Producing biofuels via the sugar platform

Article · March 2015

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Producing Biofuels via the Sugar Platform

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Whether the feedstock is sugarcane, corn, or lignocellulose, the fermentation and ethanol recovery operations are similar. The differences arise in the way the sugars are released and the co-products produced.

Simple sugars (mono and disaccharides) are reactive molecules that can be converted biologically or chemically into fuels, chemicals, food, and animal feed. Sugarcane, sugar beets, sweet sorghum, and a few other types of plants contain significant quantities of simple sugars. In nature, these sugars are often polymerized as storage polysaccharides (*e.g.,* starch) in grains like corn, rice, and wheat. Worldwide, grains are the basis of most human food and are either eaten directly or fed to livestock.

Grain is a small portion of plants. Plants are composed primarily of structural components such as roots, trunks, branches, stems, and leaves. Plant cell walls are composed of lignocellulose — a composite of cellulose (glucose polysaccharide), hemicellulose (often primarily xylose polysaccharide), and lignin (aromatic polymer), along with lesser amounts of other components such as minerals and protein. These complex structural polysaccharides impart strength and rigidity to plants. Several chemical and physical barriers make it difficult to convert hemicellulose and particularly cellulose to simple sugars. This recalcitrance, or resistance to being hydrolyzed into sugars, is the primary economic barrier to low-cost products from lignocellulosic biomass.

Global production of simple sugars (*e.g.,* sucrose) is approximately 200 million m.t./yr at a price of about $400/m.t. Production of starch in the form of the three major cereal grains (wheat, rice, and corn) is about 2 billion m.t./yr with a value of $300–$600/m.t. of starch. This compares with total world oil extraction of about 4 billion m.t./yr at $400–800/m.t. (equivalent to roughly $50–100/bbl).

Other than paper pulp and lumber, large markets do not exist for most forms of lignocellulose; thus, the world utilizes far less lignocellulose than it could. Nonetheless, total lignocellulose production in the biosphere is roughly 100 billion m.t./yr. At the farm gate, some biomass varieties (*e.g.,* high-biomass sorghum) can be produced for about $60/m.t., which is equivalent to oil at about $20/bbl on an energy basis. At this volume and cost, lignocellulosic biomass has the potential to replace a substantial amount of petroleum, if it could be economically converted to sugars and then to liquid fuels. Anaerobic fermentation of sugars to fuels is attractive, because well over 90% of the energy in the sugars can be conserved in the fuels.

Figure 1 compares the three branches of the sugar platform *(1):*

a. Simple sugars from sugarcane. Sugar is extracted from sugarcane and fermented to ethanol, and the resulting ethanol-water mixture is distilled to recover ethanol.

b. Starch from corn via wet or dry milling. The corn is ground and cooked to make the starch available for amylase enzymes to hydrolyze it to sugar; the enzymatically produced sugars are then fermented, and the resulting ethanol is recovered by distillation.

c. Lignocellulose. Biomass is ground and pretreated to disrupt the plant cell walls and make them available for enzymatic conversion to sugars and then to ethanol, which is recovered by distillation.

The key to obtaining inexpensive sugars is to achieve high yields and concentrations of readily fermentable sugars from low-cost feedstocks while keeping processing costs low. Sugar and starch feedstock costs are relatively high, but their processing costs are comparatively low. Conversely, lignocellulose feedstock costs are lower, but processing costs are high primarily because of the material's recalcitrance *(2)*.

Ethanol from Brazilian sugarcane and U.S. corn

For many decades, fuel ethanol has been produced commercially by fermenting sugars derived from sugarcane and grains, primarily corn. Ethanol is also the first biologically produced liquid fuel being pursued for large-scale commercial production from lignocellulose. Lessons learned from commercial production of ethanol from sugarcane and corn apply to the emerging lignocellulosic biofuels industry.

Since the auto industry began, ethanol has been used as a fuel in motor vehicles. Henry Ford originally targeted ethanol to power his early vehicles.

Currently, ethanol comprises about 30% of the gasoline mix in Brazil (roughly 6 billion gal/yr of ethanol) and about 10% of the mix in the U.S. (about 14 billion gal/yr). In Brazil, all automobiles are designed to run on different ethanol-gasoline mixtures, and all refueling stations provide a variety of ethanol-containing fuels.

