Renewable Fuels from Biomass: Technical Hurdles and Economic Assessment of Biological Routes

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Lignocellulosic biomass is an abundant, renewable source of polysaccharides that could be available in amounts sufficient to provide a source of sugars for carbon neutral biofuel production. We review the background to biofuels production in the US from corn sugars and subsequent R and D efforts to saccharify plant biomass to provide an alternative sugar source. Research efforts and programs have generally not addressed the key technical hurdles in providing a commodity-scale supply of biomass and in developing biological routes to saccharify it at high yields. Techno-economic analyses of proposed processes highlight the importance of biomass cost, the role of pretreatment on both inhibitor generation, and the contribution of enzyme costs to saccharification. Alternatives, such as the production of fatty acids by microalgae, have comparable technical hurdles. Although there is a regulatory framework for biofuels, which is discussed, a credible biological process for large-scale, cost-effective production of lignocellulosic biofuels remains elusive. *AIChE J*, 61: 2689–2701, 2015
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Introduction

Today, the United States consumes 220 billion gallons of liquid transportation fuels annually. Since the 1950s, the United States has relied on imported oil to address its production deficit. Following World War II, global oil consumption grew by over fivefold in 25 years, with most of the imports derived from the Middle East. In October 1973, an international energy crisis resulted from an attack on Israel by Egypt, Saudi Arabia, and other Arab countries. The Organization of the Petroleum Exporting Countries (OPEC) embargoed oil exports to the United States and stated they would drop oil production by 5% per month. Oil prices in the United States quadrupled from $4.50 to 22.50 per barrel and rationing was implemented. The embargo ended in March 1974, with considerable damage to the US economy. This led to a search for alternative energy sources, including ethanol production from corn. President Nixon launched "Project Independence" in 1973 to achieve energy independence by 1980 and created the Federal Energy Office. The Department of Energy (DOE), created in 1977, combined the Federal Energy Administration, the Energy Research and Development Association (ERDA), and the Federal Energy Regulatory Commission.

A second energy crisis in 1978 resulted from a strike at Iranian oil refineries, which decreased world exports by 5%. The subsequent overthrow of the Shah in January 1979 and the Iranian revolution, together with OPEC action, led to oil price increases up to $34.50 per barrel. The US farm industry supported the production of ethanol from corn in the form of a 10% blend with petroleum (gasohol). Introduced by President Carter in 1980, a 40, 50 (1982), and 60 (1984) cents/gallon tax incentive and a tariff on imported ethanol from Latin America facilitated the growth of the US ethanol industry, producing over 1 billion gallons from corn annually by the mid 1990s. The ~13 billion gallons per year of ethanol produced from corn today represents approximately 4% of our liquid transportation fuel energy demand and consumes just under 30% of the nation's corn crop. Corn for ethanol requires 26 million acres (105,000 km^2) of farmland, which is approximately 6% of all US cropland and 1% of the total U.S. land area. These figures illustrate the nature of the problem in providing renewable fuels at scale and the inadequacy of the current approach.

US Research Activities

A research effort aimed at conversion of lignocellulosic biomass and municipal waste to ethanol was undertaken by the Department of Energy, managed by its Solar Energy

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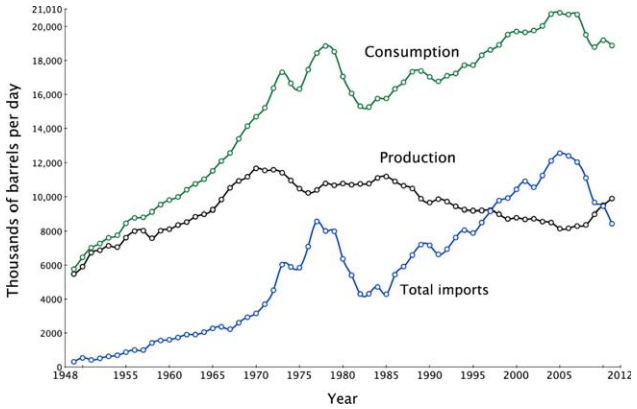

Figure 1. US oil imports, production and consumption by year (source US Energy Information Administration, www.eia.gov).

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Research Institute (SERI), from the late 1970s to mid 1980s. Funding rose from $40 million in 1977 to $115 million in 1978–1979, decreasing to $40 million by 1983. By 1989, it was less than $10 million annually. During this period, significant advances were made in developing biological routes for the enzymatic hydrolysis of the cellulose and hemicellulose content of lignocellulosic biomass to their component sugars and in the fermentation of both xylose and glucose to ethanol. A guide, published in 1980, highlights the opportunities and challenges in using biomass (both starch and cellulose based) for ethanol production and reviews decision-making processes for funding and operating ethanol plants.[1] A detailed analysis by SERI of ethanol production from aspen wood highlights the importance of raw material costs, methods of biomass pretreatment, cellulase enzyme production costs and enzyme recycle, and the ability to ferment C_5 sugars.[2] A summary of the literature on lignocellulosic biofuels production from 1901 to 1980 was made available in 1981.[3]

As a result of these ERDA and DOE research programs, by the mid 1980s, a good understanding of the key research needs and economic drivers for commercializing biological processes to convert lignocellulosic biomass to ethanol was available. The major issues facing biological routes were:

- availability and cost of lignocellulosic biomass to serve as a feedstock for large-scale ethanol plants,
- methods to effectively pretreat biomass to make it significantly more susceptible to enzymatic hydrolysis,
- cost of cellulase enzymes and methods to reduce the enzyme requirements (reduced loading) for saccharification (e.g., enzyme recycle), and
- organisms to ferment C_5 sugars (primarily xylose) to ethanol, or routes to enable cofermentation of C_5 and C_6 sugars.

In February 1995, for the first time, the United States imported more oil than it produced (see Figure 1), a situation that has only recently been reversed. Oil prices increased from 2001 onward following the attacks of September 11th, the Petróleos de Venezuela, S.A (PDVSA) strike, the Iraq war, and growth in Asia, rising from ~$30 to over $90 per barrel (in 2007 dollars). This, together with increasing concerns about the role of atmospheric CO_2 on global warming, led to renewed interest in production of transportation fuels from renewable biomass. In December 2005, the DOE held a workshop "Biomass to Biofuels: A Roadmap to the Energy Future,"[4] in response to President Bush's Advanced Energy Initiative. The Workshop was "to define barriers and challenges to a rapid expansion of cellulosic-ethanol production and ways to speed solutions through concerted application of modern biology tools as part of a joint research agenda." The major barriers identified in the report were:

- understanding plant cell-wall chemical and physical structures—how they are synthesized and can be deconstructed,
- development of innovative energy crops designed for industrial processing to biofuel, and
- new biology-based treatment and conversion methods.

