evolution, or genetically assisted breeding. The biotechnological approaches most likely to have the greatest impact on the biofuel supply chain include synthetic biology, protein design, and associated tools. Synthetic biology can be defined as the rational design of biological systems on the basis of engineering principles. Central to this approach is the capability to regulate genes in native and introduced

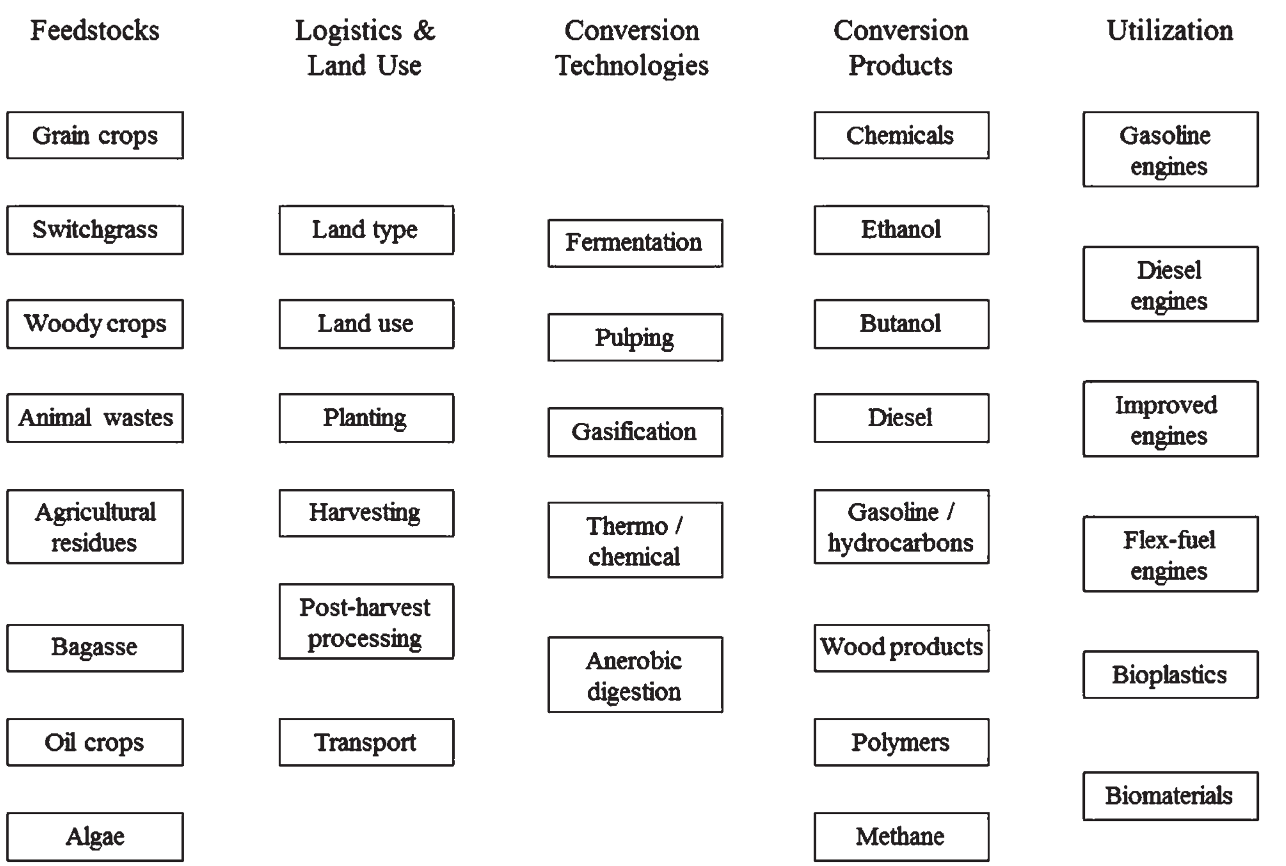

Figure 1. Schematic of major components of the biofuels supply chain.