In the U.S., most gasoline now contains about 10% ethanol, and mixtures of 85% ethanol with gasoline (E85) are

available in some states. However, the latter are not widely used due to limited numbers of flexible-fuel vehicles that can run on E85 and the limited number of fueling stations in many parts of the country. In the U.S., ethanol also suffers from an image problem based on widespread anecdotes about problems with fuel systems, lower mileage, and other perceived drawbacks that the Brazilian experience has shown can be overcome successfully. The fact that Indianapolis 500 race cars are fueled with ethanol also speaks to the excellent properties and performance achievable.

For decades, fuel ethanol has been produced in large volumes in both the U.S. and Brazil in response to three national policy goals:
• to improve national energy security and address fuel shortages such as during the mid-1970s oil embargo
• to reduce emissions of carbon monoxide and unburned hydrocarbons, particularly at high elevations during winter
• to stabilize the agricultural sector by providing another market for surplus agricultural products (sugar and corn).

In the last decade, both countries have added a fourth policy goal — to reduce greenhouse gas emissions.

In 1975, the Brazilian National Ethanol Program (PROALCOOL) was formed to directly respond to an oil price spike that caused great economic hardship. As the cumulative volume of sugarcane ethanol produced increased, the cost of production decreased almost threefold (Figure 2). Although these data are somewhat old, the figure demonstrates that the cost of production decreases as experience is gained. Because of this learning curve effect associated with extended production experience, the cost of cane sugar now represents approximately 70% of the total cost to produce ethanol — a typical percentage for a commodity product in a mature industry. Similarly, in the U.S., as corn ethanol production volume has increased, production costs have dropped substantially, and ethanol is now the low-cost source of octane for U.S. gasoline. Although additional cost reductions are likely, the corn ethanol industry is likewise mature, with net feedstock costs (feedstock less co-product credits) representing at least 70% of the cost to produce corn ethanol.

A few visionary companies have recently begun or will soon begin commercial conversion of lignocellulose to ethanol. (See the accompanying article, "Commercial-Scale Production of Lignocellulosic Biofuels," pp. 62–64.) As the lignocellulosic ethanol industry matures through extended operation of
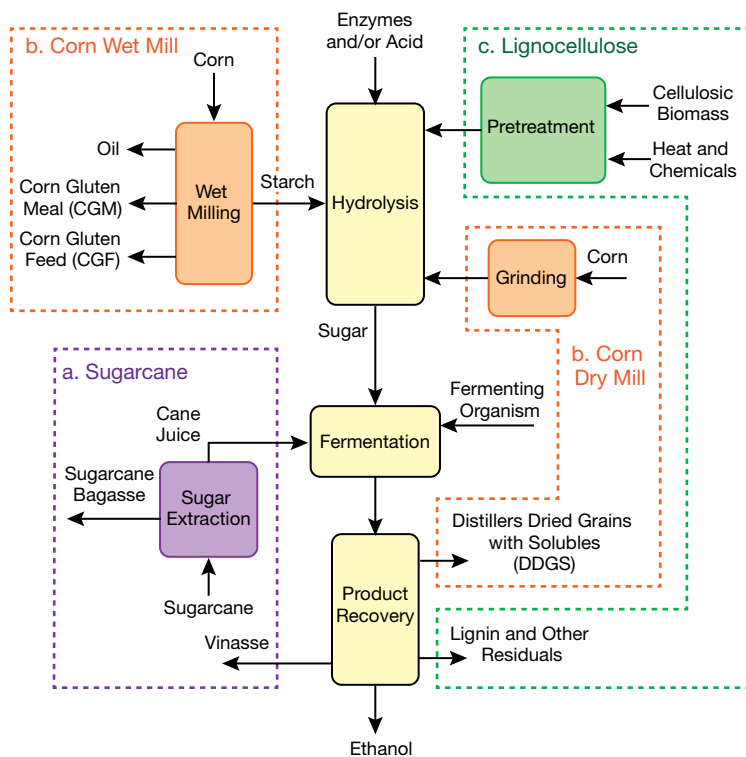
◀ **Figure 1.** The sugar platform processes for producing ethanol from sugarcane, corn, and lignocellulose have similar fermentation and ethanol recovery operations, but use different approaches to prepare sugars and generate different co-products. Source: Adapted from *(1)*.

these pioneering and subsequent plants, conversion costs will also decrease because of learning curve benefits *(3)*. Technological improvements will also drive conversion and capital costs lower and yields higher. The result will be that feedstock costs will become a much greater portion of the overall cost of making lignocellulosic ethanol. However, just as for sugarcane ethanol, corn ethanol, petroleum, and other energy sources, policy and research support have been — and will be — required to build and stabilize markets for lignocellulose-derived fuels.