Specific research objectives were provided in the Workshop report. In the first 5 years, research was direct at devising sustainable, effective and economical methods to harvest, and deconstruct and convert existing feedstocks to ethanol. This included enzymatic breakdown of cellulosic biomass to C_5 and C_6 sugars and lignin and subsequent cofermentation of these sugars to ethanol or other fuels. New energy crops were to be developed within 10 years and coupled with processes for simultaneous breakdown of biomass to sugars and cofermentation of the sugars via new biological systems [italics added]. This Roadmap formed the basis for DOE

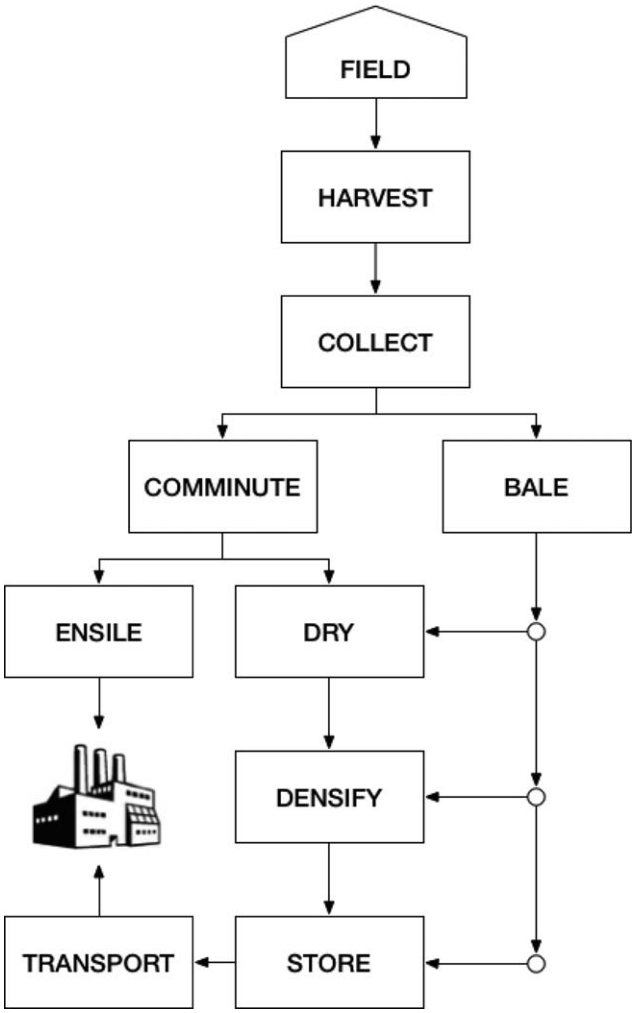

Figure 2. Simplified biomass supply chain (adapted from Ref. 14).

funding of three Bioenergy Research Centers, each supported at $25 million per year for initially 5 years (2007–2012), and subsequently an additional 5 years (2012–2017). Reference to this detailed research plan is made in subsequent sections.

The first production of fuels from lignocellulosic biomass

The hydrolysis of lignocellulosic biomass to its component sugars and their conversion to ethanol has its commercial origins at the turn of the 20th century. The first commercial plant for conversion of wood to ethanol began operation in Germany in 1898. In 1910, Ewen and Tomlinson erected a plant in Georgetown, SC for ethanol manufacture from sawmill waste using dilute sulfuric acid for holocellulose hydrolysis. It was operated by the Standard Alcohol Company and produced 5000–7000 gallons of cellulosic ethanol per day. The ethanol yield from biomass, however, was low. In Germany, the Scholler process had a better yield (60–70% of theoretical) but the resultant sugar solution was dilute (3 wt %), making ethanol distillation following fermentation very energy intensive.[5]

In contrast to these dilute sulfuric acid hydrolysis processes, concentrated hydrochloric acid (42%) can completely dissolve cellulose and hydrolyze it to glucose at nearly theoretical yields. This was the basis for the Bergius–Rheinau process, which was developed from 1916 onward, providing a total yield of 60–66% sugars from dry wood. Subsequent fermentation converted 80% of the sugar to ethanol.[5] Recovery and recycle of the HCl by vacuum distillation were critical. Recent commercial approaches using concentrated HCl have addressed this use of solvent extraction (with tertiary and quaternary amines) for HCl recovery and recycle (e.g., Virdia, ww.virdia.com). Concentrated sulfuric acid (72%) can also solubilize cellulose and has been used to release sugars from biomass. The Hokkaido process, developed in Japan in 1948, first pretreats wood with dilute H_2SO_4 (0.25 N) at 140–150°C, followed by drying and crushing of the biomass prior to contact with 90% H_2SO_4 for 30 s at room temperature. This process can recover about 85% of available glucose, but recovery of the sulfuric acid is difficult and the process has not been commercially attractive.

A variety of biomass pretreatment methods have been subsequently explored to facilitate sugar release from biomass. These include physical methods based on biomass comminution by mechanical means (knife and hammer milling), physicochemical methods such as steam explosion (based on the Masonite process[6]), ammonia fiber expansion (AFEX),[7] and hydrothermolysis. Chemical methods include the use of concentrated acids (H_2SO_4 and HCl), dilute H_2SO_4 used over a range of concentrations and exposure times, and pretreatment with bases. Biomass exposure to bases such as NaOH, hydrazine, and anhydrous NH_3 reduce cellulose crystallinity, permitting these bases to slowly penetrate further into biomass. Lignin relocalization or delignification may occur. This makes the holocellulose components of biomass more accessible to enzymatic hydrolysis. Organic solvents, such as ethanol, methanol, glycerol and a variety of ethers, ketones, and phenols in aqueous mixtures are able to hydrolyze lignin linkages and solubilize it, producing a cellulose product that can be readily hydrolyzed by dilute acids. This approach is known as organosolv pretreatment.

A recent development of the organosolv approach is the use of ionic liquids that are able to completely dissolve biomass and produce cellulose that is very readily hydrolyzed by cellulase enzymes and a soluble lignin product. These pretreatment methods have been recently reviewed and their advantages and economics assessed.[8] To date, no single method appears to be both effective in releasing all available sugars and doing so in a cost-efficient manner. It has long been apparent that biomass pretreatment is the key to the economic production of sugars from lignocellulosic biomass. Unfortunately, significant research support has not been directed toward this area (as evidenced, e.g., in the research priorities of the DOE roadmap[4]), and consequently, progress has been slow in producing sugars from biomass at a cost competitive with sugars from sucrose or starch-based sources such as corn.

The food vs. fuel debate

As a result of increased corn ethanol production to meet the US Renewable Fuel Standard (RFS), concerns were raised about the production of fuels from starch-based biomass, as it may compete with its use for the production of food and animal feed.[9] The significant cost components associated with increasing food prices in the United States have been identified as crude oil, labor, and transportation rather than any factors associated with conversion of corn from food and feed use to biofuels.[10] An increase in US corn ethanol production to 15 billion gallons by 2015 is not projected to increase conversion of nonagricultural lands to corn production but can be made possible by higher yields of corn per acre arising from advanced breeding and other efforts.[11] The United States pays farmers not to farm about 32 million acres, and this acreage could be used for biomass production for fuel.[9] The food vs. fuel aspect of biofuels production has been popularized in the press but has little basis in fact.[12]

Economic and Technical Challenges in Biofuels Production

Biomass supply and logistics

The availability of industrial quantities of lignocellulosic biomass is a prerequisite for the planning and construction of any biorefinery and supplying a constant stream of this raw material remains a key challenge in the widespread adoption of lignocellulosic biofuels. Biomass for this purpose may be forest (tree trimmings, sawdust, chipped wood, etc.) or agricultural (agricultural residues, energy crops, etc.), with different types of biomass growing in different regions and at different times during the year. Although some process technologies accommodate mixtures of biomass types,[13] most processes require a certain degree of homogeneity in the feedstock they accept. This is especially true in biochemical conversion routes compared to thermochemical conversion options. The regional and temporal variability in the supply of biomass and the continuous need for a relatively homogeneous feedstock are key considerations for building a biorefinery because a plant owner must maximize the plant operating time to minimize the burden of the capital expenditure on the biofuel production cost. These circumstances create the backdrop for the logistical challenges of biomass supply chains.

