Becoming independent of crude oil imports, mainly from the Middle East, is an urgent concern for many countries all over the world. In order to secure a sustainable energy supply, especially in the transportation sector, governments need to apply policies that promote the use of renewable energy technologies.

The dependence on crude oil imports decreases as the production of total energy from renewable sources (renewable liquid fuels) increases. There are two major factors that will influence the amount of final energy production from renewable sources.

First, sufficient quantities of biomass need to be acquired for conversion into liquid fuels. If a country cannot provide enough biomass, it has to compensate by importing it, which does not lead to energy independence. Biomass is a limited resource. Even though countries like the United States and Canada encompass large areas of rural land, agriculture and forestry have distinct impacts for the economy as well as for the environment. The use of biomass for conversion to energy competes with other purposes such as food supply and forestry products. Land availability and crop yields are key criteria in determining the quantities of biomass that can be obtained for energy use within a country. What is more, the production of biomass for energy use must be sustainable, which requires a thorough understanding of the environmental impacts and the likely demand scenarios for renewable fuels.

The amount of final energy obtained from domestic renewable energy also depends on the applied conversion technologies. The efficiency of processing biomass into liquid fuels affects the energy input-output ratio. The less energy needed to run the processes, the more that is available to enter
the market. Moreover, a change from the current conservative conversion methods to a new generation of technologies will highly influence the total amount of liquid fuels derived from biomass in the long term. A significant characteristic of the new generation technologies is the fact that a larger portion of the available biomass is convertible into biofuels.

Current Role of Biomass for Liquid Transportation Fuels

National governments, particularly in North America and Europe, are promoting the use of liquid fuels from renewable resources. Since the consumption of biofuel is mainly driven by its market price, various means of promoting biofuels intend to make them economically feasible for the customer. These means include tax reductions on gasoline and diesel blended with bioethanol and biodiesel, respectively, as well as on vehicles that utilize biofuels. Some countries, such as Brazil and the United Kingdom, enforce legislation that mandate a minimum biofuel blending level. In the long term, financial support for research and development in renewable derived fuels is important to establish a sustainable biofuel industry. The U.S. Department of Energy (DOE), for example, has been funding research centers and the development of commercial cellulosic ethanol plants since 2007.¹

Bioethanol and Biodiesel in the United States, Brazil, and Europe

An overview of the impact of renewable fuels for the transportation sector in the United States in comparison to Brazil and Europe (see table 8–1) shows U.S. bioethanol production to be comparable to Brazil, with Europe producing much less than either country. In comparison, biodiesel consumption in Europe exceeds that of the United States. Sources of feedstocks that are converted to biofuels vary by region.

<table>
<thead>
<tr>
<th>Table 8–1. Biofuel Consumption in the United States, Brazil, and Europe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bioethanol</strong></td>
</tr>
<tr>
<td>Output, 2006 (billion gallons)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Main sources</td>
</tr>
<tr>
<td>Market share</td>
</tr>
<tr>
<td><strong>Biodiesel</strong></td>
</tr>
<tr>
<td>Consumption, 2006 (million gallons)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Main sources</td>
</tr>
</tbody>
</table>
While the United States consists of a total of 2 billion acres of land, 33 percent are used as forestlands and 46 percent as agricultural lands, of which 26 percent is grassland or pasture and 20 percent is croplands. In 2006, 190 million dry tons of biomass per year were used for bioenergy and bioproducts. Most of the biomass is burned for energy; only 18 million dry tons are used to produce biofuels (mainly corn grain ethanol).\(^2\)

In 2004, 1.26 billion bushels of corn (11 percent of all harvested corn) were converted to 3.41 billion gallons of starch ethanol (one-third of world ethanol production).\(^3\) The ethanol was produced in 81 plants in 20 states. In 2006, 4.9 billion gallons of ethanol were produced,\(^4\) and a 5.5-billion-gallon capacity was planned until 2007.\(^5\) Although demand for fuel ethanol more than doubled between 2000 and 2004, ethanol satisfied only 2.9 percent of U.S. transportation energy demand in 2005.\(^6,7\) Over 95 percent of ethanol production in the United States comes from corn, with the rest made from wheat, barley, milo, cheese whey, and beverage residues.\(^8\)