The rest of this article discusses lignocellulose conversion technologies based on the sugar platform, and opportunities for improvements that would enhance process economics.

Lignocellulose

Lignocellulose is comprised of about 35–50% cellulose, about 15–25% hemicellulose, and about 10–25% lignin, with the remainder including minerals, oils, free sugars, and proteins (Figure 3) *(4)*.

Cellulose is a polymer of glucose molecules linked by beta bonds that form extended linear chains. These long chains align with each other and are connected by hydrogen bonds to form long fibers that give plants strength and rigidity. Hemicellulose can contain as many as five different sugars: arabinose, galactose, glucose, mannose, and xylose, with the latter often the most prevalent. Lignin is composed of phenyl-propene molecules that are polymerized to form a complex macromolecule.

The cellulose fibers are glued together by a lignin-hemicellulose matrix to form nature's composite material. Cellulose is analogous to the glass fiber in fiberglass composites, with the lignin-hemicellulose acting as the epoxy glue.

Although starch is also a sugar polymer, it contains only glucose molecules joined by alpha bonds that are readily hydrolyzed by dilute acids or amylase enzymes, which allows glucose to be recovered at high yields. Sugar and starch are easily metabolized for food.

In contrast, plants employ an elaborate defense mechanism to ward off attack by microorganisms and other predators that would eat the sugars in hemicellulose and cellulose. This recalcitrance has made it possible for lignocellulose such as grasses (*e.g.,* switchgrass), wood (*e.g.,* poplar), and agricultural residues (*e.g.,* wheat straw, corn stover) to grow in a variety of climates and soils around the globe. Thus, converting lignocellulose to fuels and chemicals faces additional challenges because of its recalcitrance, which is not an issue in the production of ethanol from sugarcane or corn *(2, 5)*.

Pretreatment of lignocellulose

Corn requires only mechanical milling and heating to moderate temperatures to make starch sufficiently accessible to amylase enzymes to achieve high glucose yields. In contrast, lignocellulose requires harsher pretreatment conditions to overcome its natural resistance to breakdown. To improve access for enzymes, many physical (*e.g.,* milling, radiation), chemical (*e.g.,* acids, bases, solvents), thermal (*e.g.,* heating to about 200°C), and biological (*e.g.,* fungus) pretreatments have been tested. Most pretreatments require temperatures of 120°C to 210°C to be effective *(5)*.

Adding acids or bases reduces the required pretreatment temperature and enhances overall sugar yields from the combined operations of pretreatment and subsequent enzymatic hydrolysis. However, to be affordable, these chemicals must be either very inexpensive (*e.g.,* dilute sulfuric acid) or readily recycled (*e.g.,* ammonia, sulfur dioxide).

Most pretreatments require short residence times (10–30 min), which allows them to be carried out in small vessels. However, the high pressures needed for some pretreatments and corrosion by pretreatment chemicals may require thick-walled vessels and exotic materials of construction that are more costly.