Table 1. Critical factors affecting deployment and scale-up of US bioenergy industries

Feedstocks	Logistics and land-water use	Conversion technologies	Products and utilization
Insufficient yield	*Spatially dispersed feedstock sources*	*Insufficient yield, rate, and titer*	Certification of new fuels
Tolerance to environmental stresses (e.g., drought)	*Low bulk density and high moisture content of feedstock*	*Convert all variable feedstock compositions (e.g., recalcitrance)*	*Biofuel demand –ethanol blend wall, development of new biofuels (e.g., biojet fuel)*
Amendment requirements (e.g., fertilizer)	*Biomass stability during storage*	*Marketable Co-products*	Policy stability (e.g., Renewable Fuel Standard)
Adoption of genetically modified crops	*Feedstock displacement of cropland*	High capital costs and investment risk	Compatibility with existing infrastructure
Halophytes, saline agriculture	Indirect land use change	Scalability tradeoffs	
Algae production	Scalability tradeoffs	*Direct production of drop-in fuels*	
	Water requirements		

Factors for which advanced biotechnologies are expected to make a significant impact are indicated in *italic* type.

metabolic pathways in a controlled fashion. Examples of bioengineering options include

- varying promoter activity and efficacy.
- using differently inducible promoters.
- modifying ribosome binding strength.
- exploiting messenger ribonucleic acid (mRNA) secondary structures to differentially. stabilizing specific mRNA segments.
- amplifying genes on chromosomes.
- using nonnative RNA polymerase.

Site-specific genome bioengineering tools that facilitate targeted genome editing and transcription modulation are essential for elucidating the function ofdeoxyribonucleic acid (DNA) elements. Such tools include homologous gene targeting, transposases, site-specific recombinases, meganucleases, integration of viral vectors, and artificial zinc-finger technology. When coupled to effector domains, an emerging technique – customizable transcription activator-like effectors – provides a promising platform for achieving a wide variety of targeted genome manipulations.[5] These site-specific genome bioengineering tools may achieve greater precision and predictability in modifying biological traits in feedstocks and potentially in microbial-base conversion technologies.

Variations in enzymatic function and protein features ultimately have functional relevance at the organism level and thus have been tested over evolutionary time via natural selection. The nascent field of protein engineering has been most used for sector design in microbial sciences.[6] There are two main approaches to improving or creating new protein functions: rational design of proteins and directed evolution. 'Rational design' is defined as the knowledge-based design of new proteins; 'directed evolution' refers to the generation of new protein functions through functional screening of randomly generated variant copies of the protein. DNA writing and site-specific genome engineering capabilities form the basis of the rational design approach. The directed evolution approach requires high-throughput, automated design, construction, engineering, and evaluation of tens of thousands of combinations using robotics and computational techniques.

Other important biotechnological tools include:

- molecular techniques relevant to
  - robust, reliable, and rapid single-cell genomics.
  - single-molecule detection.
  - protein profiling from very small samples.
  - DNA writing.
  - gene stacking.
  - genome engineering.
  - transient and stable transformation.
- phenotyping techniques for *in situ* measurements.
- standards-adhered reconstructions of transcriptional networks and nodes.
- annotated genome sequences from hundreds of multiple relevant species and genotypes.

The creation of transgenics or GMOs can prove the function of key genes or pathways leading to the desired phenotypes. Armed with this knowledge, genetically assisted breeding can greatly accelerate the development of specific phenotypes that are nontransgenic and thus not regulated as GMOs. This will be especially useful for improved non-GMO plant biofeedstocks, especially where the traits are either cisgenic or already within the natural variation in the population.[7]

Feedstocks

Biotechnology can improve the feedstock component of the supply chain in multiple ways:

- increasing feedstock yields
- improving robustness and reducing amendment (e.g., fertilizer) requirements
- increasing tolerance for environmental and biological stresses such as drought and pest attacks
- modifying feedstock composition (e.g., to reduce biomass recalcitrance to conversion).

Improvements will likely occur through genetic understanding that affects the phenotypic characteristics of interest. Potential useful manipulations include breeding efforts that select for traits of interest and direct genetic modifications. Traditional breeding will transform into genetically assisted breeding using biotechnological tools to rapidly identify desired traits in plants at earlier stages. Plant biotechnology will have a major impact (although field trials slow the impact of new discoveries). Some concepts in yield and robustness are already in field trials, and research into composition will follow.