U.S. biodiesel consumption was 75 million gallons in 2005\(^9\) and 263 million gallons in 2006.\(^10\) Total diesel fuel consumption in 2006 was 63 billion gallons. While blended in conventional diesel fuel, the most important biodiesel source is soybean.\(^11\)

Brazil reached a bioethanol output of 4.7 billion gallons in 2006, contributing to one-third of world ethanol production.\(^12,13,14\) Ethanol currently comprises about 40 percent of the total vehicle fuel used in the country.\(^15\) Brazil accounted for almost 90 percent of the ethanol imported into the United States in 2005.\(^16\)

European countries produced 13 percent of the world's ethanol. In 2006, approximately 416 million gallons were consumed (= 557,000 tons/year).\(^17\) Germany, Spain, and France were the main producers, using wheat and sugar beet as the predominant crops.\(^18,19\) Biodiesel consumption was approximately 1.2 billion gallons in 2006 (= 3,852,000 tons/year).\(^20\) Rapeseed (84 percent) and sunflower (13 percent) were the main feedstock.\(^21\) In 2005, the overall transportation energy use of liquid fuels was 290,000,000 tons/year.\(^22\)

Figure 8–1 displays the high growth rate for biofuels in Europe and the dominant role of biodiesel. Policies in Europe vary among countries, but leading biofuel consuming countries have set targets to reach a total market share of about 6 percent within the next 3 years. Current market share is about 2 percent. There are significant differences in composition of market share within Europe. However, biodiesel has the biggest share in most European countries. Only a few countries, including Poland, Sweden, and Spain, consume more bioethanol than biodiesel. Sweden and Germany consume notable amounts of biogas (methane generated by anaerobic digestion) and pure vegetable oil for transportation use, respectively.\(^23\)
Prospects of Biomass Use for Liquid Fuels within the United States

In 2006, 142 million dry tons of biomass were already used by the forest products industry for bioenergy and bioproducts. Residues from the industry include tree bark, woodchips, shavings, sawdust, miscellaneous scrap wood, and black liquor, a byproduct of pulp and paper processing. Other potential forestry biomass resources include logging and site-clearing residues (such as unmerchantable tree tops), forest thinning, fuel wood (roundwood or logs for space heating or other energy uses), and urban wood residues such as municipal solid waste (discarded furniture and packing material).

Agricultural biomass resources include annual crop residues, perennial crops, miscellaneous process residues, and grain (primarily corn). Annual crop residues are mainly stems and leaves (such as corn stover and wheat straw) from corn, wheat, soybeans, and other crops grown for food and fiber. Perennial crops comprise grasses or fast-growing trees grown specifically for bioenergy.

Figure 8-1. Production of Biodiesel and Bioethanol in the European Union 15*

* Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, The Netherlands, Portugal, Spain, Sweden, United Kingdom.

** Data for 2004 relates to EU25.
Potential of Cellulose

A DOE and U.S. Department of Agriculture joint study examined whether land resources in the United States are sufficient to harvest over 1 billion dry tons of biomass annually, which would be enough to displace 30 percent or more of the Nation's liquid transportation fuels. This study projected that 1.366 billion dry tons of biomass could be available for large-scale bioenergy and biorefinery industries by the mid-21st century while still meeting demand for forestry products, food, and fiber. Of the 1.366 billion dry tons of biomass, 368 million dry tons are obtained from forests, and 998 million dry tons are sourced from agriculture.

The potential of the "billion-ton" study is based upon conservative assumptions. The scenario displayed in this study assumes "business as usual" and therefore does not mark an upper limit. The study assumes continuing improvements, such as in yields per acre and harvesting technologies. It does not take future technologies, particularly cellulose-to-ethanol, into account. Cellulosic ethanol has the potential to meet most, if not all, transportation fuel need since it is potentially possible to harvest the 1.366 billion tons of biomass required on an annual basis. Cellulosic material, available in large quantities, will provide the feedstocks needed for biomass processing (see figure 8-2).