Supply chain challenges depend on the type of biomass, though some are common to all (see Figure 2). A main limitation is related to the low-bulk density of the feedstock (Table 1). Low-bulk density is a challenge because it increases the expense of all logistical steps per unit of energy delivered to the biorefinery. Furthermore, the sheer volume of

Table 1. Bulk Density of Various Preprocessed Feedstocks (Data from Refs. 14–16)

Biomass	Processing	Bulk Density (kg/m^3)
Straw	Loose	20–40
Straw	Hammer-milled	20–110
Straw	Baled	110–200
Switchgrass	Hammer-milled	115–180
Wood	Chipped	180–230
Wood	Sawdust	120–200
Wood	Pellets	500–700

biomass that must be moved to establish a global biofuel industry is immense, and it is in itself a considerable problem. Richard (2010) estimated that to reduce global green house gas (GHG) emissions by 50% through lignocellulosic biofuels, the volume of baled biomass that would require handling would exceed the world grain trade volume by ~50-fold (in 2010).[17] Other logistical challenges are associated with the different supply chain steps. In general, from field to biorefinery, biomass must be harvested, collected, preprocessed, transported, and stored. The most common preprocessing operations involve size reduction (comminution), drying, and densification. Some steps happen simultaneously (e.g., collection and comminution) and others may happen at various intermediate points along the supply chain (e.g., storage and transport). Even the relatively straightforward steps in the chain may become problematic at the scale needed for a global biofuel industry, we focus on the more salient points in this discussion.

Preprocessing of biomass makes feedstock handling easier and even though it may reduce the cost of handling, it increases the cost of the feedstock itself. Pelletization, for example, which achieves drying and densification, may increase the cost of the feedstock by up to 50% compared to the unprocessed raw material.[14,18] Although cost remains the fundamental consideration, some preprocessing steps may be critical depending on the type of biomass and local conditions. Controlling the moisture content, for example, is key, because biomass with more than 15–20% moisture is considered aerobically unstable, which presents a problem during transport and storage. Therefore, biomass with >20% moisture must be either dried to below the threshold or ensilaged for anaerobic storage.[14] Storage itself may present problems even if the biomass is relatively dry. Long-term storage may lead to loss of dry matter, reduction in quality (biomass with lower-biofuel yield), safety hazards due to self-ignition, health hazards from fungal spore dispersion, and so forth.[19] Lastly, transport is a crucial operation in the biomass supply chain, not only due to its cost but also because the distance needed for transport determines the optimal scale of the biorefinery. The larger the supply radius, the higher the transport costs, but also the larger the biorefinery can be. Because the biorefinery capital costs are generally subject to economies of scale, the optimal process capacity is inpart determined by the cost of the transport operation.[20,21] Regardless of scale- and distance-related considerations, large plant capacities are problematic because the infrastructure for transport (e.g., roads) and biomass handling (e.g., unloading docks) may become limiting.[22,23]

Ultimately, the logistical challenges of the biomass supply chain are relevant because biomass represents one of the main contributions to the biofuel production cost.[8,24,25] Indeed, biomass prices are hard to estimate and significant disagreement remains regarding market prices. A brief (and incomplete) review of the literature quickly reveals that estimates differ dramatically across studies, regions, and biomass types, with ranges from ~$30 to >230/ton.[8,21,26,27] For comparison, hay prices according to the United States Department of Agriculture (USDA) ranged from $40 to 75/ton on the week of October 4, 2014 in Iowa, but they were as high as $350–380/ton in California.[28] Part of the difference is due to the fact that estimates vary by mode of computation and by what the prices include (i.e., farm-gate vs. delivered, dry vs. in-field, preprocessed vs. unprocessed, etc.), but much of it remains because large-scale trade of biomass has not begun and thus standards have not been adopted. This has prompted the establishment of some as-yet limited commodity-type exchanges (e.g., The Minneapolis Biomass Exchange, https://www.mbioex.com). This and other exchanges are likely to have limited reach, however, because of the relative importance of storage and transport costs on the delivered price of biomass. As a result of this, the price of biomass has remained an assumption for most published techno-economic assessments of biofuel production, which in turn limits the reliability of biofuel production cost estimates needed to guide investment, policy, and research.

The local nature of the biomass market has other pernicious consequences. For example, the significance of transport costs and the interdependence between biomass growers and buyers may invite market distortions (monopolies, monopsonies, price volatility in feedstocks and products, etc.). Once a facility is established, the biomass supplier has significant influence over the cost of production of the biofuel, which may leave the biofuel producers vulnerable. Conversely, the opportunity cost of land exposes the grower, because if other uses of the land become more profitable (e.g., if corn or soybean prices increase), biomass production becomes a hindrance to the farmer. This is particularly true for some energy crops: once planted, switchgrass will regrow for 10 years, and Miscanthus for 15 years. This implies that sustained high prices for the biomass are needed to ensure that the cost of establishing the crop plus a reasonable margin is recovered over its lifetime. In other words, what is best for the grower may not be best for the biofuel producer. Legal arrangements can be used to ensure an alignment of purpose, but a market cannot be sustained purely on contractual obligations; economic benefits for all players must be at work. This fact points to the need for vertical integration, profit sharing, or cooperative business structures, all of which are already practiced and may become more widespread in the future.

Biomass pretreatment: The key to saccharification economics

There are two main approaches to convert lignocellulosic biomass to fuels; chemical routes involving biomass pyrolysis or liquefaction and biological routes that rely on the microbial production of fuels from biomass saccharides. As observed earlier, the pretreatment of biomass prior to enzymatic saccharification is regarded as the most challenging step in the biological approach. For cost-effective fermentation for fuel production (e.g., ethanol, butanol or other higher carbon number blendstocks), a saccharide concentration of over 100 g/L in the fermentation medium is desirable, requiring a biomass loading of 15–25 wt % in the saccharification step unless an intermediate sugar concentration step is used. Few studies have been conducted under high-loading

conditions and to date only an auger-based system appears to be satisfactory in handling these high-biomass solids loadings in a continuous manner.[29] The objective of biomass pretreatment is to facilitate the action of glycosyl hydrolases to provide near complete liberation of hexoses and pentoses from the cellulose and hemicellulose components of lignocellulosic biomass. Effective pretreatments increase biomass surface area, decrystallize cellulose, remove associated lignin, and may partially hydrolyze hemicellulose.[30] However, certain pretreatments, for example, dilute acid hydrolysis, also liberate compounds such as acetate, furfural, and phenolics from hemicellulose and lignin. These are inhibitory to the subsequent fuel fermentation, and minimizing production of these inhibitors from the biomass is thus desired.