Yield, robustness, and planting requirements

Agricultural and forest residues are critical feedstocks for the first biofuel conversion facilities.[8] Corn stover, a particularly important agricultural residue, is the feedstock source for cellulosic ethanol facilities currently under construction by POET[9] and DuPont.[10] But because corn grain probably will continue to be a higher-value commodity than stover for bioenergy, most biotechnology efforts to improve corn focus on optimizing grain yield and quality. Average grain yields have been projected to approach 250 bu/ac in 2030,[11] up from an average 160 bu/ac in 2013.[12] This increase could have the paradoxical effect of actually reducing corn stover feedstocks. The total amount of stover produced depends on the harvest index (ratio of grain mass to total aboveground crop mass). Currently, the harvest index averages 0.5 (half of the above ground plant mass is grain); but genetic selection to increase grain yields could increase the harvest index to as much as 0.7,[11] greatly reducing the amount of corn stover available. For example, if the corn grain yield were 250 bu/ac with a harvest index of 0.5, the pre-harvest stover yield would be 15.7 Mg/ha (7 ton/ac). If the harvest index increased to 0.7, the stover yield would drop to 6.7 Mg/ha (3 ton/ac). However, the harvest index can be modified to increase biomass production by advanced breeding and biotechnology if the markets make such efforts worthwhile.[11] Similar changes might also be seen in residues from other food crops.

Biotechnology will be used to improve robustness and decrease water/nutrient requirements for energy crops. Two active avenues are in control of the plant (such as osmotic capacities, transpiration, nutrient transporters) and manipulation and control of the rhizosphere, where certain microbes have been shown to enhance drought resistance in switchgrass.[13]

Leading candidate crops that may supply second-generation feedstocks for lignocellulosic ethanol include perennial grasses such as switchgrass, Miscanthus, energy cane, and short-rotation woody crops like poplar and willow. Perennial oilseed species such as Jatropha may serve as biodiesel feedstocks,[14] and algae may eventually become a major feedstock.

The attractiveness of microalgae stems from their rapid growth under a broad variety of conditions, including in saline or nutrient-poor water or waste water. They can be grown in open ponds, closed chambers, or vertical stacks, eliminating the use of arable land. However, there are barriers to making algal biofuels economically viable, including high infrastructure and energy costs for growth and harvest; the necessity of year-round warm weather; and a high possibility of contamination, evaporation, or inconsistent mixing.[15] Additional barriers are outlined in Cheng and Timilsina[16] and in the DOE algal fuels roadmap.[17] Traditional biology or advanced biotechnology may be able to improve the salt tolerance of algae, allowing the use of brackish water for growth. Most research targets improvements in eukaryotic algae, especially those that naturally accumulate large quantities of lipids under stress. Recent advances suggest a combination of metabolic engineering and process control can alleviate algal challenges. Further discussion of how biotechnology could advance algal feasibility is in supplemental material section S2.

Feedstock composition

Lignocellulosic-biomass-based biofuels would use dedicated energy crops such as switchgrass, along with green waste or residue from food or nonfood crops (e.g., corn, wheat, rice, sorghum). The lignin and cellulose, their organization and quality, structural features of the plant cell wall, and patterns of cell wall development directly affect biomass properties and in turn the sugar yield and the potential for producing liquid transportation fuels.

A major goal of feedstock biotechnology efforts is making more easily convertible plants, for instance by reducing lignin content. Early work dispelled the intuitive idea that reducing lignin content would reduce the strength of plants and lead to lodging (i.e., plants falling over in the fields, making them difficult to harvest).[18] Instead, *increased* lignin was found to be more likely to cause lodging, possibly by making plants more brittle. As researchers continue to develop plants with lower lignin content, there may be a point at which improvements in digestibility are not worth the reductions in strength, water movement or pest resistance. A review by Pedersen *et al.*[19] found that, although results were mixed, reduced lignin tends to decrease the agricultural fitness of plants. However, they noted the significance of reduced lignin content is strongly affected by interactions with the environment and genetic background. Lignin has been shown to play a role in plant shear strength;[20] since grinding equipment designed to exert shear forces on biomass is more energy-efficient, reducing lignin could in turn reduce the energy required for grinding. As adequate quantities of modified bioenergy crop material become available in field trials, experiments are needed to test the relationship between lignin content and grinding energy.