However, the second-generation conversion technologies that are implemented in demonstration facilities are not yet economically feasible.

figure 8-2. COMPARISON OF FOSSIL ENERGY RATIO FROM FOUR DIFFERENT ENERGY SOURCES
Today's cellulosic biorefineries convert the biomass in numerous complex, costly, and energy-intensive steps.

**Production of Cellulosic Ethanol**

Research on processing cellulosic material to ethanol has been conducted for many years. The following section gives an overview of its state of the art and will serve as an introduction to display some barriers in commercialization.

**Characteristics of Lignocellulose**

The strength and the complexity of lignocellulosic material, which makes hydrolysis difficult, are a result of its structure and composition. The strength of the cell wall is created by the network formed of hemicellulose and cellulose and the presence of lignin. The variability of these characteristics accounts for the varying digestibility between different sources of lignocellulosic biomass, which mainly consists of the polymers cellulose and hemicellulose, as well as lignin, which is nonfermentable.

Since cellulose is a heterogeneous substrate, it makes cellulolytic enzyme hydrolysis difficult to model. Cellulose is composed of chains of glucose (arranged in layered sheets) connected by beta 1–4 glycosidic bonds. The two most dominating characteristics of cellulose are as follows. The specific surface area (SSA) is defined as the amount of surface area per mass of cellulose. SSA is significant because it determines the number of enzymes that are adsorbed by the substrate. An increase in SSA leads to an increasing rate of hydrolysis. The crystallinity index states the relative amount of crystalline cellulose as opposed to the amount of amorphous cellulose. When endoglucanases (Egs) attack the amorphous regions, the crystallinity index increases.

By using enzymes to break down the polymers, nearly theoretical yields of sugars can be obtained. However, a pretreatment step is essential to achieve these high yields. In this step, cellulose is liberated from the lignin seal and from its crystalline structure, rendering it accessible for the enzymes during the hydrolysis (see figure 8–3).

**Cellulose-to-Ethanol Process Overview**

The first step of the process is biomass handling, where the size of the lignocellulose is reduced to make handling easier and ethanol production more efficient. During pretreatment, the lignin seal is broken, the hemicellulose is partially removed, and SSA is increased. After pretreatment, the cellulose and hemicellulose fractions are more accessible to enzymes in the forms of polymers and oligomers. Hydrolysis then breaks the chains into monomers.
The monomers can be fermented by natural yeast (glucose fermentation) or by genetically engineered bacteria (pentose fermentation). The result of fermentation is a mixture of water, ethanol, and residues, with carbon dioxide being formed and removed as a gas from the fermentation (see figure 8-4).

Finally, ethanol is purified in a distillation column while the residues can be either burned to power the process or converted to co-products.

Hydrolysis and fermentation can be done separately or in the same tank, which reduces costs. In a biorefinery, nonfermentable material, such as lignin, is used to power the process. Furthermore, expensive catalysts are recovered for reuse. Carbon dioxide is recycled into plant matter through production agriculture. Figure 8–5 shows the process sequence of a biorefinery.

High concentrations of sugar and ethanol should be aimed at, so that the amount of energy needed for distillation can be minimized.

**Feedstock Preparation and Pretreatment**

Handling of the feedstock is an important step because it facilitates the biochemical conversion of lignocellulosic biomass. Size reduction makes the feedstock more accessible to process media and therefore reduces enzyme loadings as well as the amounts of water and chemicals needed. After the mechanical process of milling, the biomass particles are pretreated. The two main goals of the pretreatment step are improving the rate (amount of converted monomers per time unit) of enzyme hydrolysis, and increasing the yield of fermentable sugars from cellulose or hemicellulose.

Pretreatment is one of many steps in the cellulose-to-ethanol process, but it currently represents a critical and expensive step that must take place for hydrolysis to occur. Therefore, it cannot be engineered without regard to the other steps. An effective pretreatment has several attributes, including reducing particle size and conditions that avoid degradation of pentose from hemicellulose or glucose from cellulose, and limiting formation of degradation products that inhibit growth of fermentative microorganisms (which are needed for the fermentation process). Pretreatments should also limit energy, chemical, and/or enzyme usage in order to limit the cost of the pretreatment process itself.29

![Figure 8-5. UNIT OPERATIONS OF BIOREFINERY](image-url)
The question arises of how much economic effort should be devoted to pretreatment. To answer this question, the process parameters must be balanced against their impact on the cost of all other process steps before and after pretreatment. In addition, a tradeoff between operating costs, capital costs, and biomass costs have to be taken into account.