A significant research effort over the past 7 years has been devoted to the study of the plant cell wall—its biosynthesis, structure and function, in efforts to subsequently genetically modify the cell wall to improve its ability to be biologically deconstructed.[31–33] Unfortunately, these efforts remain specific to the type of pretreatment used (e.g., AFEX[34] or alkaline hydrogen peroxide[35]) and generally have not yielded the enhancement in cell wall breakdown that would be required for significant commercial impact on the cost of biomass saccharification. However, a promising pretreatment approach is biomass solvation in certain ionic liquids, which has the advantage that it is equally effective for both woody biomass and grasses and provides a cellulose stream that can readily be saccharified. Modifications to the lignin component of biomass, by the addition of hydrolyzable ester linkages to make it more readily fragmented, have been successful in enhancing biomass pretreatment with ionic liquids.[36,37]

Ionic Liquids for Pretreatment. The observation of near-complete dissolution of biomass in imidazolium-based ionic liquids[38] has led to a large number of studies directed at using this approach for biomass pretreatment. Dissolution of biomass in certain ionic liquids, followed by the addition of an antisolvent, such as water, alcohol/water, or acetone/water mixtures, results in the precipitation (regeneration) of the cellulose component of biomass. After washing, this cellulose phase is readily enzymatically saccharified,[39,40] providing saccharification rates that are nearly an order of magnitude higher than those obtained with other pretreatment approaches. The lignin component of biomass remains mainly dissolved in the ionic liquid.[41] The addition of an aqueous solution containing a kosmotropic anion (e.g., K_3PO_4) or a base results in the mutual near immiscibility of the ionic liquid and aqueous solution, potentially providing a route to recover and recycle the ionic liquid.[39] The increased hydrolysis rate and sugar yield in enzymatic saccharification following ionic liquid pretreatment have been attributed to delignification, decreased cellulose crystallinity and increased cellulose surface area available to the cellulases.[40]

Biomass dissolution in ionic liquids thus provides a number of advantages as a pretreatment for subsequent enzymatic or chemical depolymerization of all of the biomass polymers. By partially fractionating these components, higher rates of saccharification of the holocellulose content can be obtained, and the amount of enzyme required (loading) can be significantly reduced. As enzymes cost represents the second largest raw material cost in biofuels production from lignocellulose, this can have considerable economic impact.[24,42] However, the price of the ionic liquids that best dissolve lignocellulose is high, and very effective recovery and recycle of these solvents is crucial, as shown in a recent economic assessment of biofuels production using ionic liquid pretreatment.[43] The use of quaternary ammonium cations, replacing the more expensive imidazolium-based ionic liquids that are the most effective lignocellulose solvents to date, may provide a route to reduce the costs of ionic liquids substantially.[44]

Biomass saccharification

The Cost of Cellulase Enzymes. The enzymatic hydrolysis operation is at the heart of the economic viability of the biorefinery, for two main reasons. First, the efficiency of hydrolysis influences the yield of biofuel from feedstock, which in turn is a principal determinant of the biofuel production cost.[24] Second, the enzymes themselves are a main contributor to the variable operating cost of the biorefinery.[42] These two factors are linked and centrally important to their tradeoffs is the unit cost of enzyme.

Despite the importance of the cost contribution of enzymes on the viability of biorefineries, this area has received only cursory attention in the published literature. Some authors emphasize the importance of this economic driver, whereas others imply through assumptions or simplifications that this parameter is not particularly influential. The cost contribution of enzymes, assumed or calculated, ranges enormously in the literature, from $<$\$0.30[45–47] to more than 1/gal.[42] This disagreement emanates largely from the fact that there are few studies in the literature that focus explicitly on the cost contribution of enzymes on the production of lignocellulosic biofuels. Furthermore, the cost contribution depends not only on the cost of producing or sourcing the enzymes but also on the operational parameters and performance of the biorefinery, which has accentuated the differences across studies. Although several authors have published detailed studies on the cost of on-site enzyme production,[47,48] the industrial trend has so far been on supply by a third party.

To better understand this problem, our group published an open-access process model for cellulase production, which we used to calculate the cost contribution of enzymes on the production of ethanol.[42] We determined that the minimum selling price of enzyme based on the process model was $\sim$\$10/kg, which we then contextualized as "dollars per gallon of biofuel" based on a corn stover-to-ethanol biorefinery. Depending on the overall biofuel yield at the biorefinery and enzyme loading during saccharification, the cost contribution ranged from \$0.34/gal (maximum theoretical yield, 5 filter paper units (FPU)/g loading) to \$1.47/gal (typical biorefinery yield, 10 FPU/g loading). These figures were based on enzyme production by the current commercial organism, *Trichoderma reesei*, producing 100 g cellulase/L in a 192-h fermentation. In summary, the cost contribution of enzymes is significant and is not independent from the operational characteristics of the biorefinery, as is many times presented. In particular, the overall yield and the enzyme loading are key parameters that must be considered.

Although measures to increase yield are likely to be biorefinery-wide, lower enzyme loading is directly related to the saccharification step. Several strategies have emerged or been proposed to this end. For example, Kim et al. (2011) showed that the by-products of various pretreatment methods could inhibit not only fermentation (see below) but also

saccharification.[49] They showed that by selectively removing phenolic compounds from the hydrolysis feed, a lower-enzyme dosage could be used. Better or more stable enzymes or enzyme adjuvants could also be discovered or engineered to lower the enzyme loading.[50,51] Certain pretreatment technologies can reduce the required enzyme loading or forego the use of enzymes altogether, for example, by significantly improving enzyme accessibility to the cellulose through biomass solubilization (reviewed by Ref. 52) or using enzyme-free acidolysis followed by sugar extraction.[53,54] Feedstocks can also be made more amenable to saccharification by modifying plant metabolism. Lignin is a key biomass component contributing to its recalcitrance,[55] and engineering of the lignin biosynthetic pathways has led to feedstocks that are easier to deconstruct (reviewed by Ref. 36). Process modifications can be implemented to reduce the enzyme loading, such as increasing the residence time of the hydrolysis operation or reduce the solids loading. The first strategy has limited impact because the conversion curve saturates with time regardless of conditions[56,57] and because a higher residence time translates into a higher capital cost for this operation. The second strategy is hampered by the fact that lower solids loading leads to increased capital cost because dilute systems require larger facilities (per unit of biofuel produced) than concentrated systems.[58] In summary, strategies to moderate the impact of enzymes on the cost of biofuel production have been proposed and should be pursued, but a closer look into the tradeoffs of saccharification imply that this operation must be optimized through a systems approach and in the context of other operations in the biorefinery.