Although pretreatment improves enzyme effectiveness,
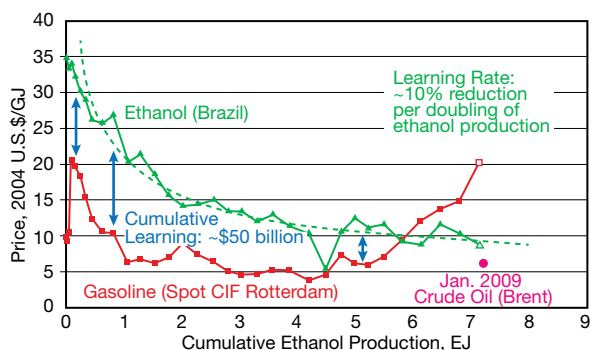
▲ **Figure 2.** With operating experience, the price of Brazilian ethanol has declined and is comparable to the cost of gasoline on an energy basis. Source: *(22)*.
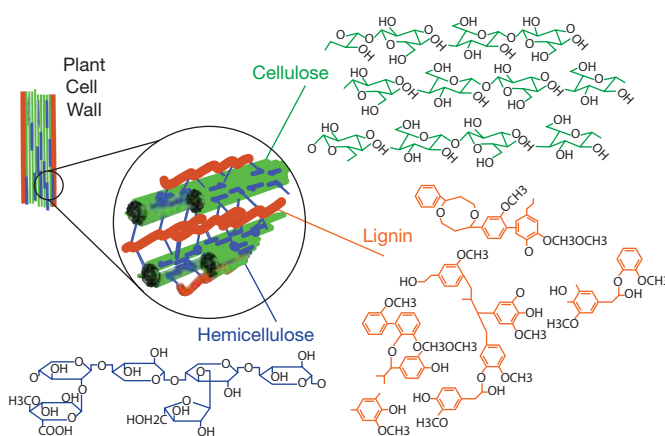
▲ **Figure 3.** Lignocellulose consists of cellulose, hemicellulose, and lignin.

> Lignocellulose requires harsher pretreatment conditions than corn to overcome its resistance to breakdown.

the enzyme doses required to achieve high yields are still very costly *(6)*. Thus, biological conversion of lignocellulose would benefit greatly from pretreatments that use low-cost and/or recyclable chemicals, require short residence times, allow processing in low-cost vessels, degrade little or no sugar during pretreatment, and produce solids that low enzyme loadings can convert to sugars at high yields *(7, 8)*.

Enzymatic hydrolysis

As illustrated in Figure 1, biological conversion of starch and lignocellulose to sugars requires hydrolysis of the polysaccharides to form simple sugars. Polysaccharide hydrolysis can be performed with mineral acids (*e.g.,* sulfuric); however, acid hydrolysis of cellulose suffers from high acid costs or low yields. As a result, to produce simple sugars, all commercial biological processes that use starch and lignocellulose employ enzymes.

Although the enzymatic hydrolysis of starch and lignocellulose are conceptually similar, cellulose is far more resistant to enzymatic hydrolysis than starch. Starch hydrolysis is readily achieved using amylase enzymes. In contrast, hydrolyzing cellulose and hemicellulose to fermentable sugars requires complex mixtures of cellulases, hemicellulases, and other enzymes *(4, 9)*. Furthermore, because of the recalcitrance of lignocellulose, much more enzyme — 10 to 100 times more — is required to achieve high sugar yields.

In addition, the complex lignocellulose structure (lignin, cellulose, hemicellulose, and other components) creates obstacles that impede enzyme hydrolysis and reduce sugar release rates significantly. The amount of cellulase enzymes needed to achieve high yields costs about $1.00/gal ethanol ($1.50/gal equivalent gasoline) *(6)*. Thus, the key opportunities are in:

• improving pretreatments to reduce enzyme requirements
  • recycling enzymes inexpensively
  • reducing enzyme production costs
  • significantly enhancing enzyme effectiveness.

For over three decades, considerable work has been devoted to the latter two strategies. Thus, at this point, efforts aimed at inexpensive enzyme recycling and improving pretreatments may produce bigger impacts. In addition, major dividends will result from devoting more systematic attention to understanding mechanisms by which pretreatment overcomes biomass recalcitrance, as well as improving pretreatment technologies to make the pretreated solids more amenable to hydrolysis by much lower enzyme doses *(7, 8)*.

To this end, a pretreatment strategy using tetrahydrofuran (THF) as a miscible cosolvent in water containing

very dilute acid has achieved close to theoretical yields. The combined pretreatment and enzymatic hydrolysis of the pretreated solids recovered nearly all of the sugars that could be produced from the cellulose and hemicellulose using less than one-tenth the enzyme doses needed with conventional pretreatments *(10)*. This novel pretreatment, called cosolvent enhanced lignocellulosic fractionation (CELF), recovers virtually all of the sugars from hemicellulose in solution while also removing about 90% of the lignin from biomass to produce solids that are highly enriched in cellulose. Because THF is far more volatile than water, it can be readily recycled to keep operating costs low. The high degree of lignin and hemicellulose removal during CELF appears to be at least partially responsible for such enhanced enzyme effectiveness at low loadings, but further research is in progress to better understand controlling mechanisms.