There are two different pathways for pretreatment: physical and chemical. Comminution, or mechanically reducing the particle size, and steam explosion (see below) are physical means. Chemical pathways are adding solvents to dissolve the cellulose (an effective but expensive method) or using other chemicals.

Each pretreatment pathway has distinct effects on the chemical composition and chemical/physical structure of lignocellulosic biomass. Table 8-2 gives an overview of the effects caused by different pretreatment methods. One effect is the increase of SSA by creating pores in the cellulose structure, thus improving the micro-accessibility of the cellulases. Decrystalization of cellulose refers to disrupting the hydrogen bonds that tie up the cellulose chains. Decrystalization increases the macro-accessibility of the cellulases. The removal of both hemicellulose and lignin weakens the structure of lignocellulose, because it eliminates part of the network that accounts for the strength of the cell wall. The enzymes’ accessibility to the cellulose fibrils is

<table>
<thead>
<tr>
<th>Pretreatment Method</th>
<th>Increases specific surface area</th>
<th>Decrystalizes cellulose</th>
<th>Removes hemicellulose</th>
<th>Removes lignin</th>
<th>Alters lignin structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncatalyzed steam explosion</td>
<td>X</td>
<td>X</td>
<td>ND</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Liquid hot water</td>
<td>X</td>
<td>X</td>
<td>ND</td>
<td>X</td>
<td>*</td>
</tr>
<tr>
<td>pH controlled hot water</td>
<td>X</td>
<td>X</td>
<td>ND</td>
<td>X</td>
<td>ND</td>
</tr>
<tr>
<td>Flowthrough liquid hot water</td>
<td>X</td>
<td>X</td>
<td>ND</td>
<td>X</td>
<td>*</td>
</tr>
<tr>
<td>Dilute acid</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>*</td>
</tr>
<tr>
<td>Flowthrough acid</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ammonia fiber explosion</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ammonia recycled percolation</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Lime</td>
<td>X</td>
<td>ND</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

X = Major effect  
* = Minor effect  
ND = Not determined

improved when hemicellulose is solubilized and lignin is partially solubilized. Altering the lignin structure means partially cracking it and breaking the bonds between cellulose and its lignin sheathing. Different pretreatment methods emphasize certain effects, and the combinations of these effects increase the overall efficiency of the pretreatment.31

Pretreatment increases the yields of sugars and ethanol during hydrolysis and fermentation, respectively, when operated at optimal conditions. However, biomass pretreated at overly harsh conditions can lead to formation of degradation products, which decreases sugar and ethanol yields. The pretreatment model in figure 8–6 illustrates the process of cellulose saccharification and degradation.

Pretreatment renders the recalcitrant cellulose fraction of the biomass more accessible to enzymes by altering its structure. During this step, crystalline (C) and amorphous (C*) cellulose is converted into glucose oligomers (Gₙ). If the process conditions are too severe, the bonds in the cellulose chain will break further into sugar monomers (G), which will eventually degrade to toxic substances. The constant k represents the rate of reaction at each stage. The rates and the exposure time of the cellulose to the pretreatment environment determine the level of degradation.32

Pretreatment Technologies

Uncatalyzed steam explosion. High-pressure steam is applied to the biomass without addition of chemicals.33 The rapid thermal expansion following the compression opens up the particle structure. Then, the water itself or SO₂ acts as an acid at high temperatures. During steam explosion, acids are released. This leads to removal of hemicellulose (from the complex lignocellulosic structure), improving their accessibility to the enzymes.

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Liquid hot water pretreatments. Cooking the biomass in hot water breaks lignocellulosic particles without adding acid. Pressure is needed for this process in order to maintain the water in a liquid state. However, water pretreatment requires no neutralization or conditioning of chemicals because of the absence of acid. A highly digestible cellulose results when enzyme is added, high yields of sugars from hemicellulose occur, and more than half of the lignin is dissolved during pretreatment. By maintaining the pH constant between 5 and 7, the hemicellulose fraction is converted into oligosaccharides while minimizing the formation of monomeric sugars. Thus, degradation reactions, which would lower the yield of fermentable sugars, are hindered.