There has been a rapid rise in reports on genetic and molecular underpinnings of biomass properties in both woody and herbaceous plants. Several excellent recent reviews are available on genomics and biotechnological approaches to improving plant cell wall characteristics and saccharification efficiency.[21–27] These reviews report that changes in cellulose, xylan, and pectin can all have beneficial impacts.

Another goal of feedstock-related biotechnology is increasing plant-oil production in natural oil-seed crops, algae, or new plant species. These plant oils can be directly made into biodiesel or upgraded into a biojet fuel, as several recent reviews report.[28–30]

Barriers to improvement of feedstocks

A nearly universal goal of plant biotechnology research is increased yields, in terms of overall plant growth (height or mass) and/or the plant component needed for the feed, food, or fiber market. It remains a colossal challenge to precisely define the genetic basis of a plant trait. The more complex the trait, the more factors there are that control the trait, making it harder to pin down targets for transformation.

Recent biotechnological approaches include studying pathways and genome-wide-omics*, but when it comes to validating a hypothesis for a role or function, one gene is manipulated at a time. The manipulation of one gene often has both expected and unexpected pleiotropic and undesirable effects on the plants. The paucity of publications reporting dramatic improvements may be due to the failure to target the right combination of gene(s) and promoter elements, and to the lack of understanding of the correlation between protein structure and the most effective enzyme biochemistry. Therefore, a whole pathway or systems view is needed. These bottlenecks to realizing biotechnological crop advancements can be addressed with

- robust statistical analysis methods to handle complex and diverse datasets.
- plant models with shorter life cycles.
- field trials coordinated tightly with greenhouse assessments.
- parallel and concerted investment by industry.

Logistics and land and water use

Efforts to genetically enhance energy crop production and conversion will affect supply-chain logistics (i.e., harvest, storage, transport, and size reduction) and resource utilization. Speculative biotechnological improvements that would improve logistics include increased biomass density, improved storability, decreased variability, and reduced ash content (especially for thermochemical processes). Higher yields for biomass crops – a primary goal of feedstock biotechnology research – typically help reduce crop production and logistics costs. Sokhansanj *et al.*[31] show that increasing switchgrass yields from 4.5 to 13.4 ton/ac dropped production costs by more than 50% (from $37.66 to $17.37 ton/ac).

Although many biotechnological advances, such as increasing yields, will improve logistical efficiency and reduce costs, others may increase the difficulty of building supply chains capable of delivering high-quality year-round feedstocks. For example, how will genetic improvements that make plants more easily convertible impact size-reduction and storage operations? More discussion is in the supplemental material, sections S3 and S4.

Competition between bioenergy crops and food/feed production (real and perceived) for land and water is a challenge. Measures to reduce land competition will include increasing biomass yield (dry mass produced per unit of land per unit of time), without harming soil health,[32] to minimize the land area required for bioenergy feedstocks,[33] and designing plants that tolerate environmental stresses such as drought and salt and could be grown on currently unproductive or underused lands.[33,34] Strategies such as double-cropping, either seasonal

*The term '-omics' refers to the growing suite of large-data biological analyses, including genomics, transcriptomics, proteomics, and metabolomics.

(planting biomass as a winter cover crop) or spatial (planting grasses between rows of a tree plantation) can help energy crops coexist with food crops. Some energy crops, particularly trees, appear to be able to tolerate saline soils that are not suitable for food crop production. It has been estimated that trees selected or designed for ability to grow on salt-affected land could supply up to 8% of global primary energy consumption.[35,36] Miscanthus, which can substitute for corn in ethanol production, requires less land and water than corn. Carefully selecting and transforming crops for higher yields and lower water use will be necessary to achieve sustainability in the use of basic resources such as land and water.

Expanded research to improve the productivity of bioenergy crops with low or no chemical inputs will achieve the dual goals of expanding the production and use of biomass and improving sustainability.[32]

Conversion technologies

Advanced biotechnology is critical to developing and deploying solutions for biomass conversion. There are two aspects of this issue: (1) modification of the microbes used in conversion processes, via metabolic engineering or synthetic biology, to produce new or improved products and (2) improvement in the key factors of bioconversion: yield, titer, and rate.

Biotechnology has the potential to enhance three primary aspects of conversion:

- additional feasible feedstock streams
- product diversification
- process technologies and efficiencies

Metabolic engineering, synthetic biology, and other biotechnological advances will allow more rapid optimization of conversion processes by rational enzyme engineering, by introduction of new pathways and regulation, and by control of the flux.