Fermentation

Figure 1 suggests that fermenting sugars from lignocellulose is similar to fermenting sugars from sugarcane and corn starch. This is an oversimplification. Cane sugar contains predominantly sucrose with small amounts of glucose and fructose, all of which are readily fermented. Similarly, starch contains only glucose. In contrast, lignocellulose contains large amounts of hemicellulose, which is comprised of several different sugars. Conventional yeast or other fermentative organisms cannot fully convert the five-carbon sugars arabinose and xylose to ethanol. Fortunately, microorganisms have been genetically modified so that this obstacle has been largely overcome *(11)*.

However, another important obstacle remains: ethanol concentration. Cane and corn sugars can be readily mixed at sufficiently high concentrations in fermenters to reach ethanol concentrations of up to about 15%, which is the approximate upper limit of ethanol tolerance by yeast. At this concentration, ethanol recovery by distillation is attractive.

In contrast, loose, uncompacted lignocellulose solids cannot be readily mixed at high concentrations. For example, a mixture of about 10% loose biomass (*e.g.,* straw) and water contains no free water, and cannot be stirred or pumped effectively. Furthermore, the solids contain only about two-thirds carbohydrates, which limits fermentation yields to about 5% ethanol or less at mixable overall solids levels *(12)*. Although 5% ethanol concentrations can be recovered at reasonable cost, significant advantages would accrue if ethanol concentrations could be increased to approximately 12%.

Fed-batch and continuous fermentations can approach this target, because enzymatic hydrolysis of solid lignocellulose releases soluble sugars (glucose and others) that keep the suspended solids concentrations within limits amenable to mixing, provided solid feed rates are properly controlled. However, most fermentation research is con-

ducted in a simple batch mode in which all of the ingredients are added at the start. Unfortunately, very little attention has been focused on developing and improving continuous or fed-batch fermentations to reach higher ethanol concentrations, at least partly due to the complexity of running continuous fermentations in conjunction with continuous feeding of solids *(13)*.

In this regard, ammonia fiber expansion (AFEX) pretreatment can achieve high solids loadings that enable high ethanol concentrations. AFEX treats damp lignocellulose with ammonia for a few minutes at elevated temperatures, and then rapidly releases the pressure to recover and recycle the ammonia. After AFEX pretreatment, the biomass is easily pelletized without requiring high temperatures or added binders. The resulting pellets are very durable and can be handled, shipped, and stored like corn. Importantly, AFEX biomass pellets do not absorb nearly as much water as loose biomass. Pellets of AFEX-treated corn stover (straw) have been effectively hydrolyzed at solids loadings of up to 36% *(14)*.

Enzymatic hydrolysis and fermentation may be conducted in series, as separate hydrolysis and fermentation (SHF). However, enzymes are inhibited by sugars released during pretreatment and enzymatic hydrolysis. Thus, to realize acceptable yields, sugar concentrations and therefore ethanol concentrations must be kept low, or even more enzyme must be added.

Decades ago, researchers found that adding yeast along with enzymes would convert glucose and other sugars to ethanol virtually as soon as they were released by enzymatic hydrolysis *(15)*. Because ethanol is far less inhibitory to enzymes than are sugars or sugar oligomers, the result was higher ethanol concentrations, faster rates, and higher yields for a given enzyme loading by this so-called simultaneous saccharification and fermentation (SSF) route. Most SSF experiments are conducted in a batch mode, but fed-batch or continuous operations could make lower enzyme loadings effective, because the lower amount of unreacted lignocellulose in a continuous stirred tank reactor would need less enzyme to achieve high conversions *(13)*.