There are three methods of transporting the hot water through the biomass: co-current, countercurrent, and flowthrough. This leads to diverse appliance constructions with distinct yields and concentrations of sugar.

Experiments with corn stover have shown that about 40 percent of the lignocellulose can be dissolved during pretreatment releasing sugar oligomers. In the following hydrolysis of the pretreated biomass, nearly 80 percent of the cellulose and 70 percent of the hemicellulose have been converted into glucose and pentose, respectively. This is due to the change of the chemical composition of corn stover. In addition, the physical appearance of corn stover is affected in such a way that the surface becomes perforated. The pores increase the enzyme-accessible surface area, which increases the enzyme digestibility. The result of pretreatment, in terms of yield and avoiding degradation, is mainly a function of temperature, pressure, pH, and time. While high temperature tends to reduce the required time for pretreatment, lower temperature may be preferred since the operational pressure, and therefore the energy demand, is lower. The relevance of pH and the effect of degradation will be discussed in detail.

Acid pretreatment. Lignin is disrupted and hemicellulose is removed when dilute sulfuric acid is added to the heated biomass, enhancing digestibility of cellulose in the residual solids. The yields are very high, but so are the costs. The latter is due to:

- expensive construction materials (because of corrosion)
- the acid neutralization step (acid degrades the sugars)
- the release of natural biomass fermentation inhibitors
- the disposal of neutralization salts
- long reaction time
- high enzyme demand
- high energy demand for the cellulose grinding.

Flow-through acid pretreatment. Most of the disadvantages of acid pretreatment can be avoided when configuring the acid level at lower than 0.1
percent. The sugar yields are very high, and only few acids are released during the reaction. However, the system requires significant energy for pretreatment and product recovery as a result of the great amount of water processed.\textsuperscript{37}

**Lime pretreatment.** Lime, calcium oxide, is an alkali and can be dissolved in water, where it becomes a medium strength base. Lignocellulose can be treated with lime water at ambient conditions. Low temperature and pressure will result in long reaction times, while high temperature and pressure will accelerate the reaction. The major effect of this technology is the removal of lignin from the biomass. A limitation occurs because some of the alkali is converted to irrecoverable salts or incorporated as salts into the biomass.\textsuperscript{38}

**Ammonia pretreatment.** Liquid ammonia causes swelling of the lignocellulose, which increases SSA. In ammonia recycled percolation, ammonia reacts primarily with lignin. The ammonia depolymerizes the lignin and breaks the lignin-cellulose linkages. Ammonia fiber/freeze explosion simultaneously reduces lignin content and removes some hemicellulose while decrystallizing cellulose.

While the cost of ammonia and especially ammonia recovery drives the cost of this pretreatment, the sugar yields are very high and the enzyme loadings as well as the degradation are low.\textsuperscript{39} Table 8–3 shows the main characteristics of the technologies described above.

table 8–3. REPRESENTATIVE PRETREATMENT CONDITIONS OF CONSIDERED TECHNOLOGIES

<table>
<thead>
<tr>
<th>Pretreatment technology</th>
<th>Chemicals used</th>
<th>Temperature, °C</th>
<th>Standard atmospheric pressure, absolute</th>
<th>Reaction times (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncatalyzed steam explosion</td>
<td>Water</td>
<td>240</td>
<td>40</td>
<td>&lt;15</td>
</tr>
<tr>
<td>Dilute sulfuric acid</td>
<td>0.5%–3% sulfuric acid</td>
<td>130–200</td>
<td>3–15</td>
<td>2–30</td>
</tr>
<tr>
<td>Flowthrough acid</td>
<td>0.0%–0.1% sulfuric acid</td>
<td>190–200</td>
<td>20–24</td>
<td>12–24</td>
</tr>
<tr>
<td>Lime</td>
<td>0.05 gram–0.15 gram Ca(OH)\textsubscript{2}/gram biomass</td>
<td>70–130</td>
<td>1–6</td>
<td>60–360</td>
</tr>
<tr>
<td>Ammonia recycled percolation</td>
<td>10–15 weight percent ammonia</td>
<td>150–170</td>
<td>9–17</td>
<td>10–20</td>
</tr>
<tr>
<td>Ammonia fiber explosion</td>
<td>100% (1:1) anhydrous ammonia</td>
<td>70–90</td>
<td>15–20</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>
pH Monitoring during Pretreatment