Conversion of additional feedstocks and components

The most critical bioenergy feedstocks will be cellulosic and hemicellulosic sugars from plant biomass. Several companies (Abengoa, Beta Renewables, and DuPont[37]) are using conversion processes driven by separately produced cellulolytic enzymes [e.g., simultaneous saccharification and fermentation (SSF)]. Enzyme engineering will lead to advances in rational improvement of hydrolytic cellulolytic enzyme activity and will help lower cellulase production costs. Enzyme cocktails have steadily improved over the past two decades and probably will continue to do so, but major SSF breakthroughs are unlikely.

Consolidated bioprocessing (CBP) uses microorganisms that produce their own hydrolytic enzymes and complete fermentation into the product in one unit operation. CBP probably will be realized in some form within the next 10 years; hybrid CBP/SSF approaches also will be deployed that combine cellulase-expressing industrial microbes that are incapable or poorly capable of converting plant biomass alone, but that require less added cellulase than current strains. There has been progress both in engineering cellulolytic microbes to make fuels[38,39] and engineering fuel-making microbes to degrade cellulose.[40–42] Synthetic biology and metabolic engineering tools have been brought to bear on issues such as co-utilization of glucose (the sole component of cellulose) and xylose (the primary component of hemicellulose) in a variety of organisms.[43–46] Further near-term progress toward efficient co-utilization is certain. Non-xylose hemicellulosic sugars such as rhamnose, arabinose, and galactose are underutilized; advanced synthetic biology tools for metabolic control are being developed[47] that will enable them to be used more efficiently, thus increasing the fuel/chemical yield per ton of biomass without decreasing the fitness or robustness of the CBP micro-organism.

Preliminary reports suggest un-pretreated biomass could be a feasible substrate for bioconversion.[48] If biotechnology can improve the yield and rate of product formation from un-pretreated biomass, it would be a game-changing technology because it would eliminate the need for costly chemical pretreatment.

Planned waste streams from bioprocessing – including lignin, acetic acid, and glycerol – need to be utilized to add value for biorefineries. Biotechnology could help remedy the underutilization of lignin,[49] a highly amorphous and hydrophobic polymeric network of substituted aromatic compounds that accounts for approximately 25% of plant biomass.[50] It is typically burned for process heat and electricity, but it could be used to make fine and/or bulk chemicals to increase the economic viability of biorefineries. One challenging potential solution would be engineering a microbe to depolymerize lignin and convert it to a fuel or a bulk chemical. Given the vast quantities of lignin that would be produced by a mature biofuels industry, the target products would need pre-existing large markets to prevent market saturation and enable rapid commercialization.

Glycerol is a by-product of both biodiesel and bioethanol production. Both native and engineered organisms have been demonstrated to convert crude glycerol to value-added products such as succinic acid, propanediol, and polyhydroxyalkanoates (PHAs).[51] Acetic acid, a low-value or waste substance present in plant biomass in the form of acetylated xylan, is also a product of microbial metabolism. Microbes will be engineered to convert acetic acid to value-added products rather than allowing the carbon to go to waste. Recently, *Saccharomyces cerevisiaewas* engineered to consume acetic acid.[52] It is unclear whether substantial value will be added to these waste streams within 10 years, but the rapid progress of synthetic biology makes these high-risk, high-reward projects more feasible.

Increased product diversification

New biomass-derived fuels, chemicals, and other products are likely to become more prominent over the next decade. Cellulose-derived short-chain alcohols such as ethanol will be the first to be commercially successful; other products will follow. There are substantial cost barriers to the commercialization of microbially produced hydrocarbons (biogasoline, biojet fuel) and medium-to long-chain fatty acids (for esterification to biodiesel) as fuels, but an intense research effort over the next decade would have a moderate chance of reducing the cost to a competitive range. Even if costs remain too high to allow their use as fuels, many of these compounds are potential high-value co-products. For example, farnesene can be sold as a high-value precursor for cosmetics and other applications, and Amyris's business model targets doing so in the near term. Even bio-compounds such as n-butanol and isobutanol are current feedstocks for the chemical industry and may begin displacing petroleum-derived compounds. Other compounds with high potential for deployment within the next decade include organic acids (e.g. succinic acid, malic acid, lactic acid, adipic acid), diols (e.g. propanediols, butanediols), and PHAs, which add to the current biocommodities of acetic acid and 1,3-propanediol.