Improving Cellulase Enzymes. Cellulases are glycosyl hydrolases that cleave the β-1,4 glycosidic linkage of the cellobiose monomer units of cellulose. They do so through a highly conserved kinetic mechanism, using two acidic residues (glutamate and/or aspartate), one of which is protonated and the other unprotonated. The mechanism is either a single-displacement inverting mechanism or a double-displacement retaining mechanism.[59] In contrast to most enzyme substrates, lignocellulose is a solid and the cellulases must first find and bind to a free end (exocellulases) or a binding site along the cellulose chain (endocellulases). Given the wide variety of plant biomass, it is not surprizing that a large number of glycosyl hydrolases have evolved in bacteria and fungi. However, all use the same chemistry to break down and release glucose (and other sugars) from the cellulosic component of biomass. As the cellulose substrate entering the active site of the enzyme remains the same, it would be surprizing to find or evolve a new cellulase that used a different hydrolytic mechanism. The major differences in cellulase activity result from the physicochemical nature of the lignocellulosic substrate, primarily the extent of crystallinity and the amount and relocalization of lignin, which affect enzyme binding and diffusion to the sites of hydrolysis. The optimal enzyme mixture (cocktail; ratios of exo, endocellulase and β–glucosidase) is dependent on the type of biomass and how it was pretreated.[60] With extremely efficient pretreatment, it might be anticipated that glucose release could depend solely on the kinetics of enzyme binding to cellulose and formation of the transition-state complex,[60,61] a situation approaching cellulase "intrinsic" kinetics.

Opportunities to discover or evolve cellulases with increased rates of glucose release are thus constrained. If the pretreatment is held constant, then cellulases with reduced levels of cellobiose or glucose inhibition can show improvement, as will cellulases with improved thermal stability (longer half-lives), as the duration of saccharification can range up to several days. Efforts to improve the hydrolytic activity of cellulases by selection or evolution have not been successful as this is not the rate-limiting step in cellulose hydrolysis. Typical activities (turnover numbers) of individual cellulase enzymes on soluble substrates are over 30-fold higher than those on solid substrates, reflecting the requirements for adsorption, binding, and diffusion events on the solid. Although there have been reports of improved enzyme activities at elevated temperatures, most have compared activities between variants and the native enzyme—with all enzymes being produced in heterologous hosts (bacteria and yeast).[62,63] Typically, no comparison is made with the activity of the corresponding commercial enzyme produced in its native host (e.g., *T. reesei*), which is many times more active. There is a large reduction in activity of cellulase enzymes produced heterologously in bacteria and yeast, that is, due to the lack of N-terminal glutamine cyclization that occurs in fungi. Treatment with glutaminyl cyclase in vitro restores the activity of enzyme variants expressed in yeast or bacteria to levels closer to those of the corresponding native enzymes expressed in *Trichoderma* or *Neurospora*.[64]

The cost of producing cellulases in any organisms other than fungi, such as *Trichoderma, Humicola,* or *Aspergillus,* is extremely high as the native enzyme concentrations that can be obtained with fungi are over 100 g/L. Production by aerobic bacteria such as *Cellulomonas* does not reach these levels. Producing heterologous cellulase enzymes in yeast (glycosylated) or bacteria (unglycosylated) is extremely costly, as concentrations that can be obtained are typically only hundreds of milligrams to grams per liter, over 100-fold less than with fungi. Thus, the cost of heterologously-expressed cellulases or their variants will be one or more orders of magnitude greater than the cost of commercial enzymes produced by *Trichoderma*. In addition, the intrinsic activity of enzyme variants or newly discovered enzymes is unlikely to be different from existing enzymes for the reasons outlined above. Differences are likely to simply result from biomass pretreatment prior to saccharification. Thus, new enzyme discovery is only likely to find enzymes with higher thermal stability, and such enzymes would be extremely expensive to produce. Most reports of a reduction in cellulase costs reflect a lowering of the fermentation cost (e.g., reduced inducer requirements) rather than any improvement in the specific activity of individual enzymes.

Nevertheless, there has been considerable effort devoted to discovery of more active cellulases. For example, termites are prolific digesters of lignocellulose and do so through a multistep process. Lignocellulose is first macerated to 20 (foregut) and 10 (midgut) micron-sized particles,[65] a process that would be commercially cost prohibitive. The pH of the midgut–hindgut junction is among the most alkaline recorded in biological systems (pH 11–12.5).[66] Thus the micron-sized lignocellulose particles are effectively alkaline pretreated prior to attack by the cellulases produced by the gut microbiota. Their small particle size and the high-pH pretreatment would make cellulose readily digestable, far more so than might be accomplished with current commercial processes. The cellulases produced by microbial symbionts in the gut would thus not require capabilities beyond known cellulases to hydrolyze cellulose. Even if a cellulase system of high

activity were discovered, such enzymes would be extremely costly and difficult to produce. Nonetheless, extensive metagenomic analysis of termite hindguts has been undertaken and, unsurprisingly, a variety of cellulase genes found.[67] This and similar studies of cow rumen microorganisms have not produced any commercially useful enzymes, and bring into question the wisdom of enzyme discovery without any means available to produce "discovered" enzymes at scale.

Cellulosomal Systems. Some anaerobic bacteria are able to degrade lignocellulose by secretion of a multienzyme complex, the cellulosome. The cellulosome typically remains attached to the cell and contains cellulases, hemicellulases, and esterases linked along a scaffolding protein. *Clostridium thermocellum* is a mesophile that degrades cellulose by a cellulosomal system, producing ethanol and acetate by a mixed acid fermentation.[68] It has thus been examined for direct production of ethanol from biomass. Cellulosomes have been of interest, as they appear to be a "molecular machine," with enzymatic subunits in close proximity on the scaffolding subunit and a cellulose-binding module to anchor the complex to the cellulosic substrate. Much of the DOE Roadmap section on deconstruction was devoted to cellulosome design for cellulose hydrolysis and sugar production.[4] From a practical perspective, however, cellulosomal systems or proposed "designer" cellulosomes would be exceedingly expensive to produce in heterologous hosts, and when produced in native hosts, they are not present at concentrations sufficient to enable rapid cellulose hydrolysis. It was thus unfortunate that the DOE Roadmap emphasized further research on cellulosomal systems. Commercial efforts to use *C. thermocellum* for ethanol production have been subject to long fermentation cycles arising from low levels of cellulosome production and ethanol inhibition of these cellulolytic bacteria at concentrations over 25–30 g/L. Therefore, it is not likely they will play a role in commercial biomass saccharification. Despite this, *C. thermocellum* has been widely explored for one-pot cellulose hydrolysis with simultaneous fermentation of the resultant sugars to ethanol (consolidated bioprocessing or CBP). Although CBP is widely cited to be the most cost-efficient commercial route for biofuels production, there are no economic comparisons in the literature of CBP with lignocellulose pretreatment and saccharification using commercial enzymes at their optimal conditions (50°C, pH5) with subsequent fermentation of sugars to ethanol by *Saccharomyces cerevisiae*.[48] An experimental comparison of CPB using *C. phytofermentans* with simultaneous saccharification and fermentation (SSF) of AFEX-treated corn stover at 30°C indicated that SSF was more effective than CBP,[69] although in both cases ethanol concentrations observed were very low (<4 g/L). The large difference in timescales between biomass saccharification using commercial enzymes under optimal conditions and that of CBP, which is significantly longer due to the time required to grow the organism and produce cellulosomes, will result in very large differences in capital requirements and make CBP less attractive.