Harsh pretreatment environments, like liquid water temperatures above 200°C and pH below 5, decrease the yield of fermentable sugars and thus the yield of ethanol. The pH decreases when the temperature decreases during pretreatment. At low pH, organic acids can form, thus leading to dissociation of hydrogen ions. The existence of hydrogen ions during pretreatment promotes autohydrolysis, or hydrolysis of cellulose to oligosaccharides and glucose in absence of enzymes. Autohydrolysis has a negative impact on the entire process for two reasons:

- Glucose tends to degrade to organic acids in the harsh pretreatment conditions. The organic acids such as furfur then form more hydrogen ions, which further accelerates autohydrolysis.
- Degradation decreases the extent of enzymatic conversion of polysaccharides and oligosaccharides to monosaccharides, which are needed for fermentation.

The reduction of pH can be avoided by means of adding KOH, which is a strong base. Controlling the pH at about 7 (neutral) results in a higher percentage of solid cellulose and a lower concentration of hydrogen ions after the pretreatment. A higher pH leads to bacterial contamination and must be avoided as well.

The formation of organic acids is relevant only to the cellulose fraction of biomass. The solubilization of hemicellulose during pretreatment, which occurs to almost 100 percent, does not result in the formation of significant amounts of acids.

The choice of chemicals used, pH, temperature, and pressure applied:

- affects the construction design of the pretreatment unit (cost of pretreatment itself)
- influences the yields from cellulose and hemicellulose
- releases cellulose and hemicellulose in distinct states of polymerization.

Due to the varying structure and composition of different lignocellulosic resources, the pretreatment methods applied should depend on the raw material used.

Hydrolysis

The rigid crystalline structure of cellulose needs to be decrystallized in order to produce ethanol. There are two pathways of hydrolyzing: the use of acids and of enzymes (the latter being focused on here). Enzymatic hydrolysis has the potential to make ethanol, derived from cellulose biomass, competitive when compared to other liquid fuels on a large scale. Cellulolytic enzymes, evolved from fungi and bacteria, are used in these processes as well as in other
industries (such as the textile industry) to liberate the individual glucose monomers that will then be fermented to ethanol. Hydrolysis refers to a mechanism of breaking the bonds of the glucose chain by adding water.\textsuperscript{42}

For improving the efficiency of the hydrolysis, it is important to gain knowledge in the substrate parameters (different crystal forms, varying degrees of crystallinity) and the enzymatic system applied (number of enzymes, synergistic effects, and product inhibition). Due to the high cost of cellulolytic enzymes, the enzymatic system needs to be optimized. The enzyme loading also depends on the results of pretreatment. The more accessible the cellulose is for the enzymes, the lower is the required enzyme loading.\textsuperscript{43}

Cellulases are enzymes that catalyze cellulolysis (hydrolysis of cellulose). Cellulases are classified into three main groups: cellobiohydrolases (CBHs), endoglucanases, and beta-glucosidases. These groups play distinct roles in the hydrolysis. While CBHs attack the chain ends of the cellulose polymers, Egs attack the amorphous regions of the cellulose chains. After breaking the cellulose, beta-glucosidase hydrolyzes the generated cellobiose to glucose.\textsuperscript{44}

The three types of enzymes operate synergistically. The effect of the most obvious synergism, called endo-exo, is that Egs generate more chain ends by random scission for the CBHs to attack.\textsuperscript{45}