A long-range, high-risk application of synthetic biology would be to combine the metabolic engineering approaches used to convert sugars to useful products with the engineering of photosynthetic microbes to provide direct conversion of sunlight into chemical products, fuels, or electricity.[53] However, this combines the challenges of efficient bioproduct formation with the major challenges of algal bioprocessing and is seen as unlikely in the next decade.

Improved process technologies and efficiencies

The key factors in a bioconversion process are yield, titer, and rate. Synthetic biology and metabolic engineering are the main drivers of increased yield and will be required for either expanded feedstock streams or new products to be economically viable. Additional barriers are outlined in Cheng and Timilsina.[16]

The final titer of a desired product (e.g., ethanol) is often controlled by product inhibition or tolerance. Much research in a variety of organisms has targeted understanding and mitigating the toxicity of process inhibitors, including alcohols, hydrocarbons, phenolic compounds, and organic acids.[54–57] Most efforts to increase product tolerance involve adaptation and evolution; learning how to truly *engineer* tolerance would be a major advance. High-titer (10% to 20%) soluble products are usually separated via distillation. Direct production of insoluble hydrocarbons provides a distinct biotechnology-driven advantage for processing because the initial process can be a liquid/liquid phase separation. However, insoluble solvents can cause cellular disruption and inhibition in many microorganisms. Moderate but important advances are likely in the realm of tolerance of products and other inhibitors, which will allow higher titer production. Rational decoupling of growth from metabolic flux has the potential to increase yield by eliminating the flux of substrate to production of new cells while also increasing titer by reducing product inhibition.[58] Previous technologies include microbial or biocatalyst retention processes such as cell recycling or immobilization to maintain a high rate. Future developments in synthetic biology to control cellular physiology have a moderate chance of enabling the rational decoupling of growth and metabolism. However, economical decoupling with a concomitant increase in yield and titer would result in substantial process improvements.

Challenges associated with growing and processing algae – including concerns over water use, pond contamination, use of GMOs in open ponds, photobioreactor scale-up, product harvest, and product dewatering – will continue to be barriers[17] and will be difficult to address using biotechnology only. In this case, issues associated with phototrophic growth are combined with the challenge of genetic manipulation of non-model bacteria.

Current bioprocesses tend to rely on mesophilic microorganisms (20 to 45°C), but the ability to operate under extreme conditions could simplify process operations and lower costs. Extremophiles can tolerate high levels of temperature (up to 100°C), pH, or salts, and thermophilic microbes often operate at increased rates.[59] Thermophilic processes are speculated to improve separations (i.e., *in situ* vapor extraction) and lower heating and cooling costs. Partial analyses show generally small advantages because some heating, cooling or product separations will still be required. The greater advantage of extremophilic processes is indirect – they are likely to resist contamination by undesired microbes. In addition, feed stream cleanup for these processes (e.g., for salts or low pH) would be less stringent. However, extensive biotechnological modification will be required either to use most current extremophiles (likely for selected extremophiles) or to make current robust microbial hosts into extremophiles (unlikely soon).

Biotechnology can accelerate thermochemical conversion of plant material and municipal waste into syngas followed by syngas bioconversion. Bacterial strain engineering and development by companies such as LanzaTech have demonstrated the potential for bioconversion of syngas to a suite of fuels and chemicals. Although biotechnology is unlikely to significantly overcome all limitations of syngas as a feedstock, such as mass transfer of CO, the product diversification barrier is ripe for biotechnological innovation.

In addition to using microbes alone for bioconversion, an alternate approach is in vitro product formation with enzymes alone. It removes the desired enzyme pathway from the living microorganism and uses a mixture of specific enzymes. Pathways of more than ten enzymes have been devised and tested.[60] Breakthroughs are needed in enzyme stability and cofactors, as well as in lowering the cost of enzyme production. Improved in vitro enzymatic processes are likely but will be cost prohibitive for most commodities.