A new bioconversion process that reduces the amount of unreacted lignocellulose is called rapid bioconversion with integrated recycle technology (RaBIT) *(16)*. RaBIT takes advantage of the fact that enzymatic hydrolysis is rapid during the first 24 hr or so, and then slows dramatically as unreacted cellulose accumulates. To take advantage of the initial high-rate period, the hydrolysis mixture is centrifuged after 24 hr to remove unreacted solids and to recover a clean sugar stream containing no solids. Unreacted solids (about 40–50% of the initial mass) are then mixed with fresh enzyme (about half the initial amount) and additional fresh pretreated biomass to continue the hydrolysis. After another 24 hr of hydrolysis, the centrifugation and addition of fresh

> ## Metabolic engineering aims to change cellular pathways so organisms can both produce appropriate enzymes and ferment the sugars to ethanol at high yields.

biomass and enzyme are repeated for as many cycles as desired. The hydrolyzed sugars are fermented at high cell loadings to achieve rapid fermentation. Because both hydrolysis and fermentation are conducted at high rates, reaction vessels are much smaller.

RaBIT also permits easy recycle of about half of the enzyme, which is adsorbed to the unhydrolyzed solids. Excess cell mass can be easily separated from the clean sugar stream and has potential commercial use as animal feed. In contrast, when cells are mixed with residual biomass solids, using excess cell mass is much more difficult. Despite process improvements such as SSF and RaBIT, enzyme loadings needed to achieve high yields are still quite high and must be further reduced, perhaps by improved pretreatments.

Enzyme production

The most common source of cellulase and hemicellulase enzymes is an aerobic fungus (*Trichoderma ressei*), which the U.S. Army first isolated from rotting cotton shelter halves in the South Pacific after World War II. Production of fungal enzymes is expensive, because the fermentation is slow and requires considerable power to introduce and disperse small air bubbles.

An alternative strategy employs anaerobic fermentative organisms that produce their own enzymes, thereby combining enzyme production, enzymatic hydrolysis, and sugar fermentation to reduce capital costs and power requirements *(17, 18)*. For example, this consolidated bioprocessing (CBP) approach has achieved very high conversion (about 90%) of cellulose in poplar wood pretreated with only hot water in reasonable time periods (about seven days) without adding any external enzymes — much higher yields than are possible with moderate enzyme loadings in a conventional approach.

Conventional fermentative organisms (*e.g.,* yeast) that produce ethanol at high yields and concentrations cannot produce appropriate enzymes that hydrolyze lignocellulose. Conversely, naturally occurring fermentative organisms that do produce cellulase and hemicellulase do not produce ethanol at high yields and concentrations.

To overcome this challenge, metabolic engineering of organisms aims to change cellular pathways so that organisms can both produce appropriate enzymes and ferment the sugars to ethanol at high yields. With some success, enzyme production pathways have been introduced into yeast *(19)*, but making the complex array of enzymes required is very challenging. In an alternative approach, the bacterium

Clostridium thermocellum produces a cellulosome, a teth-ered array of enzymes that is more effective than mixtures of fungal enzymes.

A promising enzymatic route to overcoming biomass recalcitrance appears to be modifying *C. thermocellum* or other effective CBP organisms that produce their own highly effective enzymes anaerobically and also effectively ferment the sugar product to realize high ethanol yields. Similarly, combining CBP organisms with effective pretreatment, hydrolysis, and fermentation approaches (*e.g.,* RaBIT or THF CELF) is very promising.

Product recovery

The final processing step of all three sugar platform pathways is distillation of the fermentation broth to recover virtually pure ethanol in the overhead, and solids, water, and other low-volatility compounds in the bottoms. However, once again, important distinctions exist. With cane sugar and glucose from wet milling of corn, it is relatively simple to recover ethanol from the fermentation broths, because the liquid contains primarily water, ethanol, yeast, and nutrients. In contrast, the distillation process for lignocellulose-derived ethanol is more complex (Figure 4).

Regardless of the feedstock, ethanol is concentrated to its azeotrope (about 95% ethanol by weight) in the rectifica-tion column above the feed tray, and the remaining water is typically removed from the azeotropic solution by molecular sieves to produce fuel-grade ethanol — which must be virtu-ally water-free to prevent phase separation in gasoline. Other materials entering from the fermenters drop down from the feed tray through the beer column to the reboiler.