The majority of the cellulases (CBHs and Egs) are modular proteins with two distinct, independent domains. The catalytic core is responsible for the hydrolysis of cellobiose from the cellulose chain. The cellulose binding domain (CBD) has a dual activity. The CBD is in charge of binding the enzyme to the cellulose layer, and it also affects the cellulose structure. After the enzyme has bound to the cellulose, it reduces the cellulose's particle size, therefore increasing SSA. Since the enzyme is connected to the cellulose layer via the CBD, the catalytic core can work independently from the CBD. This molecular architecture allows the enzyme to release the product while remaining bound to the cellulose chain. A linker region joins the independent catalytic core to the CBD (see figure 8–7).\textsuperscript{46}

**Fermentation and Separation**

The glucose monomers that are released during hydrolysis can be fermented to ethanol using yeast. Saccharomoyces are natural yeasts that feed on the glucose to produce ethanol and are currently applied in the large-scale corn-to-ethanol and sugar cane-to-ethanol industries. However, ethanol production from lignocellulose requires fermentation of not only glucose, but also pentose sugars. Saccharomoyces are not able to ferment pentose. One way to manage fermentation of pentose is the utilization of genetically modified yeasts, specifically engineered for this purpose.\textsuperscript{47}

Hydrolysis performed separately from fermentation is known as separate hydrolysis and fermentation. Cellulose hydrolysis carried out in the presence
of the fermentative organism is referred to as *simultaneous saccharification and fermentation*. Simultaneous saccharification of both cellulose (to glucose) and hemicellulose (to pentose) and co-fermentation of both glucose and pentose are realized by genetically engineered microbes.\

Ethanol is recovered from the fermentation broth by distillation. The residual lignin, unreacted cellulose and hemicellulose, ash, enzyme, organisms, and other components end up in the bottom of the distillation column. These materials may be concentrated and burned as fuel to power the process, or converted to various coproducts.

**Challenges**

Fundamental research in biochemistry has been conducted in order to achieve the state-of-the-art technologies. However, feedstocks, pretreatment, and hydrolysis as well as the use of enzymes and yeast are not yet fully understood individually and as a system. This knowledge is critical in order to make current technologies more efficient so that cellulosic ethanol can be produced on a commercial scale.

**Guideline and Time Frame for Establishing a Biofuel Industry**

The usage of cellulose biomass has the potential to become the key source for meeting America's future transportation fuel needs. As mentioned above, a sustainable biofuel industry is based on supply of sufficient biomass and on cost- and energy-efficient conversion technologies. In 2006, DOE set up a technical strategy to develop a viable cellulosic biomass to biofuel industry over the next
5 to 15 years. This strategy includes concurrent development of innovative energy crops with new biorefinery treatment and conversion processes.

The development is built on three successive phases. The research phase will provide the basis of knowledge in the fields of agriculture and biochemistry. This knowledge will be transferred into technology modules in the technology deployment phase. In the systems integration phase, all modules will be consolidated as a system to ensure productivity and sustainability. The roadmap is summarized in figure 8–8.

Research Phase

Fundamental knowledge in three critical areas—plant design, deconstruction of lignocellulose, and fermentation and recovery—has to be acquired.

The traits of current energy crops have to be optimized, since the crops play an important role in the bioenergy system. The biofuel industry strives for crops with high yields and improved robustness that are also uncomplicated to process. However, growing such crops in large quantities will require a sustainable soil ecosystem. Research in plant design will enhance understanding of cell-wall polymer organization and interactions.

Further research in pretreatment of cellulosic biomass will focus on improving hydrothermal and mechanical pathways. Also, a deeper understanding of enzyme function is required. This will lead to higher hydrolysis rates and increased microsysy robustness. A goal is to be able to redesign cellulosome (containing the full repertoire of degrading various polysaccharides) for a diversity of feedstock.

An organism needs to be found that allows simultaneous saccharification and co-fermentation—that is, not only co-fermentation of pentose and hexose, but also incorporating hydrolysis and co-fermentation in one single step, thus reducing the number of processing steps. Due to the nature of the process, such an organism must have high tolerance to stress, inhibitors, and high alcohol concentrations.