Microbial communities may be assembled to perform desired functions. However, nearly all industrial product formation uses single isolated microbes or enzymes – pure microbial cultures – as opposed to mixed cultures. Exceptions are in food production or waste treatment (e.g., biomethane production) and are driven by consumption for growth. A major breakthrough would be the ability to design, assemble, and control a mixed culture to produce a specific desired product (such as a biofuel). However, many challenges in pure culture apply also to mixed populations, making this approach unlikely in the next decade.

Barriers to improving conversion processes

The major barriers to biotechnological advancements in conversion of additional feedstock streams and new products are limited knowledge of microbial metabolism and difficulty in controlling metabolic flux. Advances in synthetic biology, particularly, will begin to enable more dynamic control of metabolic flux via coupling sensing of the environment with gene expression, translational and posttranslational control, and allosteric regulation. At the same time, fundamental studies will continue to provide an intellectual foundation for future work. This might include discovery of unique enzymatic activities or pathways that could be harnessed for biotechnology, along the lines of the recently discovered enzyme fatty aldehyde decarbonylase[61] that produces hydrocarbons from fatty acid derivatives.

Often, a microbe newly isolated from the environment has desirable properties but a lack of genetic tools hinders its development as an industrial biocatalyst. Typically, several years of intensive trial-and-error genetic tool development are needed to genetically engineer any novel microbe. More rational, reliable approaches to developing genetic tools and cultivation methods would allow the use of many unique features of novel microbes (such as extremophiles or microbes that can consume complex substrates or produce novel products). This development is only moderately likely over the next decade but could be a game-changer if accomplished. Genetic tools for these non-model organisms are improving.[62]

Products

Potential applications of biotechnology to by-product production are primarily in

- altering feedstock characteristics so by-products are more suitable for the intended use.
- altering enzymes, bacteria, or yeast used in the fermentation process to yield higher-value or more consistent co-products.

Given the scale of biofuel production necessary to make an impact on the market, co-products must be useful in massive quantities to avoid collapsing their market prices. For example, a major by-product of bioenergy production, particularly biodiesel, is glycerin. Glycerin is valuable because it is useful in producing hundreds of products; however, because of increased biofuel production, the glycerin supply has exceeded demand and the price has collapsed.[63] This example illustrates the problem of targeting specialty chemicals as co-products of biofuel production.

At least two by-products, distillers' grain and lignin, could supply a large market and thus improve the economics of biofuel production. The use of distillers' grain in animal feed is a proven contributor to the economics of biofuel production from corn. Lignin has potential uses as a precursor in carbon fiber production or as an additive to plastics to improve the qualities of plastic products.[49]

US exports of distillers' grain as an animal feed have risen sevenfold since 2005/2006[12] with the rise in ethanol production from corn. Distillers' grain is a good source of animal feed, and using it as such improves the greenhouse gas benefits of biofuel production. It is often blended with other plant materials and supplemented with specific amino acids for optimal nutritional quality. Because there is little information on equivalent materials that will come from cellulosic biofuel plants, research is needed on potential issues with using them, including nutritional quality, presence of toxic products, and predictability of the content. Feedstock characteristics and the processes used in preprocessing it will affect the utility of the by-products as an animal feed. Genetic manipulation of the feedstock could increase the value of the distillers' grain by increasing total protein content and the content of amino acids that might be lacking (e.g., lysine). Alternatively, the fermentative microorganisms could be engineered to do the same, improving the value of the product. However, given the time scale of deploying engineered plants and the current lack of nutritional data, this has a low probability of completion within the next 10 years.

Potential uses of lignin include use as a feedstock for structural materials such as carbon fiber[64] and materials for energy-related applications, such as anodes for lithium ion batteries.[65] The current price of carbon fiber limits its use to specialty applications, but a price drop would open new markets for it as a structural material.[66] One challenge is the variability of the structure and composition of lignin,[65] and no one has demonstrated cost-effective production of carbon fiber from lignin that meets the strength requirements.[66] Ongoing research into how genetic variation in the feedstock affects the suitability of the lignin for use as a carbon fiber precursor could lay the foundation for future plant-engineering strategies for improving lignin conversion to carbon fiber. Lignin pathways are being altered in bioenergy crops to increase conversion. These same plants will likely also be assessed for the effect of lignin composition on the production of useful products, including carbon fiber.