In the sugarcane pathway, the stream leaving the bottom of the beer column is called vinasse, which can be spread on fields to provide nutrients and water for growing new crops. In the corn wet mill pathway, the yeast and nutrients in the column bottoms are added to animal feed. In the corn dry mill pathway, all of the ingredients in the corn kernel that are not fermented to ethanol (protein, corn oil, fiber, residual starch) leave the bottom of the beer column and are dried to produce distillers dried grains with solubles (DDGS), an animal feed that contains about 27% to 30% protein.

Current lignocellulose-to-ethanol processes also employ distillation to recover fuel-grade ethanol. However, an important distinction is that the fermentation broth in these processes typically contains unconverted cellulose and hemicellulose, as well

as lignin, fermentation organisms, hydrolysis enzymes, minerals, fermentation nutrients, unrecovered pretreatment chemicals, and other components that depend on feedstock choice and upstream process features. To avoid severe plant maintenance issues, the challenges these components pres-ent for distillation must be fully addressed. For example, some pretreatments use sulfuric acid, which is subsequently neutralized with lime to produce gypsum. Gypsum (which has a reverse solubility curve) can foul heat exchanger tubes. The solids can be partially dried and used as boiler fuel to produce heat and electricity for the process *(20, 21)*. It should be noted that the broths produced by the RaBIT and the THF CELF processes consist primarily of water, ethanol, yeast, and nutrients; their similarity to corn and sugarcane ethanol avoids many of the complexities associ-ated with traditional paths to lignocellulosic ethanol.

Providing heat and power

An important distinction among the three sugar-platform pathways that has implications for energy balances and greenhouse gas emissions is how the different processes are powered.

The bagasse residues left after the sugar has been extracted from the sugarcane are burned to provide all the heat and power needed to run the fermentation facility. Because limited fossil fuel inputs are needed to produce sugarcane ethanol, the ratio of ethanol energy output to fos-sil energy inputs is very high. Furthermore, carbon dioxide released from bagasse combustion is biologically sourced, and therefore is recycled by photosynthesis without net accumulation of carbon dioxide in the atmosphere.

In contrast, the solid residues from corn ethanol production are typically used as animal feed, and the process
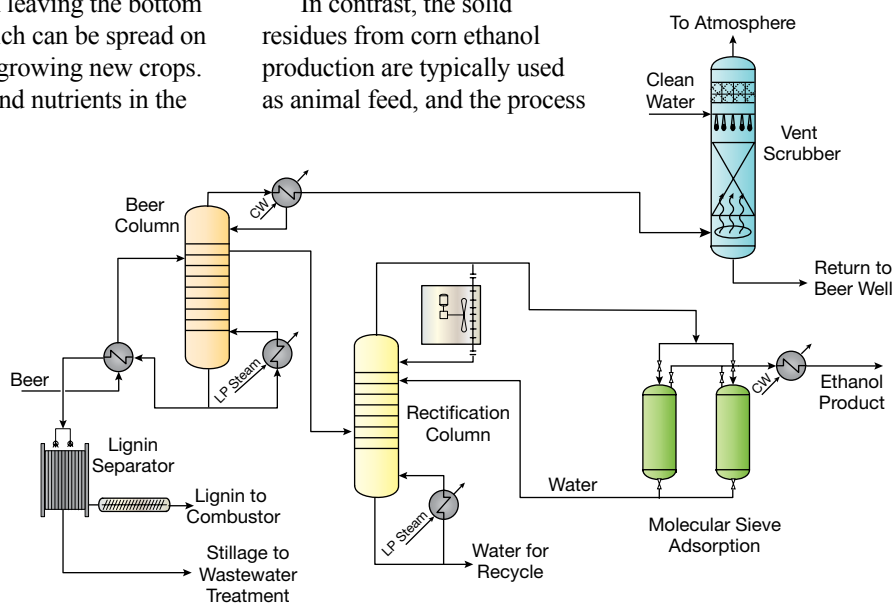
▲ **Figure 4.** Ethanol is recovered through distillation and molecular sieve adsorption. Source: *(21)*.

is fueled by burning natural gas or coal. Thus, compared to sugarcane, the ratio of ethanol energy output to fossil energy inputs is less favorable.