Technology Deployment Phase

In this phase, plant domestication technologies will reach a mature state. This includes availability of multiple crops for distinct regional and global agroecosystems. Furthermore, improved pretreatment procedures, enzymes, and fermentation organisms will be deployed in existing biorefineries. First results of the concurrent development of feedstock and processing technologies will be recognizable. Deconstruction of lignocellulose should become easier to handle due to the domestication of the energy crops.
Systems Integration Phase

Feedstocks and processes will be fully consolidated as a bioenergy system and will accelerate and simplify the end-to-end production of ethanol. Bioenergy systems do not only function in a single area. They also are tailored for specific regional climate and soil characteristics. Toolkits will be made available that enable rapid diagnosis and manipulation of all critical aspects of the biosystem (field and facilities).
Conclusion

Successfully establishing a cellulose-to-biofuel industry requires understanding of a complex system of parameters. Biomass availability, technological progress, international fuel markets, and fuel utilization are parameters that will play major roles in the biofuel area.

In order to make biofuels economically feasible, several requirements have to be met. First, large quantities of biomass must be made available for biofuel use in order to reduce competition with other agricultural industries (food and feed), while high crop yields will reduce agricultural land use. Sufficient production of biomass and efficient land usage are important factors for avoiding the effect of price increase for feedstock. Technological progress in both plant domestication and conversion processes will drive this development and ensure sustainability.

Biofuel production and trade have to be considered on an international stage since production externalities affect the market price. Production costs vary among countries due to different fuel sources (such as sugar cane, bagasse, corn stover, sugar beet), land availability, labor cost, and so forth. Producing biofuels in an environmentally friendly and sustainable manner is important but also cost effective. However, there are not yet international legislations or incentive measures to ensure equal market opportunities for all producing countries. Low-cost biofuel imports to the United States will therefore have an impact on the feasibility of domestic production.

Action within the automotive industry can accelerate the biofuel market share. This includes providing the vehicle market with engines that are able to drive with a variety of fuels. Due to the nature of the conversion processes, biorefineries are economically feasible when they can generate a great diversity of products, including a variety of transportation fuels. However, this variety of liquid fuels is only marketable if there is a demand for a range of biofuels. Continuing the development of flexible fuel vehicles and promoting their acceptance in society will therefore be a key in establishing a biofuel industry.

NOTES


2 Ibid., 9.


5 Breaking the Biological Barriers to Cellulosic Ethanol, 12.

6 Ibid.

7 “Petroleum Navigator.”


10 F.O. Lichts, World Ethanol and Biofuels Report 6, no. 1 (September 6, 2007).

11 de Guzman.

12 Solomon, Barnes, and Halvorsen.

13 “Bioethanol and the World.”


15 Solomon, Barnes, and Halvorsen.

16 Ibid.

17 877,000 tons/year = 36,831,000 gigajoules (GJ)/year (1 ton = 42 GJ); energy content of ethanol (LHV) = 0.0234 GJ/liter; 3.785 liters = 1 gallon.


19 “Bioethanol and the World.”

20 3,852,000 tons/year = 161,787,000 gigajoules (GJ)/year (1 ton = 42 GJ); energy content of biodiesel (LHV) = 0.034 GJ/liter; 3.785 liters = 1 gallon.

21 “Biofuels Barometer 2007.”


23 Ibid.

24 Breaking the Biological Barriers to Cellulosic Ethanol.

25 Ibid.

26 Ibid.


30 Ibid., 676.

31 Ibid.

33 Cristobal Cara et al., "Influence of Solid Loading on Enzymatic Hydrolysis of Steam Exploded or Liquid Hot Water Pretreated Olive Tree Biomass," *Process Biochemistry* 42, no. 6 (June 2007), available at [www.sciencedirect.com/science/article/B6THB-4NC5GST2/2/66e5b58a04c97941b13dd5c22f](www.sciencedirect.com/science/article/B6THB-4NC5GST2/2/66e5b58a04c97941b13dd5c22f).


35 Wyman et al.

36 Mosier et al., "Features of Promising Technologies."

37 Ibid.

38 Ibid.

39 Ibid.


42 Mosier et al., "Reaction Kinetics, Molecular Action, and Mechanisms of Cellulytic Proteins."

43 *Breaking the Biological Barriers to Cellulosic Ethanol*, 31–32.


46 Ibid., 33–36.


51 *Breaking the Biological Barriers to Cellulosic Ethanol.*