Crosscut impacts on supply chain

It is essential to study, understand, and improve the environmental and ecological sustainability of future bioenergy crops that may be planted at the scale of millions of hectares of land. The ideotypic or most desirable bioenergy plant will need to be productive on marginal lands (land having poor soil structure, nutrient composition, and moisture) and in fluctuating weather conditions and will need to be resilient in the face of various abiotic stresses (e.g., water, nutrients, and temperature) and biotic stresses (e.g., pathogens or altered composition of soil symbionts). Biotechnological improvements in these areas will reduce plants' dependence on fertilizers, pesticides, and other agrochemicals. This in turn is expected to result in (i) reduced pollution and (ii) reduced production costs; however, these sustainability factors will need to be measured.[67]

If microbes can be engineered to efficiently convert cellulosic material into liquid fuels and chemicals from unmodified plants, this would suggest that plant biomass yield per acre would become a primary plant engineering target. However, if the former goal remains out of reach, then an essential target for improvement would be to modify plants so that they are more easily converted to products. Another niche use to provide additional value within the supply chain might be the use of biorefinery wastewater for bioproduction of fuels and chemicals.[68]

Conversion will also impact how fuels are used. The choice of compounds produced is highly pertinent. If ethanol remains the dominant product, then the blend wall will continue to be a barrier and to limit the size of the biofuels market. However, if biogasoline, biodiesel, and biojet drop-in fuels become economical, then the blend wall will no longer be an issue.

Developing microbes designed for mutually beneficial interactions with plants has been identified as a way to increase crop yields, decrease nutrient applications, improve resistance to pests and diseases, and improve plant water use.[69] These symbiotic relationships are expected to increase grower profit by stabilizing yields across years with varying weather conditions, and by reducing grower costs. They should help address the challenges of greenhouse gas emissions and competition for land by improving yields with reduced inputs. There are other important questions related to the sustainability of biofuel plantations: we do not understand how the biota of ecosystems (e.g. pollinators, aphids, birds, small mammals) will be affected by the cultivation of plants improved using advanced biotechnology, in nonnative or untested geographical niches, and the related land use changes. The impacts of improved biofuel crops on the carbon cycle, biosequestration,[70] and the promise of biofuel as a carbon-neutral fuel option need to be demonstrated. The impacts of changes in atmospheric CO_2 levels, precipitation regimes, and temperatures require that bioenergy plant performance and sustainability be assessed within the context of climate change models.

The ultimate success will come from combining advances that result from biotechnology approaches with more traditional chemical engineering to develop cost-effective processes.

Conclusions

Projections based on current research and development indicate that biotechnology will continue to improve biofuel supply chains. Over the next 10 years, most biotechnological advances will be in conversion processes, followed over a slightly longer period by feedstock improvements.

In conversion, genetic engineering of microbes and enzymes, combined with other biotech process modifications, will continue to improve yields, rates, and titers. Improved microbes and enzymes will more completely convert biomass feedstocks and handle a broader variety of biofeedstocks. There will be rapid advances in developing and deploying additional marketable co-products from biorefineries – including fuel blendstocks beyond ethanol (e.g., butanol or hydrocarbons) to get past the ethanol 'blend-wall' – as well as commodity chemicals to improve the biorefinery economy.

For feedstocks, advances are anticipated in yield, nutrient uptake (lower fertilizer use), and tolerance of environmental stresses (e.g., drought). These probably will require the adoption of (GMOs). Because GMO crops have longer field testing and deployment cycles, and because of societal reservations about GMOs, deployment of these improvements is likely later within the 10-year timeframe.

A biofuels industry of the scale required to produce enough energy to make a major impact will also generate massive quantities of co-products and by-products. Biotechnology can be useful in tailoring feedstocks so that they produce by-products suitable for widespread use and in altering conversion processes to yield consistently high-value co-products.

Some biotechnology fields could allow more rapid impacts and should be monitored for breakthroughs. Strategic and knowledge gaps in advanced biotechnology related to bioenergy are primarily in two scientific areas: (i) understanding of underlying biology to allow rational changes and (ii) improvement of tools for implementing changes.

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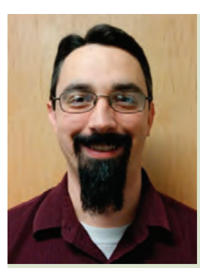

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