In summary, the main challenges in biomass saccharification are:

- development of an effective pretreatment that facilitates enzymatic saccharification and reduces enzyme loading,
- reduction in the inhibition of cellulases by cellobiose and glucose,
- improvements in thermal stability of cellulases, particularly β-glucosidase, and
- routes to recycle enzymes and decrease enzyme loading (e.g., membrane separations or continuous countercurrent enzyme/solids systems).

Conversion of biomass-derived sugars to fuels

Today, ethanol production from corn provides the largest source of biofuels in the United States. Current production is ~1.18 billion gallons/month, roughly 10% of the 12 billion gallons/month of gasoline consumed. Ethanol is produced by the yeast *S. cerevisiae* in a nonaseptic fermentation of starch-derived glucose. A large inoculum results in a rapid fermentation, high-ethanol concentrations and lowering of the fermentation pH. These, together with anaerobic conditions, contribute to reducing opportunistic infection. *S. cerevisiae* does not natively ferment pentose sugars, and incorporation of xylose metabolism was one of the early challenges in genetic engineering of this yeast for conversion of biomass sugars.[70] The inhibition of D-xylose transporters by D-glucose remains a significant problem in cometabolism of both sugars, although some progress has been made.[71] Xylose uptake and ethanol production in industrial organisms is also affected by inhibitors present in lignocellulosic hydrolyzates and further progress is required to reach industrial ethanol production levels comparable to those of glucose.[72] The bacterium *E. coli* is able to sequentially metabolize both glucose and xylose and is readily engineered but is significantly inhibited by compounds produced from biomass processing and by ethanol itself.[73] Simultaneous fermentation of both hexose and pentose sugars for ethanol production from biomass still remains a challenge.

An alternative to the ethanol fermentation is the solventogenic fermentation of sugars by *Clostridia*. Acetone, butanol, and ethanol (ABE) production by *C acetobutylicum* has been practiced commercially for over one hundred years. Clostridia are able to anaerobically ferment a diverse range of substrates, including glucose, xylose, sucrose, fructose, lactose, and short chain fatty acids including acetic, butyric, and lactic acids.[74] They are thus ideal organisms for conversion of biomass to fuels but lag in the development of genetic tools, although recent advances in the chromosomal manipulation of *Clostridia* have been reported.[74] However, by integrating extractive fermentation with chemical catalysis, the ABE fermentation products can be converted from C_7 to C_{11} and higher ketones. This route can provide petrol, jet, and diesel blendstocks from both lignocellulosics and cane sugars at near theoretical yields.[75] By altering the ratio of acetone to butanol, and reducing the formation of ethanol, higher chain length ($>C_{11}$) ketones can be produced at high titers and yields.[76] Inhibitors generated by biomass pretreatment can be removed from the fermentation by the extractant, enabling high-volumetric productivities to be achieved in the fermentation. This hybrid route appears very promising as it achieves near theoretical yields without the necessity of metabolic engineering of the fuel-producing organism.[77]

Efforts have also been directed toward the direct microbial production of fungible (or nearly-fungible) fuels. These include n-butanol, iso-butanol, C_6–C_{10} alcohols, fatty acids and fatty acid ethyl esters (FAEEs), methyl ketones, farnesol, and bisabolene. Most have been produced in *E. coli* under (micro) aerobic conditions, although efforts have been made to address cofactor availability nicotinamide adenine dinucleotide (NADH) for anaerobic production.[78] Yields of fuel products are pathway dependent. For example, farnesol

production by the mevalonate pathway has a yield of 0.272 g/g glucose and 0.324 g/g via the 1-deoxy-D-xylulose-5-phosphate (DXP) pathway. Butanol production in *S. cerevisiae* is dependent on the isomer produced and the pathway.[79] Energetic efficiency of metabolic pathways and stoichiometry can be used to determine optimal molecules for targets as fuels. Typical pathways generate metabolic intermediates that are more oxidized than the substrate (glucose) and produce reducing equivalents (e.g., NADH/NADPH). These metabolic intermediates are then reduced by the reducing equivalents formed in the first step. The balance of production and consumption of reducing equivalents is reflected in the efficiency of the pathway.[80] This provides a useful means to evaluate alternative substrates, cosubstrates, and yields, which impact the variable operating costs for biofuel production. The capital costs are related to the volumetric productivity of the fermentation and the product titer. To date, volumetric productivities of fungible fuels are very low in *E. coli*: those of fatty acids and FAEEs are less than 0.2 g/L.h and farnesol and bisabolene are below 0.02 g/L.h.[73] By comparison, ethanol productivity in *S. cerevisiae* is about 10 g/L.h, indicative of the large improvements in both titer, yield and fermentation time that must be made for production of fungible fuels in *E. coli* to be cost effective.

The imbalance in generation and subsequent use of reducing equivalents in engineered metabolic routes to fuels typically results in the necessity for aerobic or microaerobic growth conditions for the host organism, as this provides a route to react excess reducing equivalents to form water and capture energy. Large-scale microaerobic fermentation is challenging, as elimination of oxygen gradients in a large vessel is difficult. Regions of either excess or depleted oxygen can alter the metabolism of the organism, resulting in decreases in product yields. A redox-balanced metabolic pathway permits anaerobic growth, which eliminates this problem and additionally decreases the amount of sugar substrate that is converted to cell mass (aerobic cell yields are typically 0.5 g cells/g sugar; anaerobic yields are 0.1 g/g). Thus, anaerobic production of ethanol by yeast or bacteria and the ABE fermentation by *Clostridia* appear to be optimal choices for biofuels production as they have near-theoretical yields and high rates.

Effect of Inhibitors Generated by Pretreatment on Fermentation. Given the high cost of enzymatic saccharification, the pretreatment process is typically aggressive to maximize the effect of enzymes during the subsequent operation. The generally severe conditions of pretreatment not only accomplish the partial deconstruction of the biomass but also produce inhibitors that can hamper the saccharification and fermentation operations. The by-products of pretreatment depend both on the type of biomass that is processed and the parameters of the pretreatment method (time, temperature, pressure, pH, added chemicals, etc.). These inhibitors are most often derivatives of sugars or the phenolic monomers of lignin, although they can also result as a consequence of subsequent neutralization operations (e.g., high salts[81]).

The most common sugar by-products include furfural (from C_5 sugars) and hydroxymethyl furfural (HMF; from C_6 sugars), and their derivatives such as levulinic acid.[82,83] These are commonly formed during severe acid-mediated pretreatments, such as steam explosion or dilute acid.[84] Conversely, alkaline pretreatments result in the formation of toxic hydroxyl-monocarboxylic acids, such as glycolic and lactic acids.[85,86] Aromatic inhibitors other than furfural and HMF are typically derivatives of lignin monomers, and include phenol, and various aromatic aldehydes, ketones, and acids. Examples include vanillin, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, coniferyl aldehyde, and syringic acid.[87,88] Finally, acetic acid is generally formed during deconstruction because the hemicellulose and to a lesser extent the lignin components are acetylated.