For lignocellulosic ethanol, burning lignin and undigested cellulosic components can provide all the heat and power needed, with excess electrical power available to export into the grid. Thus, beyond the limited fossil fuels that may be needed to transport feedstock, manufacture fertilizer, and meet other incidental needs, lignocellulosic ethanol has favorable energy ratios and significantly reduces greenhouse gas emissions compared to the use of fossil fuels *(20)*.

Economics of cellulosic ethanol production

To estimate the cost of producing cellulosic ethanol, the National Renewable Energy Laboratory (NREL) has conducted a series of techno-economic studies *(21)*. Some important findings are summarized here.

First, high yields are critical to lower costs and are a necessary, although not sufficient, requirement. For example, for a feedstock that costs about $80/dry ton at the plant gate, an ethanol yield of 80 gal/dry ton translates into a cost of about $1.00/gal ethanol (about $1.50/gal gasoline equivalent).

Because fuel ethanol is a commodity product, feedstock costs are expected to dominate; therefore, to maintain profitability, other costs must be kept low. Thus, it is important to minimize chemical usage for pretreatment, neutralization, pH adjustment, fermentation nutrients, etc. Furthermore, enzyme cost — whether the enzymes are purchased or produced onsite — must be kept low. Current costs are up to about $10/kg of enzyme protein. For typical enzyme loadings and resulting ethanol yields, this enzyme cost translates to about $1.00–$1.50/gal ethanol ($1.50–$2.25/gal gasoline equivalent) *(6)*. This projected enzyme cost is as high as or higher than the anticipated cost of the feedstock (about $1/gal), and is much higher than one would expect to pay for a catalyst used to produce a commodity product. Clearly, enzyme costs remain a challenge.

The cost of lignocellulosic ethanol can also be lowered substantially by developing CBP organisms that can achieve high product yields without added enzymes.

To achieve truly competitive fuel ethanol costs, capital costs must also be kept low. NREL capital investment projections are about $7/annual ethanol gallon ($10.40/annual gallon of gasoline equivalent) *(21),* including onsite enzyme production. Amortizing these costs would contribute approximately $1.00/gal ethanol ($1.50/gal gasoline equivalent).

Pretreatment, enzyme production, enzymatic hydrolysis, and fermentation combined are responsible for the largest portion of this overall capital cost (about 36%), with pretreatment and enzymatic hydrolysis (which are needed to overcome lignocellulose recalcitrance) the largest contributors. In addition, making or purchasing enzymes adds sig-

nificant costs. The cost of overcoming biomass recalcitrance, therefore, dominates overall processing costs.

Equipment to produce electrical power from lignin and unconverted lignocellulose accounts for another 28% of the total capital cost. Wastewater-treatment costs are significant (21%), whereas distillation, product purification, and solids recovery capital costs are low (about 10%). The remaining capital costs are for storage and utilities (5%).

Overall, these estimates highlight the importance of reducing capital costs for deconstructing lignocellulose to sugars. They also show that lignin utilization (by combustion) and wastewater treatment require significant capital investments.

In total, the combined cost of feedstock, enzymes, labor, taxes, etc., and allowing for profit and return on total capital investment, has been estimated to be about $2.15/gal ethanol ($3.27/gal gasoline equivalent) *(21)*. However, it is important to keep in mind that cost estimates are very specific to the technology, the site, and the practitioner's risk tolerance, and actual costs can vary considerably from the estimates presented here.

Closing thoughts

Lignocellulosic ethanol offers important advantages for domestic production of liquid transportation fuels and is now reaching commercial production. Because of the limited need for fossil fuels to convert lignocellulose to ethanol, fossil fuel inputs are highly leveraged and therefore reduce net carbon dioxide emissions.

To achieve high product yields, however, biological production of ethanol incurs significant costs for enzymes, as well as large capital and operating costs for pretreatment and enzymatic hydrolysis. Thus, significant advances in pretreatment can dramatically reduce processing costs and thereby accelerate commercialization of lignocellulosic ethanol. In addition, CBP is increasingly recognized as a promising path to achieve this goal by virtually eliminating enzyme production costs, provided product yields and concentrations can be improved.

Regardless of the products targeted (*e.g.,* fuels, chemicals, solvents), the same fundamental recalcitrance barriers must be overcome to achieve low costs for biological conversion of lignocellulose. **CEP**

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