The harmful effects caused by the presence of inhibitors depend on their chemical nature and the microbial response to them. The inhibitors that are produced directly in pretreatment can be broadly categorized as organic acids, furans, and aromatic compounds (or phenolics). We describe their activity here only briefly; the interested reader can find more information in extensive reviews on the topic.[82,83,89,90]

Organic acids are chemically weak, meaning that in general, they exist partially dissociated in the fermentation medium. The undissociated acid, which is neutral, can cross the microbial membrane and lower the intracellular pH. Rebalancing of the intracellular pH requires metabolic energy in the form of adenosine triphosphate (ATP) and thus imposes a toll on the fermenting microorganisms. Concomitantly to the accumulation of protons, the concentration of the corresponding anions increases. The anions then hamper the cell metabolism through their activity in intracellular reactions, through interference with the function or structure of enzymes, or other cellular components.[83,91] Furfural, a common furan oftentimes used as a model compound in toxicity studies, acts as a redox sink, oxidizing biosynthetically -produced NADH while at the same time arresting cell replication.[92] Lastly, phenolic compounds act by disrupting the integrity of biological membranes, which damages the essential physiological functions that happen through and on the membranes.[90] Detoxification of hydrolysates is possible and sometimes practiced, including through ion exchange, extraction, overlimining, and other methods.[89] All these incur additional costs, and thus the benefits of detoxification must be duly evaluated and quantified to determine its value proposition to the biorefinery.

Chemical Routes from Sugars to Fuels. Biomass-derived sugars are ideal substrates for biological conversion because these compounds are the natural carbon and energy sources for most micro-organisms used by industrial biotechnology. Sugars, however, can also serve as substrates for catalytic conversion to fuel molecules. Sugars are functionally rich, thus the aim of catalysis is to eliminate the functional groups and produce molecules that resemble the hydrocarbons that make up fossil fuels. The main reactions that are catalytically performed on sugars or sugar derivatives are hydrolysis, dehydration, isomerization, aldol condensation, hydrogenation, and hydrogenolysis.[93]

Although the number of molecules that can be theoretically produced through catalytic conversion of sugars is large, only a handful of routes have been explored. Of course, not all sugars are equivalent for catalytic conversion, even though they are chemically similar. The reaction performance (rate, selectivity, etc.) and the product composition depend on both the substrate and the catalytic system used. For example, acid-catalyzed fructose conversion to HMF has been a preferred route, as this reaction has relatively high rate and selectivity. Glucose, conversely, displays lower yields to HMF and cross-reacts with the reaction

intermediates and the HMF product.[94] Sorbitol can be converted to hexane through cycles of dehydration and hydrogenation in a catalyst system composed of a metal (e.g., Pt) and a solid acid catalyst (e.g., silica/alumina), though with a selectivity below 60%.[95]

Sugars have been the main target for catalytic conversion, but other components of biomass and biomass-derivatives have also been explored as substrates. For example, γ-valerolactone (GVL), a naturally occurring compound in plants, has been treated at elevated pressure over a silica/alumina catalyst to produce butene, which can then be condensed to alkenes that can be processed to gasoline or jet fuel.[96] The concentration of GVL in plants is low, but it can be produced from levulinic acid, which can in turn be produced catalytically from cellulose with yields of 50–70%.[97] Catalytic or thermocatalytic lignin reprocessing has also been explored, as this component makes up a significant fraction of lignocellulosic biomass (15–35%) and its use as a coproduct can substantially aid the economic performance of a biorefinery.[43] Technologies commonly used with biomass can be also used on the lignin fraction, including pyrolysis and hydropyrolysis, gasification, and catalytic hydrogenolysis.[98] These routes can produce intermediates such as bio-oil and syngas, which can be then used to make fuel products. As far as chemicals, lignin has been used to produce aromatics and synthetic alcohols, although this area is nascent and has largely remained a research endeavor.

The diversity in the chemical structure of biomass components and the reactivity of catalysts has invited the attention of a variety of researchers, but it has so far failed to deliver profitable commercial processes for production of liquid fuels. One reason is that the fractions that result from pretreating lignocellulosic biomass are extremely complex mixtures, and catalytic systems can be easily poisoned by a number of the constituent components.[99,100] This challenge must be addressed experimentally, but a review of the literature reveals that published protocols are performed with pure streams of the substrate as model systems (e.g., using glucose as a proxy for sugar or syringaldehyde as a proxy for lignin). Therefore, there is little evidence that the research community is working on addressing this challenge. In the case of sugars, the main components of biomass, an alternative could be to use microbial conversion as a first step and catalytic conversion as a subsequent operation. Microbial systems can selectively convert sugars into intermediates that can be separated and then used in purer form as substrates for chemical catalysis. A hybrid chemical-biological process could combine the best aspects of both routes, achieving high selectivity for sugars or lignin monomers in the mixed feed while maximizing the conversion rate during catalytic conversion of the intermediate product.[101,102]

Production of fatty acids from algae

Production of biofuels from algae, and in particular, of fatty acids from microalgae, has attracted significant attention in the field. In fact, some authors have touted this route to be uniquely capable of delivering a carbon-neutral alternative to fossil liquid fuels at a large scale.[103–106] Specifically, microalgae have been considered the ideal production platform for biofuels because they can grow in brackish or sea water, do not require arable land, they can accumulate lipids, have shown high-photosynthetic efficiency, and can fix CO_2 in a single unit operation (either in ponds or in photobioreactors, or a combination of both). It should be noted that microalgae is not a single organism that can be put to the service of biotechnology, and that many practical challenges arise when considering microalgae as a diverse group instead.[107] This fact notwithstanding, it is conceivable in theory that many of the advantageous characteristics found in microalgae could be found or engineered in a single host. Therefore, and for argumentative purposes only, we refer to microalgae as a platform for biofuel production.

Despite their numerous advantages, production of drop-in biofuels from microalgae has been criticized on two main grounds. First, the energy balance for production of microalgal biofuels has been often questioned, in particular when compared to alternatives.[108–113] Slade (2013), for example, conducted an extensive review on the topic and concluded that the incomplete nature of many life cycle assessment (LCA) studies in the field hampers an appropriate comparison of the arguments from various sources[110] The system boundaries, unit operations considered, assumptions, and LCA methodologies vary across studies. In spite of this, the authors conclude that unless significant technology advances are brought about, the energy balance of microalgal systems will not be positive. Cox et al. (2014) calculate that the fossil energy ratio, the energy output divided by the fossil energy input, is 1.0, close to that of fossil kerosene, at 0.92.[108] To be fair, the topic is far from settled in the public literature. The discrepancy between supporting and critical studies can be simplistically reduced to the fact that, although the theoretical potential of microalgae systems in terms of productivity and energy efficiency may be many times larger than can be achieved with other photosynthetic systems (e.g., terrestrial crops), the performance that has been observed in practice falls very much short of the potential.[111,113]

The second front on which microalgal biofuels have been doubted is related to their economic viability. Again, studies reach disparate conclusions based on different process designs, assumptions, and system specifications. In this case, the aforementioned simplification of treating microalgae as a single platform for biofuel production is particularly dangerous because detailed process economic studies require species-dependent parameters. Examples include the salt concentration of the water where the biomass is grown (which dictates allowable recycle rates, materials of construction, etc.), cell dimensions (which dictate the specifications of the harvesting and extraction operations), oil content (which dictate the specifications of all operations downstream of biomass growth), and so forth. These and other required parameters are not only species-dependent but they also vary by location and time of the year. Ultimately, it is hard to pinpoint the cost of producing microalgal biofuels simply because there are still too many unknowns for accurate numbers to be calculated. Therefore, although several studies point to the economic challenges of growing microalgae for production of liquid biofuels,[113,114] the best evidence comes from the fact that despite decades of research and sizable investments, commercial production of microalgal biomass remains a niche endeavor.[111,115,116] Furthermore, to the authors' knowledge, profitable (i.e., money-making) production of microalgal biofuels has never been sustained. Therefore, given the arguments found in the literature and summarized here, unless groundbreaking technological advances are made, it is unlikely that microalgal biofuels will make a significant dent on fossil fuel consumption in the foreseeable future.

Legislative and Economic Hurdles to Biofuels Production

Advised by academics and commercially motivated industry representatives who provide an optimistic view of the cost of biofuels, governments, hoping to reduce dependence on foreign oil, increase energy security, and tackle climate change concerns are implementing policies that support, encourage, or mandate the use of biofuels. We give a simplified description of the U.S. case in the following paragraphs, and we focus on the biofuels that are currently available in the market (ethanol and, to a lesser extent, biodiesel). The U.S. Congress has encouraged the use of biofuels through various policies. The Energy Policy Act of 2005, and its expanded version, the Energy Independence and Security Act of 2007, codified into existence the RFS program. The later version of the RFS, sometimes called RFS2, mandated the use of 36 billion gallons of renewable fuels to be blended with gasoline and diesel by 2022. The U.S. Environmental Protection Agency (EPA), which administers and implements the RFS program, determines each year the Renewable Volume Obligation (RVO) for the parties that are required to participate in the RFS program by law (the so-called obligated parties). The obligated parties are importers or producers of gasoline or diesel, and their individual mandated contribution to the RVO is calculated on a prorated basis.

To keep track of the flow of renewable fuels in the economy, the EPA created the Renewable Identification Number (RIN) system. A RIN is a 38-digit numeric code that singly identifies each gallon of renewable fuel produced in or imported into the U.S. The RINs are linked to the fuel throughout the supply chain, and are separated from it when the renewable fuel is blended with either gasoline or diesel. At that point, the RINs can be used to comply with the RFS mandate or can be traded. The obligated parties must accumulate their prorated amount of RINs either through blending or trading, so the RIN system in essence translates the specifications of the RFS into economic incentives. Naturally, the RFS program has created tensions between the renewable fuel and the fossil fuel producers and importers, although parties on both sides of the supply chain benefit to some extent from the program.[117] Regardless, refiners argue that consumers should freely choose the fuel they use without government interference and that the mandate creates problems because the infrastructure cannot support ethanol blends higher than 10% (the so-called "blend wall").[118] Biofuel supporters argue that incentives are needed to offset some of the unaccounted fossil fuel externalities and that higher ethanol blends are possible and even favorable.[118,119] The antagonism between these groups is exacerbated by the fact that the EPA has been slow to decide on the RVO: as of mid-October 2014, the agency had not decided on the renewable fuel targets for the year[120] and subsequently decided not to finalize RVO numbers until 2015.

In addition to political and legislative challenges, economic hurdles limit the expansion of the biofuel market. In particular, the capital needed to produce, distribute, and utilize the biofuels is significant. Assuming a capital cost of \$2.20/annual gallon for corn ethanol facilities,[102] the 15 billion gallon RFS target for 2022 would translate into ~\$30 billion in investment, although the majority of this has already been made (according to the Renewable Fuels Association, the United States produced 13.3 billion gallons of ethanol in 2013). Lignocellulosic ethanol plants are significantly more expensive: meeting the 16 billion gallons annually by 2022 would take an investment of \$160–260 billion (estimates from Ref. 100). The transportation and distribution infrastructure, together with the fuel station and vehicle retrofits or modifications needed, add to this considerable investment. The numbers for the infrastructure costs are hard to obtain or estimate, although some authors have provided guidelines. Spatari et al. (2009), for example, calculate that the non-biorefinery investment required is in the order of \$0.50–3.00/annual gallon.[121] In all, the investments required are significant because fuel margins are small, and there is thus little money to pay back the original investment and allow for a return large enough to mitigate the investment risk. Further, the 13.3billion gallons now produced has approximately the energy equivalent of 8.9 billion gallons of diesel or gasoline, which is a small (4%) fraction of US annual 220 billion gallon liquid fuel consumption. Although helpful, there is no evidence that biofuels, implemented according to the legislative framework using any known technology, will be able to address society's transportation fuel sustainability challenges.

Complicating the matter is the fact that different parties would have to spend in different parts of the supply chain (production, distribution, end-use). Therefore, for the investment of one party to be productive (i.e., to make a return), investment of other parties must be in place and be productive as well. For example, for a biofuel producer to be profitable after the blend wall is reached, more fuel stations must carry higher biofuel blends, car manufacturers must make more fuel-flexible cars, and consumers must buy the cars and the biofuel blends. Although the EPA has allowed the use of E15 blends, the ruling is still being debated by the courts,[122] and even if it is upheld, this will not be more than a temporary solution. This is a key argument in support of drop-in biofuels, that is, those that would not require a change in the distribution and consumption infrastructure, and although new technologies are emerging, commercial production of drop-in biofuels has not materialized at any appreciable scale.

Hastening the advent of "advanced" biofuels will, therefore, require the support of parties along the supply chain and from the policymakers. In the case of the research community, it is our responsibility to develop technologies that bring about tangible commercial successes in the field. In this regard, cellulosic ethanol offers an interesting case: although this route is still touted to be expensive compared to first-generation ethanol, a few players have started to operate commercial-scale biorefineries. For example, Iogen and Raizen recently announced production of cellulosic ethanol at their 22-million gallon facility in São Paulo, Brazil.[123] Similarly, POET-DSM's Project Liberty, a 20-million gallon facility located in Iowa, began production in September 2014, and Abengoa Bioenergy opened a 25-million gallon facility in Kansas in October 2014.[124] Although the economic profitability of these projects is not proven or guaranteed, the fact that they are coming online is a clear sign that research advances and innovations are slowly turning into commercially-relevant technologies.

In all, the above discussion points to, at best, a rocky pathway for the widespread use of renewable fuels, and although future technological solutions might enable that movement, legal and political battles will undoubtedly play a crucial role in these developments. A rethinking of current

research and development priorities for biofuels pathways is needed.

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